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Research Article

Phenolic Compounds from *Caesalpinia sappan*

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ABSTRACT

Introduction: *Caesalpinia sappan* L., a traditional ingredient of food and beverages in South East Asia, was investigated for its chemical constituents. **Methods:** The compounds were isolated by column chromatography and their chemical structures were elucidated by NMR spectroscopy and confirmed by comparison of their NMR data with literature data. **Results:** Repeated column chromatography of the EtOAc-soluble fraction from the heartwood of *C. sappan* resulted in the isolation of sappanchalcone (1), caesalpiniaphenol G (2), and quercetin (3). **Conclusion:** Three phenolic compounds have been successfully isolated from *C. sappan*.

Key words: *Caesalpinia sappan*; Caesalpiniaceae; Analgesic; Homoisoflavonoids.

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INTRODUCTION

Caesalpinia sappan Linn., belonging to family Caesalpiniaceae, is distributed widely in the tropical and subtropical regions of Southeast Asia.[1] The dried heartwood of the plant, named Sappan Lignum, has been used in folk medicines as a blood tonic, expectorant, and has exhibited a wide range of activities, including anti-malarial, anti-inflammatory and analgesic agent in folk medicine.[2,3] Previous phytochemical investigations on the heartwood of *C. sappan*, resulted in the isolation and identification of various types of compounds, including homoisoflavonoids, chalcones, dibenzoxocins, and brazilins.[4-8] Many of these components exhibited a variety of biological activities such as anti-inflammatory,[9,10] xanthine oxidase inhibitory,[7,10] antioxidant,[11] antidiabetic,[12] hepatoprotective,[13] and vasorelaxation activities.[14] To search for novel and bioactive compounds from *C. sappan*, this study focused on phenolics, and led to the isolation and identification of three compounds (**1, 2** and **3**).

MATERIALS AND METHODS

General experimental procedure

The NMR spectra were measured using a Varian Unity-Inova 400 MHz spectrometer in DMSO-d_6 at 500 MHz for ^{1}H NMR and 125 MHz for ^{13}C NMR. Silica gel (63–200 mm; Merck, Darmstadt, Germany) and RP-18 (75 mm; Merck) were used for column chromatography. Thin layer chromatography was carried out on pre-coated silica gel 60 F254 plates and RP-18 F254 plates (both from Merck) and the plates were visualized by spraying with 10% H_2SO_4/EtOH solution followed by warming.

Sample collection

The heartwood of *Caesalpinia sappan* was collected in Hanoi, Vietnam in 2018. A voucher specimen (no. TVT-01) was deposited at the Institute of Ecology and Biological Resources, Vietnam Academy of Science and Technology, and botanical identification performed by Dr. V. H. Do from Department of Plant Resources, Institute of Ecology and Biological Resources, Vietnam Academy of Science and Technology.

Extraction and isolation of compounds from the heartwood of C. sappan

The dried heartwood of *C. sappan* (0.5 kg) were extracted three times with 95% EtOH (3 × 1.5 L) under reflux. The filtrate was evaporated under reduced pressure to afford a crude extract. That extract (15.6 g) was suspended in H_2O and then partitioned using *n*-hexane, CH_2Cl_2, and EtOAc successively. The EtOAc fraction (4.2 g) was subjected to silica gel column chromatography (100–200 mesh), eluting with a CH_2Cl_2:MeOH by gradient system (from 50:1 to 0:1, v/v) to afford five fractions (E1–E5) according their TLC profiles. Fraction E3 was subjected to reversed-phase (ODS-A) column chromatography and eluted with MeOH:H_2O (from 1:3 to 0:1, v/v) to afford three subfractions (E3-1 to E3-3). Further purification of E3-2 (58.4 mg) by Sephadex LH-20 column eluted with CH_2Cl_2:MeOH (1:1, v/v) to yield compound **1** (8.6 mg).

Fraction E4 was subjected to silica gel column chromatography eluted with CH_2Cl_2:Me_2CO (15:1–0:1) to give three subfraction (E4-1 to E4-3). Fraction E4-2 was separated by a MCI column eluted with MeOH:H_2O (from 1:1 to 1:0) to yield compounds **2** (11.0 mg) and **3** (9.7 mg).

RESULTS AND DISCUSSION

Silica gel chromatography of the EtOAc fraction of *C. sappan* has led to the isolation of sappanchalcone (**1**), caesalpiniaphenol G (**2**), and quercetin (**3**). The NMR data of **1** are in accordance with the data reported in the literature for sappanchalcone[15], **2** for caesalpiniaphenol G[16], and **3** for quercetin.[17]

Compound **1** was obtained as a yellow powder. The ^{1}H NMR spectrum of **1** showed signals for a pair of characteristic doublets corresponding to the *trans*-α, β-hydrogens [δ_H 7.40 (1H, d, J = 15.5 Hz, H-β), 7.32 (1H, d, J = 15.5 Hz, H-α)], two sets of ABX systems

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[δ_H 7.11 (1H, brs, H-2), 6.79 (1H, d, J = 8.0 Hz, H-5), 6.99 (1H, brd, J = 8.0, H-6) and 6.52 (1H, brs, H-3′), 6.46 (1H, d, J = 8.0 Hz, H-5′), 7.53 (1H, brd, J = 8.0, H-6′)] belonging to two tri-substituted benzene rings, one methoxyl group at δ_H 3.85 (3H, s). The ^{13}C NMR and HSQC spectra of **1** showed 16 carbon signals comprising 14 aromatic or olefinic carbons, a carbonyl carbon, and one methoxy carbon. The signal of the conjugated carbonyl at δ_C 188.9 and further signals for conjugated olefinic carbons at δ_C 123.9 and 141.9 were typical of chalcones. The location of methoxy group at C-2′ was confirmed by HMBC correlations of proton -OCH_3 (δ 3.85, s) to the C-2′ (160.5). On the basis of the above analysis, compound **1** was assigned structurally as sappanchalcone (**1**). Direct comparison of spectroscopic data from this compound displayed a high similarity with those previously described for 2′-methoxy-3,4,4′-trihydroxychalcone or named sappanchalcone (**1**).[15]

Compound **2** isolated as a yellow powder. The ^{1}H NMR spectrum of **2** showed an ABX spin coupled system at δ_H 7.90 (1H, d, J = 8.5 Hz), 6.72 (1H, d, J = 8.5 Hz) and 6.58 (1H, brs) was assigned to H-5, H-6 and H-8, respectively. Further it also revealed the presence of an aromatic proton at δ_H 6.53 (1H, s), which was assigned to the H-2′. In addition, the appearance of the resonances at δ_H 5.30 (2H, s, H-2) and δ_H 7.53 (1H, s, H-9) in the ^{1}H NMR spectrum of **2**, as well as the appearance of an α,β-unsaturated carbonyl carbon resonance at δ_C 182.1 (C-4) in the ^{13}C NMR spectrum, indicated the presence of a 3-benzylidenechroman-4-one moiety. Direct comparison of spectroscopic data with those described indicate that this compound corresponds to (*E*)-3-(2,3,4,5-tetrahydroxybenzylidene)-2,3-dihydro-7-hydroxychromen-4-one, also known as caesalpiniaphenol G (**2**).[16]

Compound **3** was obtained as yellow powder. The ^{1}H NMR spectrum of **3** displayed five aromatic proton signals, including two *meta*-coupled proton signals at δ_H 6.20 (1H, d, J = 2.0 Hz, H-6) and 6.41 (1H, d, J = 2.0 Hz, H-8) ascribed to the 5,7- dihydroxylated ring A from a flavonoidic skeleton, an ABX system at δ_H 7.64 (1H, dd, J = 8.5, 2.0 Hz, H-6′), 7.75 (1H, d, J = 2.0 Hz, H-2′), and 6.90 (1H, d, J = 8.5 Hz, H-5′) ascribed to a 1,3,4- trisubstituted of ring B. The ^{13}C NMR of **3** showed the presence of 15 aromatic carbon signals. Based on the NMR data and comparison of the data given in the literature, the structure of compound **3** was identified as quercetin.[17]

Although no biological activity tests were conducted on the isolated compounds, literature search revealed that sappanchalcone (**1**) exhibited anti-inflammatory,[18-20] neuroprotective,[21,22] inhibition of antigen-induced beta hexosaminidase release,[23] anti-influenza viral,[24] growth inhibition and induction of apoptosis in human oral cancer cells.[25] Caesalpiniaphenol G (**2**) showed growth inhibition and induction of apoptosis in human premyelocytic cancer cells.[15] Furthermore, quercetin (**3**) is the most widely distributed and extensively studied flavonoid found in vegetables, fruits, seeds, nuts, tea and red wine.[26] Quercetin has displayed various biological activities such as antioxidant, anti-inflammatory, antibacterial, antiviral, radical-scavenging, gastroprotective, and immune-modulatory activities (Figure 1 and Table 1).[27,28]

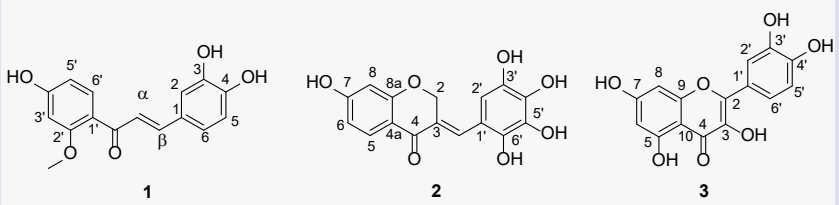

Figure 1: Chemical structure of isolated compounds (**1–3**) from *C. sappan*.

Table 1. ^{1}H NMR and ^{13}C NMR data of compounds 1–3 (δ values).

Position	Compound					
	1		2		3	
	δ_H (J in Hz)[b]	δ_C[a]	δ_H (J in Hz)[b]	δ_C[a]	δ_H (J in Hz)[b]	δ_C[a]
1		126.6	5.30, s			
2	7.11, *brs*	114.5		67.2		146.2
3		145.7		122.4		137.2
4		148.3		182.1		177.3
4a				117.5		
5	6.79, *d* (8.0)	116.0	7.90, d (8.5)	107.2		162.4
6	6.99, *brd* (8.0)	121.7	6.71, d (8.5)	108.1	6.20, d (2.0)	99.2
α	7.32, *d* (15.5)	123.9				
β	7.4, *d* (15.5)	141.9				
7				163.7		162.4
8			6.53, brs	98.9	6.41, d (2.0)	94.4
8a				160.2		
9			7.53, s	133.5		158.2
10						104.5
1′		120.3		119.2		121.7
2′		160.5	7.01, s	113.4	7.75, d (2.0)	116.0
3′	6.52, brs	99.4		162.2		146.2
4′		162.7		145.6		148.7
5′	6.46, d (8.0)	108.0		144.6	6.90, d (8.5)	116.2
6′	7.53, brd (8.0)	132.4		148.6	7.64, dd (2.0, 8.5)	121.7
–C=O		188.9				
–OCH_3	3.85, s	55.7				

[a]^{1}H NMR measured at 400 MHz in pyridine-d5.
[b]^{13}C NMR measured at 100 MHz in pyridine-d5.

CONCLUSION

It may be concluded that phenolic compounds have been successfully isolated from heartwood of *C. sappan* and on the basis of spectral data; the compounds were identified as sappanchalcone (**1**), caesalpiniaphenol G (**2**), and quercetin (**3**).

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

ABBREVIATIONS

NMR: Nuclear Magnetic Resonance; **EtOAc:** Ethyl acetate; **EtOH:** Ethanol; **CH_2Cl_2**: Dichloromethane; **CH_3OH**: Methanol, **H_2O**: Water.

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GRAPHICAL ABSTRACT

SUMMARY

Introduction: *Caesalpinia sappan* L., a traditional ingredient of food and beverages in South East Asia, was investigated for its chemical constituents. **Methods:** The compounds were isolated by column chromatography and their chemical structures were elucidated by NMR spectroscopy and confirmed by comparison of their NMR data with literature data. **Results:** Repeated column chromatography of the EtOAc-soluble fraction from the heartwood of *C. sappan* resulted in the isolation of sappanchalcone, caesalpiniaphenol G, and quercetin. **Conclusion:** This successful isolation of phenolic compounds from *C. sappan*. was carried out and their chemical structures were elucidated.

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