Chemical Composition of *Dalbergia tonkinensis* Heartwood Extracts and Comparison with *Dalbergia odorifera*

Zhongmian Wei,[a] Jia Luo,[b] Ruoke Ma,[b] and Yunlin Fu [b,*]

Dalbergia tonkinensis is very similar to *Dalbergia odorifera* in material, texture, and other macroscopic characteristics, and a comparison of the commonalities and differences in the chemical composition of the extractives may help to distinguish the two. However, the chemical composition of *Dalbergia tonkinensis* heartwood is unknown. The total flavonoid content of *Dalbergia tonkinensis* heartwood was determined to range between 49.67 to 101.95 mg, which indicated that *Dalbergia tonkinensis* is as equally rich in flavonoids as *Dalbergia odorifera*. Thirty-one flavonoids were identified *via* ultra-performance liquid chromatography-mass spectrometry in *Dalbergia tonkinensis*. Among them, the contents of 15 medicinal active flavonoids with were determined, and the liquiritigenin, naringenin, formononetin, pinocembrin, and biochanin A contents were found to be high. The 12 volatile compositions of *Dalbergia tonkinensis* heartwood identified *via* gas chromatography-mass spectrometry were very similar to those of *Dalbergia odorifera* and were dominated by trans-nerolidol, caryophyllene oxide, and eudesmol. The relationship between the chemical composition of *Dalbergia tonkinensis* and *Dalbergia odorifera* heartwood extracts was determined *via* a principal component analysis, and the results indicated that there was no significant difference in chemical composition between the two. This suggested that *Dalbergia tonkinensis* could be used as a potential substitute for *Dalbergia odorifera*.

DOI: 10.15376/biores.17.4.6108-6122

Keywords: Dalbergia tonkinensis; Dalbergia odorifera; Heartwood; Chemical composition

*Contact information: a: Nanning Arboretum, Guangxi Zhuang Autonomous Region, Nanning 530000 China; wzm123908@163.com; b: College of Forestry, Guangxi University, Nanning 530004 China; 18284905882@163.com (J. L); maruoke@163.com (R.M.); *Corresponding author: fylin@126.com*

INTRODUCTION

Dalbergia tonkinensis Prain (*D. tonkinensis*), commonly known as Vietnamese "Huanghuali", is a semi-deciduous tree of the genus *Dalbergia* in the family Papilionaceae (Wu *et al.* 2013). It is native to Vietnam, primarily in the regions of Hanoi, Quang Binh, and Ninh Binh (Ma *et al.* 2016). The wood is similar to that of *Dalbergia odorifera* T. Chen (*D. odorifera*), with a hard texture, beautiful grain, strong aroma, and abundant "ghost eyes" (Qin *et al.* 2022). It is native to Hainan Province, China, and has since been introduced to Guangxi, Guangdong, Fujian, and Yunnan (Zhao *et al.* 2020). The heartwood of *D. odorifera* is highly valued for its applications and can be used to make high-end furniture; as such, it is the most expensive wood among the 29 species of mahogany in the Chinese standard GB/T18107 (2017). The wood color, aroma, structure, density, and other

wood characteristics of *D. tonkinensis* do not considerably differ from those of *D. odorifera* and is the most similar wood to *D. odorifera* on the market. In terms of its anatomical properties, that there are no major differences in the arrangement of wood rays, distribution of ducts, and other microstructure between the two (Liu *et al.* 2021).

Heartwood is rich in a large number of extractive components, which can be chemically divided into volatile and non-volatile components. The volatile components of wood are primarily terpenoids, which are the main source of aroma for the heartwood (Jones *et al.* 2011; Moniodis *et al.* 2015; Tasnim *et al.* 2020). A comparison of the volatile components of *D. odorifera* and *D. tonkinensis* using gas chromatography-mass spectrometry (GC/MS) revealed a few differences in composition, but the formation of the chemical composition of the extracts is strongly influenced by the environment in which the trees are grown (Yang *et al.* 2016). Furthermore, the available research material is often taken from processed products of unknown origin, and the differences between the two are still not sufficiently studied. The non-volatile compound components of mahogany heartwood extractives are primarily flavonoids. The flavonoid compounds not only affect the color of the wood, but are also mostly active in traditional Chinese medicine (Ninh 2017; Shao *et al.* 2019). The flavonoid compounds in *D. odorifera* heartwood have rich biological activities, *e.g.*, anti-tumour, anti-inflammatory, anti-oxidant, anti-bacterial, anti-thrombotic, and anti-angiogenic, and have the ability to treat blood disorders, anemia, swelling, necrosis, and rheumatism (Li *et al.* 2014; Choi *et al.* 2017; Fan *et al.* 2017). The flavonoid content of *D. odorifera* heartwood extract has been previously investigated by Ma *et al.* (2022), but the effects of *D. tonkinensis* have not been reported. In addition, the flavonoid composition of *D. tonkinensis* is unknown, which also limits the application of *D. tonkinensis*.

In this study, ultra-performance liquid chromatography-mass spectrometry (UPLC/MS), and GC-MS were used to identify the characteristic flavonoid and volatile components of *D. tonkinensis* and to obtain detailed chemical information on the heartwood of *D. tonkinensis*. Secondly, from the perspective of the secondary metabolic components of the plants, the differences and similarities between the chemical composition from the heartwood extracts of *D. tonkinensis* and *D. odorifera* were compared on the basis of a previous studies on the composition of *D. odorifera*, so as to provide theoretical guidance for the rational utilization of *D. tonkinensis*.

EXPERIMENTAL

Chemical Reagents

The HPLC-grade methanol and formic acid were obtained from Shanghai Macklin Biochemical Co., Ltd (Shanghai, China). Ultrapure water was prepared using a Milli-Q system (Millipore Corp., Bedford, MA). A standard solution of (C_8 through C_{40}) *n*-alkanes was purchased from Sigma Chemical Co., St. (Louis, MO). Nerolidol, formononetin, pinocembrin, prunetin, butein, isoliquiritigenin, liquiritigenin, butin, dalbergin, eriodictyol, naringenin, genistein, daidzein, tectorigenin, biochanin A, luteolin, Calycosin, and fisetin were purchased from Shanghai Yuanye Co., Ltd (Shanghai, China).

Material Sources

The *D. tonkinensis* heartwood was collected from the Quang Binh (DT1), Quang Ninh (DT2), and Hanoi (DT3) Provinces in Vietnam. Three groups of *D. odorifera* samples were collected from the Guangxi University (DO1), Liangfengjiang Forest Park (DO2), and Pingxiang City (DO3), of the Guangxi Zhuang Autonomous Region, China. Four groups of *D. odorifera* were collected in Dongfang City (DO4), Haikou City (DO5), Anding County (DO6), and Ledong County (DO7) in Hainan Province, China. All samples were identified by the Guangxi University Quality Inspection Centre according to the macroscopic and microscopic anatomical structure of the wood. The samples were crushed by a grinder (SL-100, Zhejiang Yongkang Songqing Hardware Co.) and used for subsequent analysis.

Sample Extraction

First, 0.050 g of naturally dried *D. tonkinensis* heartwood powder was added to a grinding mouth bottle, and then 5 mL of an 80% methanol (v/v) solution was added, after which the ultrasonic (40 KHz) extraction was performed using an ultrasonic extractor (KQ2200, Kunshan Ultrasonic Instruments Co., Ltd.) for 1 h. Finally, the solution was filtered through a 0.22 μm microporous membrane and stored in a -4 °C refrigerator for subsequent analysis.

Determination of the Total Flavonoid Content

The total flavonoid content of the heartwood was determined using the method reported by Ma *et al.* (2022). Briefly, the rutin standard solution (100 ug/mL) was prepared; 0, 1, 2, 3, 4, and 5 mL of this reagent were removed in 10 mL volumetric flasks; thereafter, 0.3 mL of 5% $NaNO_2$ solution and 0.3 mL of 10% $AlCl_3$ solution were added respectively, following which the flasks were shaken well and left undisturbed. Finally, 4 mL of NaOH solution was added, the solutions were fixed, and their absorbance was measured using a UV spectrophotometer at a wavelength of 510 nm. Results were used to draw the standard working curve, as shown in Eq. 1,

$$y = 0.0095x + 0.0041, R^2=0.9997 \quad (1)$$

where y is the absorbance value of the sample and x (ug/mL) is the concentration of rutin.

For total flavonoids content in naturally dried *D. tonkinensis* heartwood, sample solution (0.3 mL) was processed according to the steps of the rutin standard curve method. Total flavonoid content was expressed as the mg of rutin equivalent (RE) / g dry weight (DW).

Characterization of the Flavonoid Components and Determination of the Characteristic Flavonoid Content

The chemical composition of the flavonoids was identified *via* ultra-performance liquid chromatography in tandem with a quadrupole-electrostatic field orbit trap high resolution mass spectrometer (UPLC-Q-EXACTIVE-MS).

The UPLC conditions were as follows: an ACQUITY UPLCBEHC18 (2.1 mm × 50 mm, 1.7 μm) column; the mobile phase: A was 0.1% formic acid water and B was methanol; the gradient elution conditions: 0 min to 4.0 min, 20% to 30% B; 4.0 min to 8.0 min, 30% to 40% B; 8.0 min to 17.0 min, 40% to 55% B, 17 min to 20 min, 55% to 70% B, 20.1 min to 23 min, 100% B and, 23.1 min to 26 min, 80% B; the flow rate was 0.3 $mL \cdot min^{-1}$, the column temperature was 30 °C, and the injection volume was 1 μL.

The high-resolution mass spectrometry conditions were as follows: an ESI ion source; a temperature of 300 °C, a transmission capillary temperature of 320 °C, sheath gas of 206 KPa, an auxiliary gas flow rate of 69 kPa, and a spray voltage of 3.0 kV in positive ion mode, a scan mode of Full MS and Full MS/dd-MS^2, and a mass range of 100 to 1000 Da.

The content of the characteristic flavonoid components was determined *via* the external standard method. The 15 (formononetin, pinocembrin, butein, isoliquiritigenin, liquiritigenin, butein, dalbergin, eriodictyol, naringenin, genistein, daidzein, tectorigenin, biochanin A, calycosin, and fisetin) flavonoid controls were precisely weighed, dissolved in methanol or dimethyl sulfoxide, and configured as a 1 mg/mL control master batch. The appropriate amount of the control master batch was pipetted into the injection bottle and diluted with methanol to make standard mixtures of known gradient concentrations. A standard working curve of the flavonoids was established by the ratio of the peak area to the sample concentration.

Analysis of Volatile Components

First, 0.1 mL of extract was diluted 10 times using methanol and dried overnight by adding 0.1 g of anhydrous sodium sulfate, and the assay sample was prepared by filtration using a 0.22 um filter membrane. The volatile components of the samples were detected using a Aglient 5975C GC/MSD gas chromatography-mass spectrometer with a TG-5MS column (30 m × 0.25 mm × 0.25 μm) with the following conditions: helium gas was used as the carrier gas, the programmed temperature rise had an initial temperature 60 ℃, increasing at a rate of 20 ℃/min up to 170 ℃, and then held for 1 min and then increasing at a rate of 5℃/min up to 220℃ and then held for 2 min; no split injection; the solvent delay time was 3 min; the injection volume was 1 μL; the flow rate was 1.0 mL/min. The mass spectrometry conditions were as follows: an EI ion source; an ionization energy of 70 eV; an ion source temperature of 230 °C; and a scan range of 50 to 500 amu. Identification of the volatile oil compounds was assigned by the comparison of their Kovats retention indices relative to the (C_8 through C_{40}) n-alkanes (Sigma, Chemical Co., St. Louis, MO) with those in the literature. Furthermore, the data obtained was confirmed by matching their recorded mass spectra with those stored in the NIST05 library and related literature.

Differences in the Chemical Composition of Heartwood of *D. tonkinensis* and *D. odorifera* Compared via Principal Component Analysis

The UPLC-MS data of the *D. odorifera* and *D. tonkinensis* samples from different sources were analyzed *via* PCA to reveal the differences and commonalities between them. Preprocessing of the raw UPLC-MS data for the peak extraction, deconvolution, peak alignment, and peak integration was performed using the Compound discover v3.1 software workstation (Thermo Fisher Scientific). All ions with missing values less than 80% of all the samples were filtered from the data sets; the remaining ions with the missing values were substituted with a minimum value. The data matrix was normalized by sum. The elution conditions of the chromatographic column were changed to mobile phase: A was 0.1% formic acid water and B was methanol; the gradient elution conditions: 0 min to 2.0 min, 10% to 20% B; 2.0 min to 12.0 min, 20% to 70% B; 12.0 min to 19.0 min, 70% to 90% B, 19 min to 22 min, 90% to 100% B; 22.0 min to 24.0 min, 100% to 95% B; and 24.1 min to 27.0 min, 10% B. The chemical composition information was collected in both

positive and negative ion mode, and the rest of the parameters were consistent with the settings in Section 2.5.

Data Processing

All experiments were carried out three times. In addition, PCA was performed using the SIMCA software package. The PCA model was generated by the NIPALS algorithm. The data was processed using SPSS 19.0 analysis, and the results were expressed as the mean ± standard deviation (mean ± sd).

RESULTS AND DISCUSSION

Total Flavonoid Content of *D. tonkinensis*

Recent studies have confirmed the safety and potential anti-inflammatory, anti-tumor, antioxidant, and other biological activities of flavonoids in wood, especially in heartwood (Celedon and Bohlmann 2018). The total flavonoid content is related to the quality of the herb, and it affects the color of the wood. There are differences in the color of the heartwood from the three origins of *D. tonkinensis*, with the heartwood from the Hanoi origin being the darkest, chestnut brown, and the heartwood from Quang Binh and Quang Ninh being light brown. The total flavonoid content of *D. tonkinensis* from three origins ranged from 49.7 mg to 102.0 mg RE/g dry weight (Fig. 1), which indicated that the heartwood of *D. tonkinensis* is relatively rich in flavonoids and has potential for use in food and pharmaceutical applications. The large variation in the total flavonoids from different origins indicated that the geographical environment has a strong influence on the synthesis of flavonoids in the heartwood extracts.

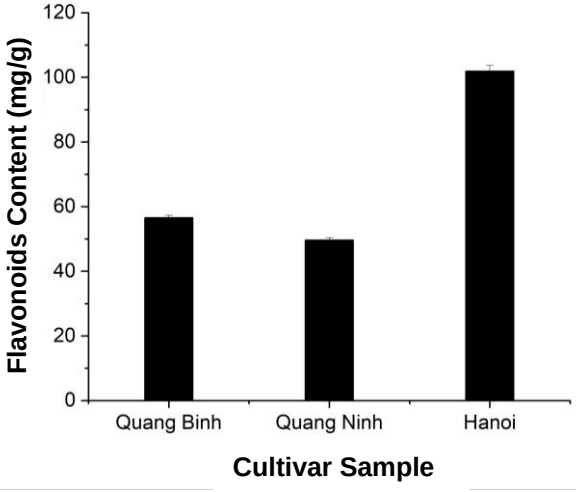

Fig. 1. Total flavonoid content of *D. tonkinensis* from different origins

Identification of the Flavonoid Components of *D. tonkinensis*

The XIC Manager module of Compound Discoverer 3.1 software was used for accurate relative molecular mass screening and analysis. In combination with the identified flavonoid components of *D. odorifera*, the mzcloud and Chemspider mass spectrometry databases were consulted to infer the possible chemical structures (as shown in Fig. 2). This analysis was based on the mass spectrometric cleavage patterns of the compounds and the mass spectra of the chemical controls, and was used to assign structural categories and

major fragment ion information (as shown in Table 1). To the best of our knowledge, this study is the first report on the flavonoid compositions of *D. tonkinensis* heartwood extracts.

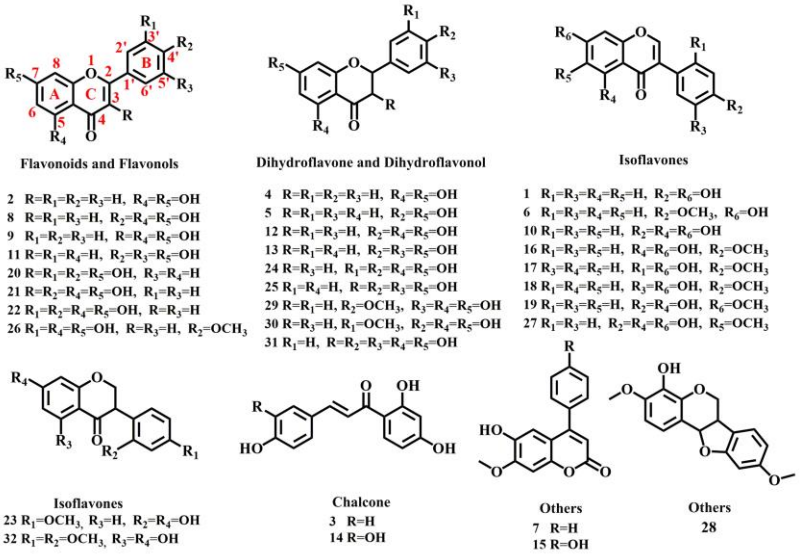

Fig. 2. The chemical structures of the compositions identified in *Dalbergia odorifera* extracts (Note: the number of the chemical components (1 through 28) corresponds to the compound numbers in Table 1)

Flavonoids and Flavonols

The mass spectral information and retention times of compound 2 (tR = 20.60), compound 20 (tR = 10.29), and compound 22 (tR = 13.76) were in general agreement with those of the Chrysin, Fisetin, and Luteolin standards control. The excimer ion peak of Chrysin was *m/z* 255.0648 $[M+H]^+$ (δ = -0.12 ppm; δ indicates the error with the theoretical molecular mass), which was cleaved by the inverse-Diels-Alder reaction (RDA) to yield flavonoid characteristic ions *m/z* 153.0181 $[A^{1,3}]^+$ and *m/z* 103.0545 $[B^{1,3}]^+$. Fisetin belongs to the flavonol group with an excimer ion peak of *m/z* 287.0544 $[M+H]^+$ (δ = -0.43 ppm); the C ring is prone to lose carbon groups and C_3 has hydroxyl substitution, which is prone to lose H_2O molecules, thus giving rise to the characteristic ion *m/z* 241.0494 $[M+H\text{-}CO\text{-}H_2O]^+$, with a continued loss of 1 molecule of CO, forming *m/z* 213.0542 $[M+H\text{-}CO\text{-}H_2O\text{-}CO]^+$, RDA cleavage characteristic ion *m/z* 151. 0069 $[A^{1,3}]^+$.

Compounds 8 (tR = 8.64), 9 (tR=12.99) and 11 (tR=16.22) are isomers with the molecular formula $C_{15}H_{10}O_5$. Compound 8 yielded ionic fragments with *m/z* 253.0492 $[M+H\text{-}H_2O]^+$, *m/z* 255.0537 $[M+H\text{-}H_2O\text{-}CO]^+$, and *m/z* 197.0589 $[M+H\text{-}H_2O\text{-}2CO]^+$, which were identified as apigenin according to the mzcloud database. Compound 9 and compound 11 were cleaved *via* RDA to yield the characteristic ions *m/z* 153.0179 $[A^{1,3}]^+$ and *m/z* 137.0594 $[A^{1,3}]^+$, which were identified as Galangin and 3',4',7-trihydroxyisoflavone, respectively, according to the reference found in Meng *et al.* (2020) and the mzcloud database.

Compound 21 (tR = 11.32) has an excimer ion peak of *m/z* 287.0546 $[M+H]^+$ (δ = -1.04 ppm), presumably with the chemical formula $C_{15}H_{10}O_6$. The loss of the CO group from the C ring forms multiple molecular fragments, including *m/z* 259.0956 $[M+H\text{-}CO]^+$, *m/z* 241.0593 $[M+H\text{-}CO\text{-}H_2O]^+$, and *m/z* 213.0538$[M+H\text{-}CO\text{-}H_2O\text{-}CO]^+$. The characteristic ion *m/z* 153.0181 $[A^{1,3}]^+$ was produced after RDA cleavage. It was identified as kaempferol based on the database information.

Compound 26 (tR=9.74), with an excimer ion peak of *m/z* 301.0699 $[M+H]^+$ (δ = -1.27 ppm), yielded characteristic ion fragments of *m/z* 286.0462 $[M+H-CH_3]^+$, *m/z* 258.0513 $[M+H-CO]^+$, *m/z* 230.1562 $[M+H-2CO]^+$, *m/z* 212.0461$[M+H-2CO-H_2O]^+$, and *m/z* 184.0514 $[M+H-2CO-H_2O-4CO]^+$, which was identified as diosmetin according to the mzcloud mass spectrometry library.

Dihydroflavone and Dihydroflavonol

Pinocembrin has an excimer ion peak at *m/z* 257.0801 $[M+H]^+$ (δ = -2.00 ppm), which upon cleavage *via* RDA yields the characteristic ion *m/z* 153.0181 $[A^{1,3}]^+$. Liquiritigenin has an excimer ion peak at *m/z* 257.0801 $[M+H]^+$ (δ = -1.60 ppm), secondary fragment-specific ions at *m/z* 239.0691 $[M+H-H_2O]^+$ and *m/z* 211.0850 $[M+H-H_2O-CO]^+$ after cleavage and characteristic ions at *m/z* 137.0230 $[A^{1,3}]$+ after RDA cleavage. Naringenin and butin excimer ion peaks both have *m/z* 273.0750$[M+H]^+$. Naringenin yielded the characteristic ion 153.0180$[A^{1,3}]^+$ after RDA cleavage. Butin was cleaved *via* collision-induced cleavage to produce characteristic ion fragments including loss of 1 molecule of H_2O to produce fragment ion *m/z* 255.0644 $[M+H-H_2O]^+$, loss of a carbon group of C ring to produce *m/z* 227.0698 $[M+H-H_2O-CO]^+$, and cleavage *via* RDA to produce characteristic ion *m/z* 137.0232 $[A^{1,3}]^+$.

Compound 24 (tR = 10.83) and compound 25 (tR = 5.00) are isomers and compound 24 was checked and identified with the mass spectral cleavage information and retention time of the Eriodictyol control. The excimer ion peak of compound 25 was *m/z* 289.0707 $[M+H]^+$ (δ=-2.92 ppm) with characteristic fragmentation information of *m/z* 271.0953 $[M+H-H_2O]^+$, *m/z* 243.0633 $[M+H-H_2O-CO]^+$, and *m/z* 215.0670 $[M+H-H_2O-2CO]^+$; the C-ring RDA cleavage yielded characteristic ion *m/z* 153.0184, which was identified as fustin according to the mzcloud database.

Compound 29 (tR = 15.68) and compound 30 (tR = 11.30) both have the molecular formula $C_{16}H_{14}O_6$ and are isomers. The excimer ion peak of compound 29 (tR = 15.68) is *m/z* 303.0860 $[M+H]^+$ (δ = -1.20 ppm) and the characteristic ions of the secondary fragments are *m/z* 285.0755 $[M+H-H_2O]^+$, *m/z* 270.0520 $[M+H-H_2O-CH_3]^+$, and *m/z* 242.0545 $[M+H-H_2O-CH_3-CO]^+$; RDA cleavage yielded the characteristic ion *m/z* 153.0387, which was tentatively identified as hesperetin according to the mzcloud database. The compound 30 excimer ion peak was *m/z* 303.0860 $[M+H]^+$ (δ = -1.97 ppm), and the secondary fragment characteristic ions were *m/z* 285.0750 $[M+H-H_2O]^+$, *m/z* 269.0438 $[M+H-H_2O-O]^+$, and *m/z* 275.0904 $[M+H-CO]^+$, which combined with the literature analysis (Liu *et al.* 2005) and data identified as homoeriodictyol. Compound 31 (tR = 5.99) was identified after comparison with the mass spectral information, retention time of taxifolin control.

Isoflavones

Compound 1 (tR = 11.78), compound 6 (tR = 17.56), compound 10 (tR = 14.22), compound 16 (tR = 20.41), compound 18 (tR = 12.80), and compound 27 (tR = 14.37) were consistent with the mass spectrometric cleavage information and retention times of the Daidzein, formononetin, genistein biochanin A, calycosin, and tectorgenin controls, respectively.

Compound 17 (tR = 14.66) and compound 19 (tR = 20.12) have excimer ion peaks *m/z* 285.0753$[M+H]^+$. The loss of the carboxyl group of the C ring of compound 17 produced *m/z* 257.0784 $[M+H-CH_3-CO]^+$ and the continued loss of carboxyl and oxygen produced fragment ion peaks *m/z* 229.0853 $[M+H-CH_3-2CO]^+$ and *m/z* 212.0539 [M+H-

CH_3-2CO-O]$^+$; RDA cleavage produced the characteristic ion *m*/*z* 137.0230. This was identified as 2'-hydroxyformononetin in combination with the mzcloud database. Compound 19 readily loses a methyl group to produce the stronger *m*/*z* 270.0515 [M+H-CH_3]$^+$, and the C ring loses a carboxyl group to produce the fragment ion peak *m*/*z* 270.0515 [M+H-CH_3-CO]$^+$, and then loses 1 molecule of H_2O to form the ion [M+H-CH_3-CO-H_2O]$^+$. Combined with the mass spectral library, it was inferred to be prunetin.

Compound 23 (tR = 11.39) has an excimer ion peak value of *m*/*z* 285.0753[M+H]$^+$ (δ = -2.03 ppm), presumably with the molecular formula $C_{16}H_{14}O_5$. The C ring loses its carboxyl group to form *m*/*z* 259.0956[M+H-CO]$^+$, which continues to lose CO and H_2O, yielding *m*/*z* 259.0956[M+H-CO-H_2O]$^+$, *m*/*z* 213.0538[M+H-CO]$^+$, which was tentatively identified as the compound vestitone in combination with references found in Liu *et al.* (2005) and databases.

Compound 32 (tR = 12.65) has an excimer ion peak equal to *m*/*z* 317.1011[M+H]$^+$ (δ = -1.49 ppm) with the presumed molecular formula $C_{17}H_{16}O_6$, yielding characteristic secondary fragments of *m*/*z* 289.1065[M+H-CO]$^+$ and *m*/*z* 257.0802[M+H-CO-CH_3OH]$^+$, 229.0855[M+H-CO-CH_3OH-CO]$^+$, 197.0595[M+H-CO-CH_3OH-CO-CH_3OH]$^+$ and 179.0697[M+H-CO-CH_3OH-CO-CH_3OH-H_2O]$^+$, respectively. The compound was tentatively identified as homoferreirin.

Chalcone and others

Compound 3 (tR = 16.72), compound 7 (tR = 16.50), and compound 14 (tR = 13.98) were identified after comparison of the mass spectral information and retention time of the isoliquiritigenin, dalbergin, and butein controls, respectively.

Compound 15 (tR = 11.92) has an excimer ion peak of *m*/*z* 317.1011 [M+H]$^+$ (δ = -1.76 ppm), with a presumed molecular formula of $C_{16}H_{12}O_5$. The secondary mass spectra with characteristic fragment ions *m*/*z* 270.0512 [M+H-CH_3]$^+$, *m*/*z* 242.0564 [M+H-CH_3-CO]$^+$, and *m*/*z* 214.0615[M+H-CH_3-2CO]$^+$, which was presumed to be melannin in combination with the literature as well as the database (Liu *et al.* 2005). Compound 28 (tR = 17.78) has an excimer ion peak *m*/*z* 301.1056 [M+H]$^+$ (δ = -2.48 ppm) and fragment ions with *m*/*z* 273.1113 [M+H-CO]$^+$ and *m*/*z* 273.1113 [M+H-CO-CH_3OH]$^+$. Combined with the reference found in Wang *et al.* (2014) and databases, the compound was tentatively identified as Melilotocarpan A.

Content of Characteristic Flavonoid Components

Flavonoids are important secondary metabolites of plants with various biological activities and are considered to be the primary active ingredients of Chinese herbal medicines (Ninh 2017). *Dalbergia tonkinensis* is rich in flavonoid components with various biological activities, and the flavonoid components in 15 heartwood with activities were selected as the characteristic substances to evaluate the medicinal quality of *D. tonkinensis* heartwood. The quantification method of flavonoids was established *via* the external standard method (the results are shown in Table 2). Naringenin, biochanin A, butein, and pinocembrin were associated with antioxidant activity; isoliquiritigenin, daidzein, formononetin, butein, and medicarpin were associated with anti-inflammatory activities; and isoliquiritigenin, formononetin, and liquiritigenin were associated with antibacterial activities. The above flavonoids were detected in the heartwood of *D. tonkinensis* from different origins, and the liquiritigenin, daidzein, naringenin, isoliquiritigenin, formononetin, pinocembrin, biochanin A content was high.

Table 1. Ultra-High Performance Liquid Chromatography-Quadrupole Time-of-Flight Mass Spectrometry (UHPLC/Q-TOF-MS) Data of the Compositions Identified in *D. tonkinensis* Heartwood Extracted *via* the Method: Water (80:20)

No.	Identification	RT	Molecular Formula	Error (ppm)	*m/z* Calculated	*m/z* Experimental	Main Fragments (m/z)
1	Daidzein*	11.78	$C_{15}H_{10}O_4$	2.35	255.0651	255.0645	227.0698, 199.0749, 137.0230, 91.0545
2	Chrysin	20.61	$C_{15}H_{10}O_4$	1.18	255.0651	255.0648	209.0595, 181.0654, 153.0181, 103.0545
3	Isoliquiritigenin*	16.72	$C_{15}H_{12}O_4$	2.72	257.0808	257.0801	239.0693, 211.0746, 137.0230, 119.0491
4	Pinocembrin*	19.48	$C_{15}H_{12}O_4$	2.72	257.0808	257.0801	153.0181
5	Liquiritigenin*	10.82	$C_{15}H_{12}O_4$	2.72	257.0808	257.0801	239.0691, 211.0750, 137.0230, 119.0491
6	Formononetin*	17.56	$C_{16}H_{12}O_4$	2.60	269.0808	269.0801	254.0555, 253.0488, 226.0617, 197.0594, 137.0230
7	Dalbergin*	16.50	$C_{16}H_{12}O_4$	2.60	269.0808	269.0801	254.0560, 253.0489, 226.0616, 198.0665
8	Apigenin	10.64	$C_{15}H_{10}O_5$	3.32	271.0601	271.0592	253.0492, 225.0537, 197.0589
9	Galangin	12.99	$C_{15}H_{10}O_5$	2.95	271.0601	271.0593	255.0642, 227.0695, 199.0748, 153.0179, 137.0230
10	Genistein*	14.22	$C_{15}H_{10}O_5$	2.21	271.0601	271.0595	243.0650, 215.0697, 197.0595, 169.0646, 153.0180, 119.0492
11	3', 4', 7-Trihydroxyisoflavone	16.22	$C_{15}H_{10}O_5$	1.84	271.0601	271.0596	243.1009, 197.0595, 137.0594, 121.0647
12	Naringenin*	13.36	$C_{15}H_{12}O_5$	3.30	273.0757	273.0748	153.018
13	Butin*	8.64	$C_{15}H_{12}O_5$	2.56	273.0757	273.0750	255.0644, 227.0698, 209.0594, 181.0644, 137.0232
14	Butein*	13.98	$C_{15}H_{12}O_5$	1.46	273.0757	273.0753	255.0647, 227.0702, 181.0647, 137.0232, 135.0440,
15	Melannin	11.92	$C_{16}H_{12}O_5$	3.16	285.0758	285.0749	270.0512, 269.0433, 242.0564, 214.0615, 213.0539

16	Biochanin A*	20.41	$C_{16}H_{12}O_5$	3.16	285.0758	285.0749	269.0442, 213.0540, 133.0645
17	2'-Hydroxyformononetin	14.66	$C_{16}H_{12}O_5$	3.16	285.0758	285.0749	270.0510, 257.0784, 229.0853, 213.0539, 137.0230
18	Calycosin*	12.80	$C_{16}H_{12}O_5$	2.81	285.0758	285.0750	270.0511, 269.0437, 253.0488, 213.0540, 197.0592, 169.0643, 153.0543, 137.0230
19	Prunetin*	20.12	$C_{16}H_{12}O_5$	1.75	285.0758	285.0753	270.0515, 242.0569, 224.0465
20	Fisetin*	10.29	$C_{15}H_{10}O_6$	2.09	287.0550	287.0544	241.0494, 213.0542, 185.0593, 157.0647, 137.0233, 121.0285
21	Kaempferol	11.32	$C_{15}H_{10}O_6$	1.39	287.0550	287.0546	259.0956, 241.0593, 213.0538, 153.0181, 125.0597, 107.0493
22	Luteolin*	13.76	$C_{15}H_{10}O_6$	1.74	287.0550	287.0545	
23	Vestitone	11.39	$C_{16}H_{14}O_5$	1.74	287.0914	287.0909	259.0956, 241.0491, 213.0538
24	Eriodictyol*	10.83	$C_{15}H_{12}O_6$	2.42	289.0707	289.0700	271.0602, 153.0181, 135.0438, 107.0492
25	(-)-Fustin	5.00	$C_{15}H_{12}O_6$	0.00	289.0707	289.0707	271.0953, 243.0633, 215.0670, 153.0184
26	Diosmetin	9.74	$C_{16}H_{12}O_6$	2.66	301.0707	301.0699	286.0462, 258.0513, 230.1562, 212.0461, 184.0514
27	Tectorgenin*	14.37	$C_{16}H_{12}O_6$	2.33	301.0707	301.0700	286.0465, 255.0642, 245.0802
28	Melilotocarpan A	17.78	$C_{17}H_{16}O_5$	4.98	301.1071	301.1056	273.1113, 241.0847
29	(+/-)-Hesperetin	15.68	$C_{16}H_{14}O_6$	0.99	303.0863	303.0860	285.0755, 270.0520, 242.0545, 151.0387, 123.0440
30	Homoeriodictyol	11.30	$C_{16}H_{14}O_6$	0.33	303.0863	303.0864	285.0750, 275.0904, 269.0438
31	Taxifolin	5.99	$C_{15}H_{12}O_7$	12.46	305.0655	305.0617	287.0536, 259.0596, 231.0650, 213.0545, 195.0290, 153.0181
32	Homoferreirin	12.65	$C_{17}H_{16}O_6$	2.52	317.1019	317.1011	299.0912, 289.1065, 257.0802, 229.0855, 197.0595, 179.0697, 163.0386
Note: *Compounds marked with an asterisk indicate that the compound was identified after comparison with the standard							

Table 2. Characteristic Flavonoid Content of the Three Origins of *D. tonkinensis*

Compound	Calibration caves	R^2	Quang Binh	Quang Ninh	Hanoi
Butin	$y = 239306*x + 3274810$	0.9980	1.642 ± 0.008	0.639 ± 0.006	0.636 ± 0.039
Liquiritigenin	$y = 441675*x + 8014080$	0.9970	3.007 ± 0.046	2.426 ± 0.033	1.348 ± 0.023
Daidzein	$y = 53546.8*x + 468109$	0.9984	2.467 ± 0.079	1.117 ± 0.031	0.524 ± 0.034
Fisetin	$y = 196400*x + 895704$	0.9980	0.144 ± 0.007	0.055 ± 0.004	0.205 ± 0.009
Erodcyol	$y = 128325*x + 843979$	0.9989	0.778 ± 0.006	0.298 ± 0.007	4.999 ± 0.176
Calycosin	$y = 740657*x + 14057900$	0.9959	1.030 ± 0.022	0.344 ± 0.011	1.194 ± 0.064
Naringenin	$y = 237970*x + 1914080$	0.9992	6.233 ± 0.033	3.317 ± 0.062	11.361 ± 0.369
Genistein	$y = 477227*x + 7308970$	0.9973	0.492 ± 0.021	0.122 ± 0.005	0.323 ± 0.019
Butein	$y = 215067*x + 870351$	0.9994	0.986 ± 0.006	0.262 ± 0.038	0.243 ± 0.007
Tectorigenin	$y = 326967*x + 3030180$	0.9988	0.478 ± 0.012	0.690 ± 0.020	1.209 ± 0.080
Dalbergin	$y = 650801*x + 7549020$	0.9985	2.452 ± 0.019	0.830 ± 0.008	0.988 ± 0.023
Isoliquiritigenin	$y = 522337*x + 4255890$	0.9981	2.168 ± 0.029	1.867 ± 0.054	0.604 ± 0.024
Formononetin	$y = 629429*x + 5283250$	0.9993	5.118 ± 0.146	3.345 ± 0.026	3.993 ± 0.181
Pinocembrin	$y = 406989*x + 5609210$	0.9994	2.447 ± 0.025	1.564 ± 0.021	29.051 ± 0.998
Biochanin A	$y = 598135*x + 8860280$	0.9993	2.094 ± 0.072	0.929 ± 0.015	5.426 ± 0.251

Analysis of the Volatile Components of *D. tonkinensis*

The identification of the volatile components in *D. tonkinensis* extracts was carried out *via* gas chromatography-mass spectrometry, and a total of 12 substances were identified in Table 1 of the supporting material. The total ion flow diagrams of the three sources of *D. tonkinensis* were more consistent (as shown in Fig. 3), and the relative areas were calculated using peak area normalization for volatile components (as shown in Table 3). The volatile components of the heartwood material from the three different origins were determined to be trans-nerolidol (32.9% to 54.7%), caryophyllene oxide (13.3% to 24.4%), eudesmol (2.53% to 8.15%), bisabolol oxide (0.88% to 3.15%), and farnesyl acetone (1.46% to 2.96%). The quantitative working curve of trans-nerolidol was established using the external standard method ($y = 600000x + 6000000$, $R^2 = 0.999$), and the trans-nerolidol peak areas of the heartwood from different sources were used as the response values to measure the contents (as shown in Table 4). The volatile components were also detected in the heartwood of *D. odorifera*, which indicated a very high similarity in the composition of the two species (Liu *et al.* 2005; Yang *et al.* 2016; Ma *et al.* 2022).

Table 3. Chemical Composition of the Volatile Compounds in the Oil Extracts of in *D. tonkinensis* Heartwood from Different Origins

RT	Compositions	Formula	RI	Quang Binh	Quang Ninh	Hanoi
10.2926	Geranyl acetone	$C_{13}H_{22}O$	1467.4975	/	/	0.28 ± 0.05
11.1397	Elemol	$C_{15}H_{26}O$	1544.4132	2.05 ± 0.07	2.71 ± 0.05	1.26 ± 0.18
11.2858	trans-Nerolidol	$C_{15}H_{26}O$	1556.8653	54.71 ± 1.54	43.90 ± 1.20	32.92 ± 0.87
	*			5.57 ± 0.21	4.81 ± 0.14	6.66 ± 0.29
11.3393	Ledol	$C_{15}H_{26}O$	1561.425	/	1.90 ± 0.10	1.99 ± 0.17
11.4513	Caryophyllene oxide	$C_{15}H_{24}O$	1570.9708	18.69 ± 0.64	24.42 ± 0.64	13.33 ± 1.00
11.5584	Globulol	$C_{15}H_{26}O$	1580.0989	/	/	1.08 ± 0.46
12.2546	Eudesmol	$C_{15}H_{24}O$	1634.3097	2.53 ± 0.12	4.24 ± 0.08	8.15 ± 0.23
12.4834	Bisabolol oxide	$C_{15}H_{26}O_2$	1651.2754	2.55 ± 0.07	3.15 ± 0.07	0.88 ± 0.04
12.6246	α-Bisabolol	$C_{15}H_{26}O$	1662	0.88 ± 0.11	1.73 ± 0.06	0.84 ± 0.07
13.8223	trans-Coniferyl alcohol	$C_{10}H_{12}O_3$	1745.3204	2.60 ± 0.17	2.45 ± 0.08	/
16.6607	Farnesyl acetone	$C_{18}H_{30}O$	1925.2319	2.56 ± 0.50	2.96 ± 0.05	1.46 ± 0.59
20.7114	Hexadecanamide	$C_{16}H_{33}NO$	2160.4745	/	1.51 ± 0.79	/
Note: *row indicates the amount of trans-nerolidol in the sample (the unit is mg/g)						

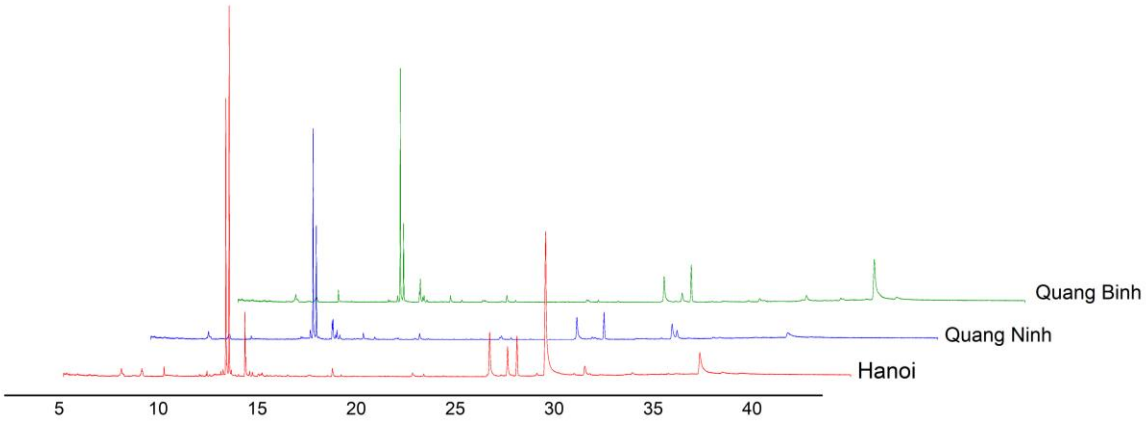

Fig. 3. Total ion chromatograms of the extracts from *D. tonkinensis* heartwood *via* GC-MS

Comparison of the Chemical Composition of the Heartwood Extract from *D. tonkinensis* and *D. odorifera*

The heartwood of *D. tonkinensis* and *D. odorifera* contained the same flavonoids (as discussed) and volatile components (as discussed) and only differed in content to effectively distinguish between the two. Furthermore, the signals of all compounds were collected *via* HPLC-TOF-MS and compared using principal component analysis (PCA) methods. In the positive ion mode (as shown in Fig. 4), the cumulative contribution of the PCA model was 74%. The *D. odorifera* heartwood, *i.e.*, the Guangxi group (Nanning-LFJ, Nanning-GXU, Pingxiang) and Hainan group (Anding, Dongfang, Haikou), were compared with *D. tonkinensis*, *i.e.*, (*D. odorifera* heartwood (Ledong) and *D. tonkinensis* (Hanoi), which were distinguished from other origins by the higher total flavonoids, nerolides, and characteristic flavonoid contents in the heartwood of these two origins. This is presumably the result of intra-species variation rather than inter-species variation. Consistent results were obtained in the negative ion model. The above results indicated that there is no significant difference in the chemical composition between *D. tonkinensis* and *D. odorifera*, which suggested that *D. tonkinensis* can be used as a potential substitute for *D. odorifera*.

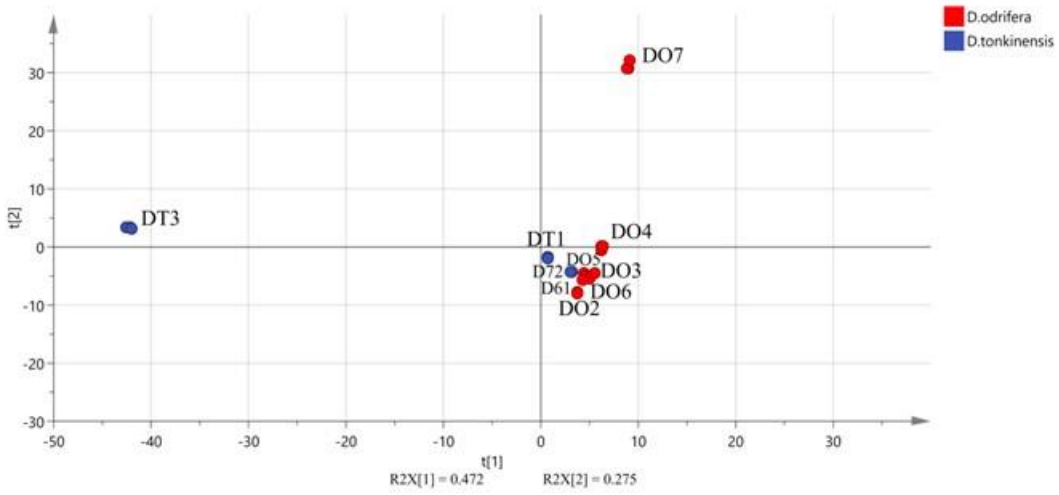

Fig. 4. Principal component analysis of the constituents of the extracts of *D. tonkinensis* and *D. odorifera*

CONCLUSIONS

1. Thirty-two flavonoids and 12 volatile components from *D. tonkinensis* heartwood were identified *via* GC-MS and HPLC-MS. The total flavonoid content was high, ranging from 49.7 mg to 102.0 mg RE/g dry weigh, which indicated that *D. tonkinensis* heartwood also contained more abundant flavonoids. Butin, liquiritigenin, daidzein, fisetin, erodcyol, calycosin, naringenin, genistein, butein, and tectorigen were used as characteristic flavonoids for the identification and evaluation index of *D. tonkinensis*. The volatile components of *D. tonkinensis* were found to be very similar to those of *D. odorifera*, with trans-Nerolidol and Caryophyllene oxide as the primary components.

2. By comparing the chemical composition of *D. tonkinensis* and *D. odorifera* heartwood *via* PCA, it was concluded that there was no significant difference between the two in terms of chemical composition, and *D. tonkinensis* could be used as a potential substitute for *D. odorifera* heartwood. In this study, the identification and more comprehensive evaluation methods of *D. tonkinensis* heartwood were established to provide a basis for its standardized utilization.

ACKNOWLEDGEMENTS

This research was supported by the National Natural Science Foundation of China (Grant No. 31870540).

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Article submitted: June 20, 2022; Peer review completed: August 28, 2022; Revised version received and accepted: September 6, 2022; Published: September 14, 2022.
DOI: 10.15376/biores.17.4.6108-6122