Study on the Characteristic Components of Distinguishing *Dalbergia cochinchinensis* from the Other Three Similar *Dalbergia* Species

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Abstract

Dalbergia cochinchinensis can be distinguished from *Dalbergia retusa*, *Dalbergia bariensis*, and *Dalbergia oliveri* quickly using infrared spectrum characteristic peaks as shown in a previous study. To investigate the components corresponding to the infrared characteristic peaks of *Dalbergia cochinchinensis*, petroleum ether, ethyl acetate, and butyl alcohol were sequentially used to extract the dispersion liquid of *D. cochinchinensis*. The petroleum ether extracts were further fractionated by column chromatography, using Fourier-transform infrared spectroscopy (FTIR) to track the characteristic components during separation. FTIR spectra of petroleum ether extractives indicated the presence of aromatic ketones and olefin compounds. The gas chromatography–mass spectrometry research showed some main components and gave possible structure. Furthermore, their detailed structures were characterized thorough a nuclear magnetic resonance approach, and then two possible components (3,5-dihydroxy-7-methoxy-2-phenylchroman-4-one and 3,5,7-trihydroxy-2-phenylchroman-4-one) were identified.

Dalbergia cochinchinensis is a species of Faboideae in the Leguminosae family that mainly grows in southeast Asia. In recent years, the global population of *D. cochinchinensis* has experienced a boom in trade, especially illegal trade. Overexploitation has resulted in *D. cochinchinensis* being threatened with extinction in the wild, and the species has been listed in CITES Appendix II in 2013 (Zhang et al. 2016). Wood samples can usually be identified to genus by morphological and anatomical classification, but the improvement of these regulations' enforcement requires that both timber and manufactured products of *D. cochinchinensis* be identified unambiguously. Chemotaxonomy methods show promise in identifying taxa to species. The science of chemical taxonomy is used for the classification of plants on the basis of a specific class of secondary metabolites and their biosynthetic pathways (Singh 2016). The chemical structure of the secondary metabolites is often specific, and these compounds are of restricted occurrence, hence very useful for chemotaxonomic classification. This method is considered better than traditional methods due to the ease of working methodology and even can detect trace amounts of chemical compounds (Ankanna et al., 2012). Some major groups of secondary metabolites, including flavonoid and alkaloids, have stability, specificity, integrality, and correlative differences, exhibiting high taxonomic value (Smith 1976).

A large number of neoflavonoid and isoflavonoid derivatives have been reported isolated from *Dalbergia* species (Seshadri 1972). The phenolic compounds isolated previously from wood of *D. cochinchinensis*, *Dalbergia retusa*, and *Dalbergia oliveri* suggest differences among the three species. The wood of *D. cochinchinensis* is reported to contain the neoflavonoid derivatives **(1)** 9-

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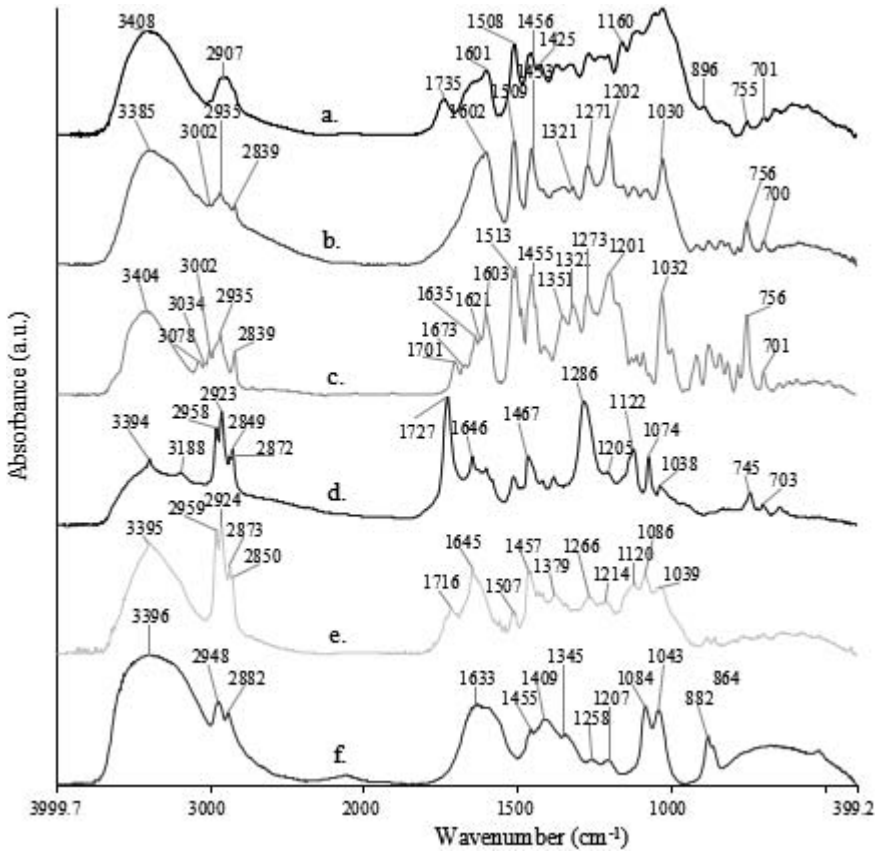

Figure 1.—FTIR spectra of (a) D. cochinchinensis *heartwood, (b) water extractives, and (c) petroleum ether, (d) ethyl acetate, (e) butyl alcohol, separated extracts from aqueous solution in turn, and (f) the residue.*

hydroxy-6,7-dimethoxydalbergiquinol, **(2)** 6-hydroxy-2,7-dimethoxyneoflavene, **(3-1)** 2,2′,5-trihydroxy-4-methoxybenzophenone (R=OH), **(3-2)** 2,5-Dihydroxy-4-methoxybenzophenone (R=H), **(4-1)** Latifolin (R=H), **(4-2)** 5-O-Methoxylatifolin (R=Me), **(5)** Methoxydalbergion, **(6)** Darbergiol and the **(7)** isoflavone Calycosin (Kuroyanagi et al. 1996, Pathak et al. 1997). Phenolic compounds reported from *D. retusa* heartwood are the neoflavonoid derivatives **(5)** 4-methoxydalbergione, obtusaquinol, **(8)** (*R*)-3-(2,5-dihydroxy-4-methoxyphenyl)-3-phenylpropene (Jurd et al., 1972b) and the isoflavones Retusin, **(9)** 7,8-dihydroxy-4′-methoxyisoflavone, **(10)** 8-O-methylretusin (Jurd et al., 1972a), and some other constituents obtusafuran, **(11)** 5-hydroxy-6-methoxy-3-methyl-2-phenyl-2,3-dihydrobenzofuran, **(12)** obtusastyrene, **(13)** obtustyrene (Gregson et al., 1978) **(14)** 4-cinnamyl-3-methoxycatechol (Manners et al. 1974) **(15)** Obtusaquinone, **(16)** O-Methylobtusaquinone (Jurd et al., 1972b). **(20)** *Dalbergia oliveri* contains an isoflavan, **(21)** isoflavanone, **(17-1)** pterocarpans (R_1=H, R_2=OMe), **(17-2)** pterocarpans (R_1R_2=OCH_2O), **(18-1)** coumestones (R_1=H, R_2=OMe), **(18-2)** coumestones (R_1R_2=OCH_2O), **(19-1)** 3-arylcoumarins (R_1=H, R_2=OMe) and **(19-2)** 3-arylcoumarins (R_1R_2=OCH_2O) (Donnelly and Kavanagh 1974, Deesamer et al. 2007). The characteristics of the Fourier-transform infrared spectroscopy (FTIR) technique are high sensitivity and selectivity, short time, and small amount of sample required for the analysis. It is a promising method used in the wood identification field. In a previous study we successfully distinguished the wood of *D. cochinchinensis* from *D. retusa*, *Dalbergia bariensis*, and *D. oliveri* using FTIR spectra (Zhang et al. 2016). In this study, the extractives of *D. cochinchinensis* heartwood were separated by organic solvents with different polarities and silica gel column chromatography and further investigated by FTIR spectra, gas chromatography–mass spectrometry (GC-MS), and nuclear magnetic resonance (NMR) techniques in order to learn the characteristic compounds that are of value as an identification aid.

Materials and Experimental

Plant material

The dried heartwood of *D. cochinchinensis* was obtained from Zhangjiagang Entry-Exit Inspection and Quarantine Bureau of P.R.C., and State Key Laboratory of Wood Identification and Quarantine.

Extraction and isolation

The heartwood of *D. cochinchinensis* was powdered and extracted with distilled cold water at room temperature for 4 days, then evaporated under reduced pressure (0.10 Mpa) to concentrate the water extracts. The aqueous solution was extracted with petroleum ether (PE), ethyl acetate, and butyl alcohol in turn to separate the water extracts. The PE-soluble extractives were fractionated by column chromatography on silica gel and eluted with petroleum ether-ethyl acetate (5:1) to yield three fractions (C-1, C-2, C-3). The second fraction C-2 was further purified by two-dimensional

thin-layer chromatography (2D TLC) using a mixture of petroleum ether and ethyl acetate (5:1) as the developing solution.

Structure characterization

Infrared (IR) spectra were recorded on a Spectrum GX FTIR system (PerkinElmer, Waltham, USA). The spectra were obtained in the scan range of 4000–400 cm^{-1} with a resolution of 4 cm^{-1} and with a total accumulation of 16 scans.

The GC-MS analysis was performed on 320-MS (Bruker Corporation, Billerica, MA, USA). A DB-5MS silica capillary column with 30 m length, 0.25 mm diameter, and 0.25 μm film thickness was used for separation. The inlet maintained at 280°C was operated in unsplit mode at a split ratio of 1:25 and 1 μL sample injection. Temperature program: 50°C (5 min)→10°C min^{-1} to 300°C (10 min). The mass spectrometer was operated in Electron Impact (EI) mode (70 eV) with a scan range from 40 to 850 m/z. Ion source and interface temperature was 250°C and 280°C, respectively. Peak assignment was accomplished by library spectra (NIST 2008). ^{1}HNMR spectra were recorded on a JEOL JNM-ECA 600 NMR spectrometer using $CDCl_3$ as the solvent.

Results and Discussion

Liquid–liquid extraction is by far the most popular separation method in many analytical techniques (Rydberg et al. 2004). Solvent extraction commonly takes place with an aqueous solution as one liquid and an organic solvent as the other, the desired components in an aqueous solution are extracted with an organic extractant. To separate the water extracts of *D. cochinchinensis*, we use solvents with different polarities, such as petroleum ether, ethyl acetate, and butyl alcohol to sequentially extract the aqueous solution.

FTIR study of extracts from different solvents

Figure 1 is the FTIR spectra of *D. cochinchinensis* heartwood, water extractives, and organic solvents extractives separated from aqueous solution and the residue. Figure 1b shows the three characteristic peaks at 1602 cm^{-1}, 756 cm^{-1}, 700 cm^{-1} that the previous study concluded were useful to distinguish *D. cochinchinensis* from the other three *Dalbergia* species that are easily mistaken (Zhang et al. 2016). After extraction by petroleum ether, these characteristic peaks disappeared. This indicated that the desired components were removed from aqueous phase by petroleum ether. Positions and assignments of IR bands of extractives are summarized in Table 1. The FTIR spectrum in Figure 1c shows typical absorption bands of aromatic skeletal vibration at 1603 cm^{-1}, 1513 cm^{-1}, and 1455 cm^{-1}, as well as C–H stretching of unsaturated hydrocarbons at 3034 and 3002 cm^{-1}. The peaks at 1701 and 1673 cm^{-1} are attributed to conjugated carbonyl stretching vibration. The peaks at 1635 and 1621 cm^{-1} are due to C=C stretching, and the peak at 3078 cm^{-1} results from $–CH_2–$ asymmetrical stretching vibrations of olefins. These characteristic absorption peaks in Figure 1c indicated the presence of aromatic ketones and olefin compounds in petroleum ether extractives. The bands at 1727 and 1286 cm^{-1} in Figure 1d correspond to the stretching vibration of carbonyl (C=O) groups and C(=O)–O, which are typical of esters. In the

Table 1.—Positions and assignments of IR bands of extractives (Hortling et al. 1997, Larkin 2011, Passauer et al. 2016).[a,b]

Band position (cm^{-1})				
PE	EA	BA	WAR	Band assignment
3,440				υ(O–H) phenyl
	3,394	3,395	3,396	υ(O–H) alcohol, phenyl
	3,188			υ(N–H) NH_2
3,078				$υ_{as}(CH_2)$ olefins
3,034				υ(C–H) aryl
3,002				
	2,958/2,872	2,959/2,873		C–H stretching vibration, methyl
			2,948/2,882	C–H stretching vibration, O-methylene
2,935	2,923	2,924		$υ_{as}$(C–H) methylene
2,839	2,849			υ(C–H) methylene
1,701	1,727	1,716		υ(unconj. C=O)
1,673				υ(conj. C=O)
	1,646	1,645		υ(C=C) olefins
1,635			1,633	
1,621				
1,603	1,601			aromatic skeletal vibration
1,513	1,513	1,507		
	1,467			bending vibration, alkanes
1,455		1,457	1,455	$δ_{as}$(C–H), CH_3, CH_2; aromatic skeletal vibration
	1,418		1,409	$δ(CH_2)$, olefins
	1,382	1,379		$δ$(C–H) CH_3
	1,286	1,266	1,258	υ(CO–O) aliphetic OAc
1,201		1,214	1,207	υ(C–OH) phenyl
	1,122	1,120		υ(C–O–C) ether
	1,074	1,086	1,084	δ(C–O) sec. alcohols, aliphatic ethers
1,032	1,038	1,039	1,043	υ(C–O–C) ether
		900–700		δ(C–H) out-of-plane

[a] PE, petroleum ether; EA, ethyl acetate; BA, butyl alcohol; WAR, water extractives residue.

[b] υ, stretching vibration; δ, deformation vibration; s, symmetrical, as asymmetrical; OMe, methoxyl.

FTIR spectrum of butyl alcohol extractives (Fig. 1e) the C–O–C asymmetrical stretching of aliphatic ethers at 1120 cm^{-1}, C–O stretching of polysaccharides at 1086 and 1039 cm^{-1}, saturated ketone C=O at 1716 cm^{-1}, and olefins C=C stretching at 1645 cm^{-1} were observed. The FTIR analysis of the residue Figure 1f revealed the presence of polysaccharides (1084 and 1043 cm^{-1}) and olefins (1633 and 1409 cm^{-1}). Analysis of extractives using FTIR allows the primary identification of phytoconstituents.

Figure 2 is the FTIR spectra of petroleum ether extracts separated by column chromatography. The three characteristic peaks at 1600 cm^{-1}, 756 cm^{-1}, and 701 cm^{-1} occurred in C-2. This indicates that the feature components were fractionated in C-2. The structural of constituents in C-2 were further studied using GC-MS.

The possible main chemical constituents of C-2 petroleum ether extractives of *D. cochinchinensis* are listed in Table 2. The major compounds of C-2 can be fit as follows: 2,6-Dimethoxyphenyl 3-phenylpropanoate (43.80%); 1,1-Diphenyl-2-propanol (10.27%); Naringenin (8.76%); Methanone, [2-hydroxy-4-(2-hydroxyethoxy)phenyl]phenyl- (5.59%); Triphenylmethane (5.23%); 5-Methoxy-6-[1-[4-

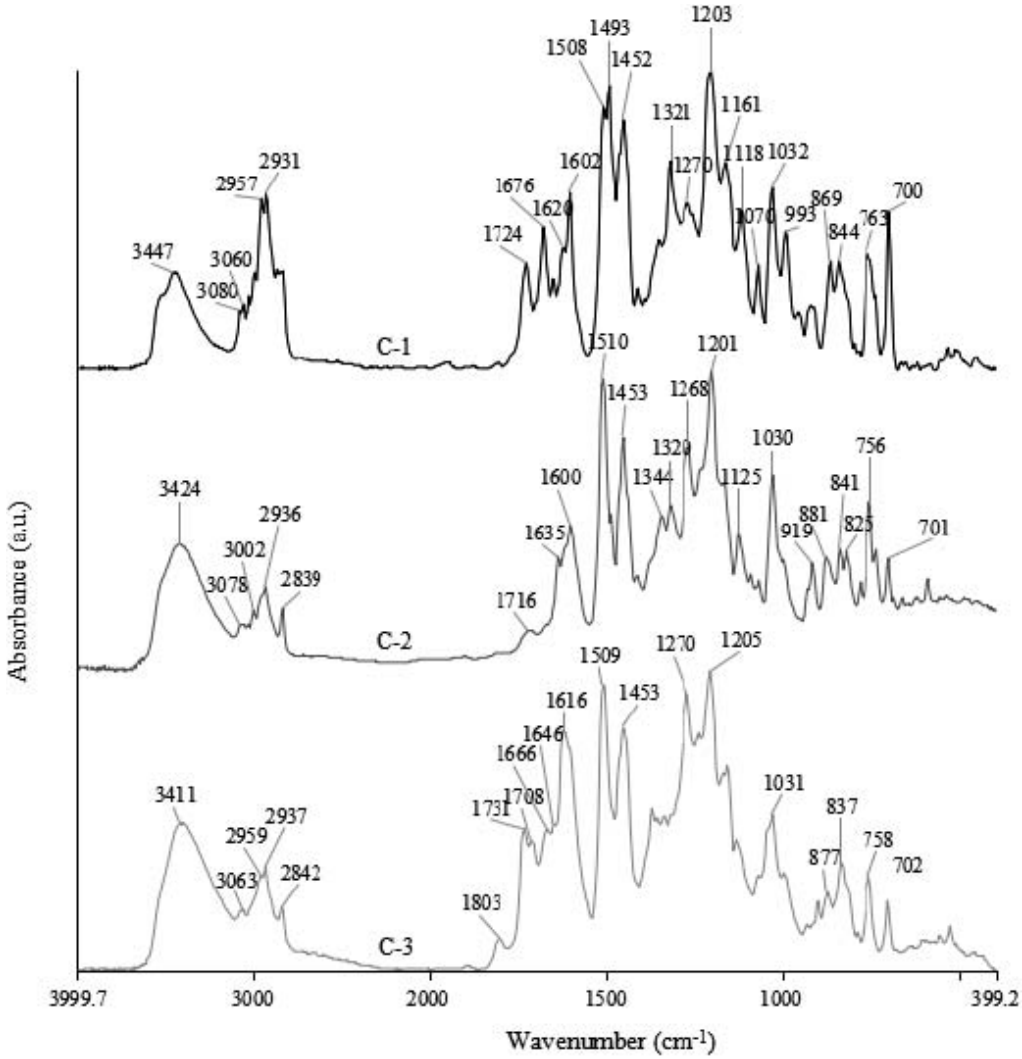

Figure 2.—FTIR spectra of petroleum ether extracts fractionated by column chromatography.

Table 2.—Chemical composition of C-2 petroleum ether extractives of Dalbergia cochinchinensis *analyzed by GC-MS.*

Label	Compound name	CAS number	Area%
1	Dibutyl phthalate	84-74-2	1.15
2	3,4-Dihydro-5-methoxy-6-methyl-2-phenyl-2H-1-benzopyran-7-ol	55670-26-3	2.54
3	Methanone, [2-hydroxy-4-(2-hydroxyethoxy)phenyl]phenyl-	16909-78-7	5.59
4	2-Vinylnaphthalene	827-54-3	0.84
5	Triphenylmethane	519-73-3	5.23
6	1,1-Diphenyl-2-propanol	29338-49-6	10.27
7	1,3-Benzenediol,1-benzoate	136-36-7	2.11
8	3-(2,5-Dimethoxyphenyl)-2-hydroxy-2,4,6-cycloheptatrien-1-one	3525-08-4	1.31
9	Dibenz[a,c]cycloheptane, 1,2,9-trimethoxy-	145068-32-2	1.72
10	2,6-Dimethoxyphenyl 3-phenylpropanoate	40123-34-0	43.8
11	2-Hydroxy-3,4-dimethoxy-alpha-(*p*-methoxyphenyl)acetophenone	3606-32-4	2.72
12	3-Hydroxy-2-(4-hydroxy-3-methoxyphenyl)-4H-chromen-4-one	159184-95-9	1.02
13	5-Methoxy-6-[1-[4-methoxyphenyl]ethyl]-1,3-benzodioxole	71712-16-8	3.12
14	9,10-Anthracenedione, 2,3-dimethoxy-	22506-55-4	1.36
15	Naringenin	480-41-1	8.76
16	7-Hydroxy-2-(3-hydroxy-4-methoxyphenyl)-4H-chromen-4-one	20575-57-9	0.26

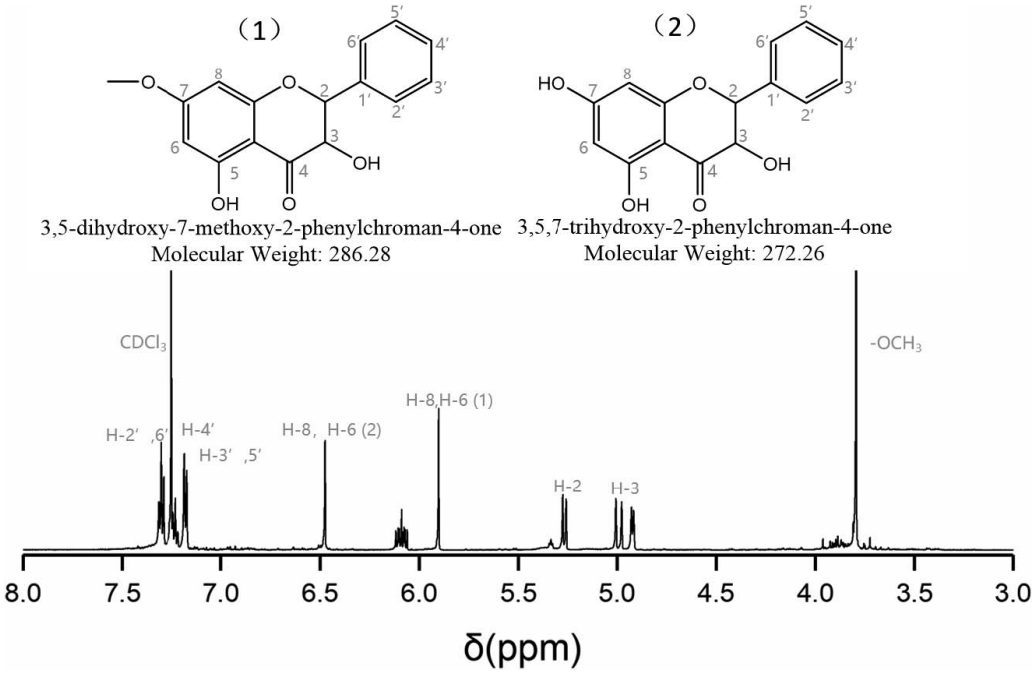

Figure 3.—^{1}H NMR spectrum (600 MHz, $CDCl_3$, 298 K) of petroleum ether extracts (C-2) fractionated by column chromatography.

methoxyphenyl]ethyl]-1,3-benzodioxole (3.12%); 2-Hydroxy-3,4-dimethoxy-alpha-(*p*-methoxyphenyl)acetophenone (2.72%); 4-Dihydro-5-methoxy-6-methyl-2-phenyl-2H-1-benzopyran-7-ol (2.54%); and 1,3-Benzenediol,1-benzoate (2.11%).

NMR spectroscopy is a powerful tool for analyzing and confirming the structure of material and has been widely used in identifying the natural products coupled with FTIR, ultraviolet-visible and GC-MS techniques. According to the results of GC-MS data, we found that the C-2 was still a that which consisted of several components. But there is a relatively large component (43.8%, molecular weight, 286) whose structures may be identified in the ^{1}H NMR spectrum. Therefore, in this work, the C-2 petroleum ether extractives were further analyzed through a JEOL JNM-ECA 600 spectrometer using the solvent peak as internal reference (Figure 3). Combining some previous literature (Harborne et al. 1975, Han et al. 2010, Schievano et al. 2019), two main components: 3,5-dihydroxy-7-methoxy-2-phenylchroman-4-one (43.8%, molecular weight, 286) and 3,5,7-trihydroxy-2-phenylchroman-4-one (8.76%, molecular weight, 272) may be confirmed. The very similar structure (B and C ring parts) of the two compounds excepting A ring will lead to the similar chemical shifts. The peaks at 7.32–7.11 were attributed to the resonance of protons of B ring part. The resonance at 6.40 and 5.90 ppm corresponds to the protons of H-8 and H-6 of (2) and (1), respectively. We think the peak at 6.40 ppm shouldn't ascribe to the flavanone (H-3) because there are no peaks near 8.00 ppm that usually ascribe to the H-2′,6′ of flavanone in $CDCl_3$. The chemical shifts of H-6,8 protons of (1) shift to a higher magnetic field, due to the presence of electron donating group (–OCH_3) that decreased the conjugation length. The peaks in range of 5.20–4.92 can be ascribed to the resonances of H-2 and H-3, while the resonance at 3.8 ppm generally come from the protons of –OCH_3.

Conclusion

The characteristic components useful to distinguish *D. cochinchinensis* from the other three *Dalbergia* species for which it is easily mistaken have been isolated from aqueous phase by petroleum ether. Primary identification of PE extractives using FTIR indicated the presence of aromatic ketones and olefin compounds. After fractionating by column chromatography, C-2 petroleum ether extractives were further studied by GC-MS. Finally, with the help of the NMR technique, the possible major compounds of these characteristic components were 3,5-dihydroxy-7-methoxy-2-phenylchroman-4-one and 3,5,7-trihydroxy-2-phenylchroman-4-one.

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