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Fatty Acids, Tocopherols, and Phytosterol Composition of Seed Oil and Phenolic Compounds and Antioxidant Activity of Fresh Seeds from Three *Dalbergia* Species Grown in Vietnam

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Abstract: This research aimed to investigate the chemical composition of seed oils extracted from three Vietnamese *Dalbergia* species (*D. tonkinensis*, *D. mammosa*, and *D. entadoides*). The fatty acid profiles and contents of tocopherols and sterols of the seed oils, and total phenolic compounds extracted from the fresh seeds were characterized using different methods. Among the examined samples, *D. tonkinensis* seed oils showed high contents of linoleic acid (64.7%), whereas in *D. mammosa*, oleic acid (51.2%) was predominant. In addition, α- and γ-tocopherol and β-sitosterol were major ingredients in the seed oils, whereas ferulic acid and rosmarinic acid are usually predominant in the seeds of these species. Regarding sterol composition, the *D. entadoides* seed oil figured for remarkably high content of Δ5,23-stigmastadienol (1735 mg/kg) and Δ7-stigmastenol (1298 mg/kg). In addition, extracts with methanol/water (80:20, *v*/*v*) of seeds displayed significant in vitro antioxidant activity which was determined by DPPH free radical scavenging assay.

Keywords: *Dalbergia* species; DPPH free radical scavenging assay; fatty acid; phytosterol; tocopherol; total phenolic compound composition

1. Introduction

Plant-derived natural products are known for their enormous health benefits and absence of side effects in humans, and therefore have been extensively studied for practical applications [1–4]. In addition, medicinal plants are considered to be a repository of bioactive compounds with a wide range of therapeutic properties. The research direction involving medicinal plants is also accentuated by the fact that approximately 80% of healthcare in developing countries relies on traditional medicine, making any progress on identification of valuable compounds from plants important and useful [5]. Vietnam possesses an enormous biodiversity with more than 10,000 plant species and a well-established

traditional medicine system. Therefore, further advances on the identification of natural compounds with beneficial properties from plants could drive the development of new drugs and open new pathways for more efficient recovery of valuable compounds [6–10].

The *Dalbergia* genus belongs to the family Fabaceae (Leguminosae), which is listed at high confidence and is composed of approximately 300 species [11]. The geographical distribution of the genus spans over various tropical and subtropical regions including Central and South America, Africa, Madagascar, and East and Southern Asia [12–14]. The heartwood and the aromatic oils obtained from species of the genus are commercially valuable materials for furniture, crafts, and treatment of diseases. For example, *Dalbergia odorifera* T. Chen, a well-researched plant with valuable timber, is also known for its abundance of aromatic oils found in the fragrant wood. In culinary use and in traditional medicine, the heartwood of *Dalbergia odorifera* is used as a spice and vulnerary to cure various diseases, including coronary artery disease and arrhythmia, cancer, diabetes, ischemia, necrosis, and blood disorders [15–17]. In addition, the bark decoction of the plant is used for the treatment of dyspepsia and the seed oil is applicable to relieve rheumatism. Such effects are mostly due to various useful bioactivities including anti-inflammatory, antioxidant, antimicrobial, and antiplatelet activity exhibited by phenolic and volatile components in *Dalbergia* species [18–21].

However, the data regarding chemical composition and biological evaluation of seed oil of *Dalbergia* species seem to be lacking. To date, the *Dalbergia* species that have been investigated for seed oil composition include only *D. melanoxylon*, *D. odorifera*, *D. paniculate,* and *D. sissoo* [22–24]. Among them, seed oil of *D. odorifera* has gained the most attention [25–27]. In addition, investigated materials in the aforementioned studies mostly comprised the *Dalbergia* species collected from India and China. Therefore, studies on Vietnamese *Dalbergia* plants and their compounds are limited [28]. One possible reason is the limited availability of *Dalbergia* plant materials in Vietnam due to overexploitation. According to the World Conservation Monitoring Centre (WCMC), the *Dalbergia* genus was categorized as vulnerable globally [29]. However, in Vietnam, the exhaustion of the plant has been increasingly alarming and some species of the genus, such as *Dalbergia tonkinensis* and *Dalbergia mammosa*, have been classified as second-grade state-protected trees [30]. Further knowledge on the chemical composition of seeds of Vietnamese *Dalbergia* species could contribute to the preservation of this huge genetic potential and allow a more sustainable and biobased utilization of the plant in agricultural and medicinal applications.

Therefore, the objective of this study was to evaluate the chemical composition of seed oil extracted from several Vietnamese species of the *Dalbergia* genus. The fatty acid, tocopherol, and phytosterol composition of seed oil, as well as the characterization of the phenolic compounds of seeds from *D. mammosa*, *D. tonkinensis,* and *D. entadoides* species were investigated. Moreover, the in vitro antioxidant activities (DPPH radical scavenging activity) of the three Vietnamese *Dalbergia* seed oils were analyzed. This study contributes to the understanding of the value of seed oils of some species belonging to the genus *Dalbergia* and provides necessary guidelines for future studies on food chemistry and industrial applications.

2. Materials and Methods

2.1. Plant Material

Three *Dalbergia* species (Fabaceae) (Table 1) were collected in southern Vietnam in 2016. Voucher specimens were kept at the Department of Organic Biochemistry, Institute of Natural Products Chemistry, Vietnam Academy of Science and Technology, Hanoi, Vietnam. Samples were identified, assigned herbarium numbers, and then stored at 4 °C for further experiments.

Table 1. List of three Vietnamese *Dalbergia* species.

	Code	Scientific Name	Collecting Place
1	VNMN-B2016.109	*D. entadoides*	Phu Quoc-Kien Giang Province
2	VNMN-B2016.114	*D. mammosa*	Cat Tien-Đong Nai Province
3	VNMN-B2016.1	*D. tonkinensis*	Dau Tieng-Binh Duong Province

2.2. Oil Extraction

Soxhlet extraction was performed to obtain the oils from three *Dalbergia* species using the modified method of ISO 659:2009 [31]. In brief, 10 g of sample material were ground in a ball mill, and then extracted in a Twisselmann apparatus for 6 h with 200 mL of petroleum ether. Afterwards, the solvent was removed by a rotary evaporator at 40 °C and 25 Torr. The oil was dried by a gentle stream of nitrogen and stored at −20 °C until use.

2.3. Analysis of Fatty Acid, Tocopherol, and Sterol Compositions

For the determination of the fatty acid composition, gas chromatography was applied following the method of ISO 5509:2000 [32]. To be specific, 10 mg of oil was dissolved in 1 mL of petroleum ether in a vial, followed by introduction of 25 µL of a methanolic solution of 2 M sodium methoxide and vigorous stirring for 1 min. Next, 20 µL of water was added and after centrifugation, the aqueous solution was removed. Then, 20 µL of 0.1 N HCl was added with methyl orange as the pH indicator. Following a thorough stirring, the lower aqueous phase was discarded, and the upper organic phase was dried by sodium sulphate. A Hewlett-Packard Gas Chromatography Instrument Model 5890 Series II/5989 A80 equipped with a 0.25 mm ZB-1 fused-silica capillary column (30 m × 0.25 µm i.d., Phenomenex, Torrance, CA, USA) was used to analyze the dried product. The carrier gas was helium at a flow rate of 1.0 mL/min.

HPLC analysis was employed to determine tocopherol according to the method of ISO 9936:2006 [33]. A Merck Hitachi low-pressure gradient system was used to analyze the sample containing 250 mg of oil dissolved in 25 mL heptane. The system was equipped with an L-6000 pump, a Merck Hitachi F-1000 fluorescence spectrophotometer (detector wavelengths at 295 nm for excitation, and at 330 nm for emission) and Chemstation integration software. A Spark marathon autosampler (Emmen, The Netherlands) was used to inject 20 µL of the sample onto a Diol phase HPLC column (250 mm × 4.6 mm i.d. Merck, Darmstadt, Germany), which was used at a flow rate of 1.3 mL/min. The mobile phase used was heptane/tert-butyl methyl ether (99 + 1, *v*/*v*). The results were given as mg vitamin E/100 g oil.

The modified method of DGF-F-III 1 (98) [34] was used to determine the phytosterol composition. First, saponification of the oil sample (250 mg) was conducted with 20 mL of 2 N ethanolic potassium hydroxide solution under reflux. The unsaponifiable components were subjected to purification by an aluminium oxide column (Merck, Darmstadt, Germany) and subsequently, by thin layer chromatography on a basic silica TLC plate (Merck, Darmstadt, Germany). GLC with betulin as the internal standard was used to determine the composition of the sterol fraction re-extracted from the TLC material. To separate the compounds, a SE 54 CB (Macherey-Nagel, Düren, Germany; 50 m long, 0.32 mm ID, 0.25 µm film thickness) was used. Parameters for the GLC included the following: hydrogen as the carrier gas, a split ratio 1:20, injection and detection temperature adjusted to 320 °C, and a temperature program 245 °C to 260 °C of 5 °C/min. For peak identification, either standard compounds (β-sitosterol, campesterol, and stigmasterol) or a mixture of sterols isolated from rapeseed oil (brassicasterol), or a mixture of sterols isolated from sunflower oil (Δ7-avenasterol, Δ7-stigmasterol, and Δ7-campesterol) was used. GC-MS was used to initially identify other sterols. Then, identification was done by comparing the retention time.

The results for fatty acids, tocopherols, and sterols were calculated on the seed oil.

2.4. Determination of Total Phenolic Compounds

Powdered seeds (1.0 g) were extracted with 5 mL methanol/water (80:20 *v*/*v*) using ultrasonic treatment (30 min, room temperature). The supernatants were filtered through a Whatman Grade 1 filter paper, and then stored at 4 °C for analysis. The Folin–Ciocalteu method [35] was adopted to determine the concentration of total phenolic compounds and the results were expressed in milligrams of gallic acid (GAE) per gram of sample. A standard curve with gallic acid was prepared from 400 to 1000 mg/L. The amount of total phenolic compounds was calculated using this standard curve. Values presented are means resulting from triplicate experiments.

The HPLC analyses were conducted using a HPLC/DAD system (VWR, Hitachi, Germany), equipped with a reversed phase C18 column (Lichrosphere 100 RP-18e (5 µm, 250 × 4 mm), Merck, Darmstadt, Germany). During the analysis, the column temperature was set to 23 °C. Water/formic acid (99.9:0.1, *v:v*) (solvent A) and acetonitrile/formic acid (99.9:0.1, *v:v*) (solvent B) was used as mobile phase at a flow rate of 1.0 mL/min with the following gradient program: 100% A, 0–5 min; 95% A/5% B, 5–35 min; 65% A/35% B, 35–45 min; 45% A/55% B, 45–55 min; 20% A/80% B, 55–60 min; 20% A/80% B, 60–63 min; and 100% A, 63–70 min. The flow rate was 1 mL/min, and the injection volume was 10 µL. The detection was conducted on a diode array detector L-2455 (Mertck Hitachi, Darmstadt, Germany) at wavelength 280 nm. The software, EZ Chrome Elite, was used for the acquisition and evaluation of the data. Quantification of phenolic compounds was achieved using a known quantity of p-hydroxycinnamic acid as the internal standard with a maximum at 280 nm.

2.5. Determination of Antioxidant Activity with the DPPH Free Radical Scavenging Method

Antiradical activity of extracts obtained with methanol/water (80:20, *v*/*v*) was measured by DPPH (2,2-diphenyl-1-picryl hydrazyl) assay and compared to that of ascorbic acid (vitamin C of Sigma, USA). Determination of the DPPH radical scavenging activity was carried out following the modified method of Saeed et al. [35]. First, 0.5 mL of 2,2'-diphenyl-1-picrylhydrazyl (DPPH) solution (50 mg/100 mL) was diluted in 4.5 mL of methanol, followed by the addition of 0.1 mL of extract at various concentrations dissolved in methanol. Then, the mixture underwent vigorous shaking, followed by incubation at RT for 45 min in the dark. A spectrophotometer was used to measure the absorbance at 517 nm against the blank (without any extract). The SC50, defined as the required concentration in which 50% of the initial DPPH radicals was quenched, was calculated from a calibration curve established with different concentrations of extracts.

2.6. Statistical Analyses

The Statistical Package for the Social Sciences (SPSS) software was used to analyze the reliability and validity of the data and to compare the differences among studied values with a significance level of $p < 0.05$. All determinations were carried out in triplicate.

3. Results and Discussion

3.1. Oil Content

Analysis of the total lipid content of all the analyzed species showed only a small amount of oil which ranged from 2.5% for *D. entadoides* and *D. tonkinensis* to 8.2% for *D. mammosa* (Table 2). This is consistent with studies of Augustus and Seiler and Badami et al. who found only small amounts of oil in seeds from two *Dalbergia* species, ranging from 4.8% (*D. sissoo*) to 7.4% (*D. paniculatae*) [22,23].

3.2. Fatty Acid, Tocopherol, and Sterol Compositions

The fatty acid compositions are summarized in Table 2. The oil of *Dalbergia* seeds is characterized by common fatty acids with 16, 18, or 20 carbon atoms. The predominant fatty acids existing in *D. mammosa* and in *D. tonkinensis* are oleic acid (51.2%) and linoleic acid (64.7%), respectively.

D. entadoides species contains comparably high amounts of oleic acid (25.1%) and linoleic acid (23.0%). However, in comparison to the other *Dalbergia species*, *D. entadoides* had a statistically significantly ($p < 0.05$) higher percentage of linolenic acid (7.3%). In another study, seed oil of *D. odorifera*, was reported to contain linoleic acid (60.0%), oleic acid (17.5%), and palmitic acid (16.7%) [27], which is similar to the current composition of *D. tonkinensis*. The fatty acid composition of *D. mammosa* was comparable to that of *D. paniculata* where palmitic acid (17.8%), oleic acid (48.2%), and linoleic acid (22.5%) were found as the main representatives [23]. One noticeable feature of *D. entadoides* seed oil is the relatively high content of behenic acid (22:0) (15.3%) over that of *D. mimosa* (3.4%), *D. tonkinensis* (0.3%), and most plant seeds except for *Arachis hypogaea* in which behenic acid accounts for 27.0% of the total lipid content [36]. The fatty acid composition of *D. tonkinensis* is very similar to that of sunflower oil which is characterized by a high content of linoleic acid, a moderate content of oleic acid, and nearly 82% total unsaturated fatty acids. In addition, there were significant differences ($p < 0.05$) of UFA components of three *Dalbergia* species. The *D. tonkinensis* species had the highest portion of UFA with 81.8%, followed by those of *D. mammosa and D. entadoides* with 74.5% and 56.7%, respectively. Similar to the UFA components, the contents of omega 3, omega 6, and omega 9 of the three *Dalbergia* species were distinctly different. The omega 6 content of *D. tonkinensis* accounted for 64.7%, which was about three times higher than those of the other two investigated *Dalbergia* species. *D. mammosa* had the highest proportion of omega 9 (52.5%), which was nearly two times higher than that of *D. entadoides* and approximately five times higher than that of *D. tonkinensis.*

Table 2. Total fat content (%) and fatty acid composition (%) of three *Dalbergia* species.

Fatty Acid Composition	*D. entadoides*	*D. mammosa*	*D. tonkinensis*
Total lipid content *	$2.7^{b} \pm 0.13$	$8.2^{a} \pm 0.09$	$2.5^{b} \pm 0.11$
16:0	$16.9^{a} \pm 0.04$	$12.0^{c} \pm 0.02$	$13.2^{b} \pm 0.02$
16:1($n-7$)	$0.5^{a} \pm 0.01$	$0.2^{b} \pm 0.001$	$0.2^{b} \pm 0.05$
17:0	$0.7^{a} \pm 0.30$	$0.2^{b} \pm 0.002$	$0.1^{b} \pm 0.03$
18:0	$6.5^{b} \pm 0.02$	$6.6^{a} \pm 0.01$	$4.5^{c} \pm 0.04$
18:1($n-9$)	$25.1^{b} \pm 0.01$	$51.2^{a} \pm 0.30$	$11.6^{c} \pm 0.10$
18:1($n-11$)	$0.5^{b} \pm 0.004$	$0.6^{b} \pm 0.002$	$3.6^{a} \pm 0.20$
18:2($n-6$)	$23.0^{b} \pm 0.02$	$20.1^{c} \pm 0.01$	$64.7^{a} \pm 0.05$
18:3($n-3$)	$7.3^{a} \pm 0.03$	$1.2^{c} \pm 0.02$	$1.5^{b} \pm 0.03$
20:0	$1.4^{b} \pm 0.05$	$1.9^{a} \pm 0.03$	$0.1^{c} \pm 0.004$
20:1($n-9$)	$0.5^{b} \pm 0.002$	$1.3^{a} \pm 0.04$	$0.1^{c} \pm 0.002$
22:0	$15.3^{a} \pm 0.10$	$3.4^{b} \pm 0.002$	$0.3^{c} \pm 0.001$
24:0	$2.5^{a} \pm 0.003$	$1.3^{b} \pm 0.01$	<LOQ
SFA	$43.3^{a} \pm 0.01$	$25.5^{b} \pm 0.04$	$18.2^{c} \pm 0.04$
UFA	$56.7^{c} \pm 0.05$	$74.5^{b} \pm 0.01$	$81.8^{a} \pm 0.05$
Omega-3 ($n-3$)	$7.3^{a} \pm 0.02$	$1.2^{c} \pm 0.03$	$1.5^{b} \pm 0.01$
Omega-6 ($n-6$)	$23.0^{c} \pm 0.30$	$20.1^{b} \pm 0.04$	$64.7^{a} \pm 0.05$
Omega-9 ($n-9$)	$25.5^{b} \pm 0.01$	$52.5^{a} \pm 0.04$	$11.7^{c} \pm 0.01$

* with regard to fresh seeds. In every row, the values with the same exponent have no statistically significant difference with $\alpha = 5\%$.

3.3. Tocopherol Composition

The total content of tocochromanols in the seed oil varied from 8.5 mg/100 g (*D. entadoides*) to 36.2 mg/100 g (*D. mamosa*) with α- and γ-tocopherol being predominant tocochromanols (Table 3). While γ-tocopherol dominated in *D. mammosa* (20.3 mg/100 g), α-tocopherol was most abundantly found in *D. tokinensis* (20.0 mg/100 g). In comparison to the two other species, *D. entadoides* seed oil contained tocochromanols in a much lower quantity with α- and γ-tocopherol detected in similar amounts (3.8 mg/100 g and 2.7 mg/100 g, respectively). γ-Tocopherol was not found only in *D. tonkinensis*. However, *D. tonkinensis* seed oil contained noticeable amounts of β-tocopherol (2.1 mg/100 g) and

δ-tocopherol (1.0 mg/100 g). Tocotrienols were also found in *D. entadoides* (1.1 mg/100 g (α-tocotrienol)) and *D. tonkinensis* (2.1 mg/100 g (β-tocotrienol)).

Table 3. Tocopherol compositions (mg/100 g) of three *Dalbergia* species, calculated for the oil extracted from the fresh seeds.

Species	α-T	α-T3	β-T	γ-T	β-T3
D. entadoides	$3.8^{c} \pm 0.05$	1.1 ± 0.03	$0.9^{b} \pm 0.003$	$2.7^{b} \pm 0.01$	<LOQ
D. mammosa	$14.9^{b} \pm 0.02$	<LOQ	$0.3^{c} \pm 0.02$	$20.3^{a} \pm 0.05$	<LOQ
D. tonkinensis	$20.9^{a} \pm 0.04$	<LOQ	$2.1^{a} \pm 0.01$	<LOQ	2.1 ± 0.01
Species	P8	γ-T3	δ-T	δ-T3	Sum
D. entadoides	<LOQ	<LOQ	<LOQ	<LOQ	$8.5^{c} \pm 0.04$
D. mammosa	0.8 ± 0.03	<LOQ	<LOQ	<LOQ	$36.2^{a} \pm 0.05$
D. tonkinensis	<LOQ	<LOQ	1.0 ± 0.02	<LOQ	$26.1^{b} \pm 0.03$

* LOQ, limit of quantitation; T, tocopherol; T3, tocotrienol; P8, plastochromanol-8. In every column, the values with the same exponent have no statistically significant difference with α = 5%.

Lianhe et al. described a high total content of tocopherols for seed oil from *D. odorifera* with 511.9 mg/kg [27]. This is much higher than the total amount found in the three *Dalbergia* species of this investigation. The pattern of tocopherols presented by Lianhe et al. for *D. odorifera* seed oil was comparable to the pattern for *D. mammosa* with a higher content of γ-tocopherol (160.8 mg/kg) and a lower amount of α-tocopherol (351.1 mg/kg). In contrast to *D. odorifera* seed, oil from *D. entadoides* and *D. tonkinensis* showed higher amounts of α-tocopherol, and lower amounts or no of γ-tocopherol.

3.4. Sterol Composition

Seed oils of *Dalbergia* species are characterized by the existence of different phytosterols including campesterol, stigmasterol, Δ5,23-stigmastadienol, β-sitosterol, sitostanol, Δ5-avenasterol, and Δ7-stigmastenol in varying amounts (Table 4). The total amount of phytosterols in the seed oils varied between 534.6 mg/kg (*D. tonkinensis*) and 6658 mg/kg (*D. entadoides*) with β-sitosterol being the major constituent in the seed oils of *D. entadoides* (1781 mg/kg) and *D. mammosa* (1878 mg/kg). In seed oil of *D. tonkinensis*, only 156.6 mg/kg of ß-sitosterol were found. Remarkably, *D. entadoides* was abundantly constituted by the high content of Δ5,23-stigmastadienol (1735 mg/kg) and Δ7-stigmastenol (1298 mg/kg), contrasted by the significantly lower amounts in the other species.

Table 4. Sterol compositions (mg/kg) of three *Dalbergia* species, calculated for the oil extracted from the fresh seeds.

Phytosterol	*D. entadoides*	*D. mammosa*	*D. tonkinensis*
Cholesterol	$19.9^{c} \pm 0.01$	$46.7^{a} \pm 0.01$	$23.6^{b} \pm 0.01$
Brassicasterol	$38.4^{a} \pm 0.05$	$7.6^{c} \pm 0.04$	$14.1^{b} \pm 0.03$
24-methylenecholesterol	$41.1^{a} \pm 0.01$	$6.5^{c} \pm 0.01$	$8.5^{a} \pm 0.01$
Campesterol	$266.3^{a} \pm 0.02$	$162.0^{b} \pm 0.01$	$29.6^{c} \pm 0.01$
Campestanol	<LOQ	12.3 ± 0.04	<LOQ
Stigmasterol	$274.3^{a} \pm 0.03$	$234.3^{b} \pm 0.03$	$50.2^{c} \pm 0.02$
Δ^{7}-Campesterol	$50.3^{a} \pm 0.04$	$19.6^{b} \pm 0.01$	$8.5^{c} \pm 0.04$
$\Delta^{5,23}$-Stigmastadienol	$1735^{a} \pm 0.01$	$29.7^{c} \pm 0.04$	$180.60^{b} \pm 0.03$
Chlerosterol	$64.3^{a} \pm 0.03$	$6.7^{c} \pm 0.02$	$49.5^{b} \pm 0.02$
β-Sitosterol	$1781^{b} \pm 0.01$	$1878^{a} \pm 0.01$	$156.6^{c} \pm 0.01$
Sitostanol	$347.2^{a} \pm 0.02$	$72.6^{b} \pm 0.03$	<LOQ
Δ^{5}-Avenasterol	$152.4^{a} \pm 0.04$	$127.8^{b} \pm 0.01$	$13.4^{c} \pm 0.02$
$\Delta^{5,24}$-Stigmastadienol	479.8 ± 0.03	<LOQ	<LOQ
Δ^{7}-Stigmastenol	$1298^{a} \pm 0.01$	$68.6^{b} \pm 0.02$	<LOQ
Δ^{7}-Avenastenol	$84.0^{a} \pm 0.01$	$14.3^{b} \pm 0.03$	<LOQ
Total amount	6658.0^{a}	2686.7^{b}	534.6^{c}

LOQ, limit of quantitation. In every row, the values with the same exponent have no statistically significant difference with α = 5%.

3.5. Content of Total Phenolic Compounds

Extraction with methanol:water (80:20 (*v*/*v*)) was more effective for seeds of *D. tonkinensis* than for seeds of *D. entadoides* and *D. mammosa* (Table 5) with respect to the total extractable compounds. To be specific, the amount of total phenolic compounds in seeds of *D. tokinensis* was three and four times, respectively, higher than those of *D. mammosa* and *D. entadoides* seeds. In addition, Folin–Ciocalteau assay showed that most of the compounds extracted from *D. tonkinensis* did not show the behavior of phenolic compounds. Although the amount of the total extractable compounds in *D. tonkinensis* was several times higher than those in the two other species, the amounts of total phenolic compounds in seeds of *D. entadoides* and *D. mammosa* were higher than that in seeds of *D. tonkinensis* with the a significant difference of 5%. In comparison to the results of Lianhe et al. [25] who found total phenolic compounds in *D. odorifera* seeds in the range from 135 to 563.2 mg/g depending on the extraction medium, the present investigation resulted in much lower total amounts of phenolic compounds. One possible reason could be the different solvents used [37]. While the present study used methanol/water (80:20 *v*/*v*) as the extraction solvent, Lianhe et al. utilized different kinds of solvents [26], resulting in varied yields due to the strong influence of composition and polarity of the solvent exerting on the yield of extractable and phenolic compounds.

Table 5. Total extractable compounds (EC) (mg/g), total phenolic compounds (PC) (mg/g), and DPPH free radical scavenging activity (SC50) (μg/mL) of extracts of fresh seeds of *Dalbergia* species obtained by methanol:water (80:20, *v:v*).

No	Species	EC	PC	DPPH Free Radical Scavenging Activity
1	*D. entadoides*	87.3^{c}	23.0^{b}	15.4^{c}
2	*D. mammosa*	144.1^{b}	24.8^{a}	18.5^{b}
3	*D. tonkinensis*	469.1^{a}	19.5^{c}	11.9^{d}
4	Vitamin C			26.3^{a}

In every column, the values with the same exponent have no statistically significant difference with $\alpha = 5\%$.

3.6. Antioxidant Activity with the DPPH Free Radical Scavenging Method

The extracts obtained by extraction of the three oil samples with methanol/water (80:20, *v*/*v*) exhibited strong antioxidant activity, as demonstrated by SC50 values ranging from 11.9 to 18.5 μg/mL. Ascorbic acid (Vitamin C), which serves as the standard compound, achieved a SC50 value of 26.32 μg/mL. Among the samples, the extract from *D. tonkinensis* showed the most promising antioxidant activity (Table 5). Previous studies have investigated antioxidant activity from several species of this genus including *D. sissoo*, *D. odorifera*, and *D. saxatilis* [26,38–40], in which *D. odorifera* was the most studied plant with antioxidant activity found in bark, roots, seeds, and heartwood [26,39,40].

3.7. Composition of Phenolic Fraction

The amounts of phenolic compounds extracted from fresh seed material of the different *Dalbergia* species measured by HPLC ranged from below limit of quantification to 34.5 mg/kg (Table 6). As the levels of phenolics were assessed only relatively, with the use of p-coumaric acid as the calibration standard, their actual contents could vary from those reported in Table 6. However, the results effectively illustrate the relative differences in the levels of individual analytes between the analyzed plants. Accordingly, the best source of taxifolin (34.5 mg/kg) and ferulic acid (23.8 mg/kg) was *D. entadoides*. Rosmarinic acid (27.4 mg/kg) and ferulic acid (21.8 mg/kg) were predominant phenolic acids in *D. mammosa.* Chlorogenic acid (19.8 mg/kg) and rosmarinic acid (10.6 mg/kg) were abundantly found in *D. tonkinensis* (Figure 1). Moreover, some other phenolic compounds were relatively abundant, such as naringinin in *D. entadoides* (6.0 mg/kg), p-coumaric acid in *D. mammosa* (6.7 mg/kg), and taxifolin in *D. tonkinensis* (9.8 mg/kg).

Table 6. Composition of phenolic compounds extracted by methanol:water (80:20, *v*/*v*) from the fresh seeds of three Dalbergia species (mg/kg).

Phenolic Acid	*D. entadoides*	*D. mammosa*	*D. tonkinensis*
Chlorogenic acid	$1.8^{b} \pm 0.04$	$0.4^{c} \pm 0.03$	$19.8^{a} \pm 0.02$
Gallic acid	$0.7^{a} \pm 0.01$	<LOQ	$0.7^{a} \pm 0.03$
Caffeic acid	<LOQ	<LOQ	<LOQ
Vanillic acid	$2.6^{a} \pm 0.05$	$0.4^{c} \pm 0.04$	$0.6^{b} \pm 0.01$
Isovanillic acid	<LOQ	<LOQ	2.0 ± 0.04
Vanillin	$0.7^{a} \pm 0.002$	<LOQ	$0.2^{b} \pm 0.02$
p-Coumaric acid	$2.3^{b} \pm 0.04$	$6.7^{a} \pm 0.02$	$0.6^{c} \pm 0.05$
Ferulic acid	$23.8^{a} \pm 0.4$	$21.8^{b} \pm 0.01$	$0.8^{c} \pm 0.002$
Taxifolin	$34.5^{a} \pm 0.3$	$3.2^{c} \pm 0.01$	$9.8^{b} \pm 0.4$
Rosmarinic acid	$0.7^{c} \pm 0.01$	$27.4^{a} \pm 0.3$	$10.6^{b} \pm 0.2$
Daidzein	$2.3^{a} \pm 0.04$	$1.9^{b} \pm 0.04$	$1.6^{c} \pm 0.01$
Cinnamic acid	$0.6^{b} \pm 0.004$	$0.7^{a} \pm 0.001$	$0.2^{c} \pm 0.03$
Naringinin	$6.0^{a} \pm 0.01$	$1.2^{c} \pm 0.03$	$1.4^{b} \pm 0.01$

LOQ, limit of quantitation. In every row, the values with the same exponent have no statistically significant difference with $\alpha = 5\%$.

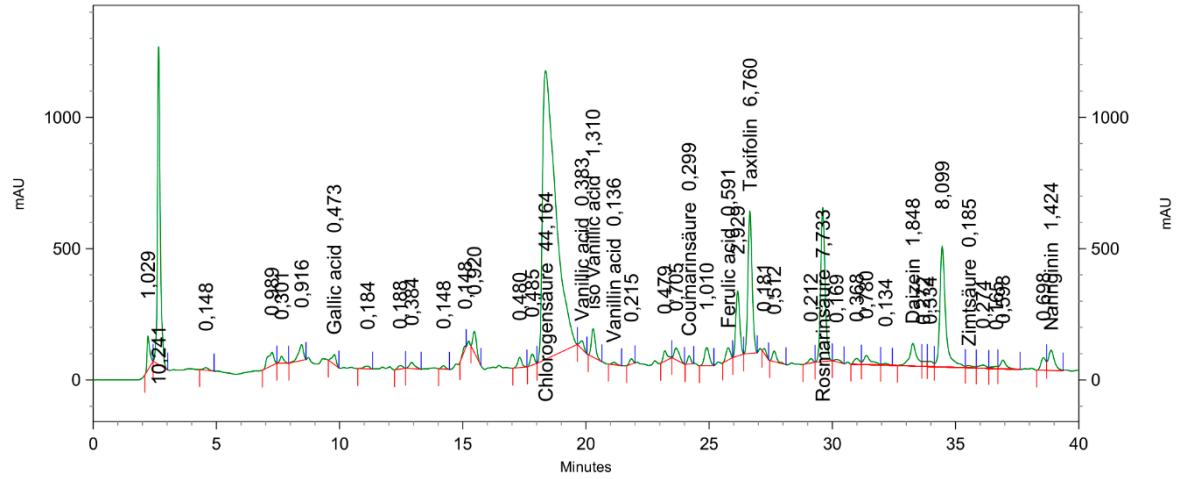

Figure 1. HPLC chromatogram of phenolic compounds of the *D. tonkinensis* seed.

In comparison to the results from the literature [37], the present work also shows the predominant position of phenolic acids as phenolic compounds found in *D. mammosa* and *D. tonkinensis*, while *D. entadoides* was characterized by a high content of flavonoids (taxifolin). In the other seed materials, significant amounts were also detected. Another flavonoid existing in the three species was naringinin, but the detected levels were low.

Overall, the composition of the phenolic fraction of fresh seeds from three *Dalbergia* species shown in the present work was characterized by multiple types of phenolic acids where rosmarinic acid, chlorogenic acid, and ferulic acid represented the highest amounts in compositions of *D. mammosa* (27.4 mg/kg), *D. tonkinensis* (19.8 mg/kg), and *D. entadoides* (23.8 mg/kg), respectively.

4. Conclusions

This study, for the first time, provides data on the fatty acid, tocopherol, sterol, and phenolic acid compositions of three *Dalbergia* seed oils grown in Vietnam. Among the examined samples, *D. tonkinensis* and *D. mammosa* seed oils showed high contents of linoleic acid and oleic acid, respectively. The α- and γ-tocopherols, β-sitosterol, ferulic acid, and rosmarinic acid are usually the major ingredients presented in these species studied. In addition, seed extracts of the *Dalbergia* species in Vietnam, including *D. entadoides*, *D. mammosa*, and *D. tonkinensis*, displayed significant antioxidant potentials

with relatively low SC50 values. Consequently, these *Dalbergia* plants should be conserved and the population should be sustained due to their potential as highly nutritional and bioactive oil sources.

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