Contribution to the study on chemical constituents from the leaves of *Cassia alata* l., (Caesalpiniaceae)

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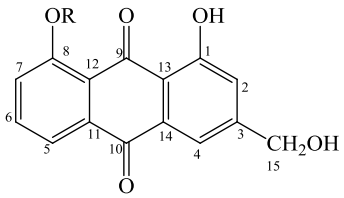
ABSTRACT

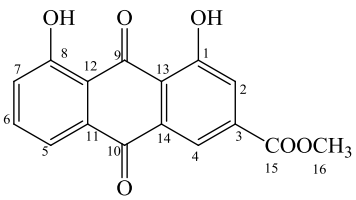
Six compounds were isolated from the leaves of Cassia alata L. (Caesalpiniaceae), including: aloe emodin (1), aloe emodin-8-O-β-glucoside (2), rhein methyl ester (3), kaempferol (4), 4-hydroxybenzoic acid (5) and phytol (6). Rhein methyl ester (3) was first isolated from the Cassia genus. Their chemical structures were elucidated by spectroscopic analysis.

Key words: *Cassia alata, anthraquinone.*

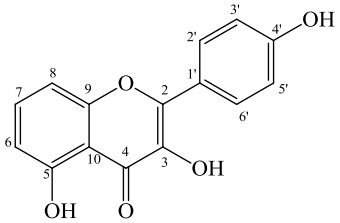


R=H Aloe emodin (**1**)

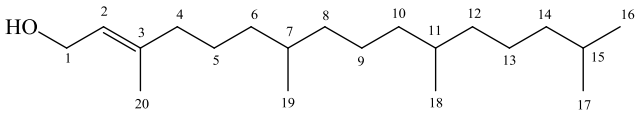
R= β-D-Glucopyranoside (**2**)



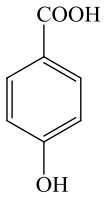
Rhein methyl ester (**3**)



Kaempferol (**4**)



Phytol (**6**)



4-Hydroxybenzoic acid (**5**)

INTRODUTION

Cassia alata L. (= *Senne alata* L.) (Fig. 1) belongs to the Caesalpiniaceae family. It is native to Central America and has been introduced into many tropical countries and islands. In Vietnam, it is widely distributed in the Middle and the South [1, 2, 3, 4].

Cassia alata L. appears in a considerable number of published ethnopharmacological studies. It is laxative, antifebrile, antiseptic and diuretic. It has been used to cure many diseases: skin rashes, constipation, herpes circine, digestive ailments… [1, 2, 3, 4].

Fig. 1. *Cassia alata* L.

MATERIALS AND METHODS

General

NMR spectra were obtained on a Bruker Avance 500 NMR spectrometer. Mass spectra were taken on a high resolution ESI Bruker Daltonics micrOTOF-Q II 10187 mass spectrometer. Chromatography column was carried out on Kieselgel 60 (63–200 mesh) Merck. Thin-layer chromatography was performed on Merck 25DC-Alufolien 20×20cm Kieselgel 60 F254 plates. 1D and 2D-NMR were recorded on Bruker Avance 500 (500 MHz for ^{1}H-NMR and 125 MHz for ^{13}C-NMR).

Plant material

Plants were collected in Binh Chanh district, Ho Chi Minh City and was identified by pharmacist Phan Duc Binh, the deputy editor of the "Medicine and Health" semi-monthly magazine.

Extraction and isolation

Plants were washed, dried, ground into powder (2.1 kg) and exhaustedly extracted by methanol at room temperature. After evaporating, the methanolic solution gave crude extract (525 g). This extract was partitioned successively in petroleum ether, chloroform, ethyl acetate and butanol to give corresponding extracts. Petroleum ether fraction and ethyl acetate fraction were chromatographied repeatedly to afford six pure compounds. Petroleum ether fraction gave (3), (6) and ethyl acetate fraction gave (1), (2), (4), (5).

Aloe emodin (**1**): lightly orange needles. ^{1}H-NMR (500 MHz, DMSO-d_6): 11.96 (2H, br, -OH), 7.82 (1H, t, J = 8.0 Hz, H6), 7.74 (1H, d, J = 7.5 Hz, H5), 7.71 (1H, brs, H4), 7.40 (1H, d, J = 8.0 Hz, H7), 7.31 (1H, brs, H2), 5.62 (2H, t, J = 6.0 Hz, 3-CH_2OH). ^{13}C-NMR (125 MHz, DMSO-d_6): 161.6 (C1), 120.7 (C2), 153.7 (C3), 117.1 (C4), 119.3 (C5), 137.3 (C6), 124.4 (C7), 161.3 (C8), 191.6 (C9), 181.5 (C10), 133.4 (C11), 115.9 (C12), 114.5 (C13), 133.1 (C14), 62.0 (C15).

Aloe emodin-8-*O*-*β*-glucoside (**2**): orange needles. ^{1}H-NMR (500 MHz, Pyridine-d_5): 13.34 (1H, br, -OH), 8.11 (1H, brs, H4), 8.04 (1H, d, J = 8.0 Hz, H5), 7.99 (1H, d, J = 8.0 Hz, H7), 7.67 (1H, brs, H2), 7.60 (1H, t, J = 8.0 Hz, H6), 5.00 (3-CH_2OH), 5.81 (1H, d, J = 7.5 Hz, H1'), 4.60 (1H, dd, J = 12.0 Hz, J = 2.5 Hz, H6'a), 4.57 (1H, t, J = 8.5 Hz, H2'), 4.43 (1H, t, J = 7.5 Hz, H4'), 4.42 (1H, dd, J = 12.5 Hz, J = 5.5 Hz, H6'b), 4.38 (1H, t, J = 8.5 Hz, H3'), 4.25 (1H, m, H5'). ^{13}C-NMR (125 MHz, Pyridine-d_5): 163.1 (C1), 121.4 (C2), 153.2 (C3), 116.7 (C4), 121.2 (C5), 135.0 (C6), 123.0 (C7), 159.4 (C8), 188.6 (C9), 182.6 (C10), 133.1 (C11), 116.0 (C12),

116.3 (C13), 127.0 (C14), 63.2 (C15), 102.7 (C1'), 74.9 (C2'), 78.4 (C3'), 71.1 (C4'), 79.4 (C5'), 62.4 (C6').

Rhein methyl ester (**3**): yellow powder. HR-ESI-MS analysis (*m/z* 299.0587 $[M+H]^+$, calcd. for $[C_{16}H_{11}O_6+H]^+$ = 299,0550). ^{1}H-NMR (500 MHz, $CDCl_3$): 12.03 (1H, s, 1-OH), 11.97 (1H, s, 8-OH), 8.43 (1H, d, *J* = 1.5 Hz, H4), 7.95 (1H, d, *J* = 1.5 Hz, H2), 7.89 (1H, dd, *J* = 7.5 Hz, *J* = 1.0 Hz, H5), 7.74 (1H, t, *J* = 8.0, H6), 7.37 (1H, dd, *J* = 8.5 Hz, *J* = 1.0 Hz, H7), 4.00 (3H, s, $-OCH_3$). ^{13}C-NMR (125 MHz, $CDCl_3$): 163.0 (C1), 125.5 (C2), 138.1 (C3), 120.4 (C4), 120.6 (C5), 137.9 (C6), 125.1 (C7), 162.6 (C8), 193.1 (C9), 181.1 (C10), 133.7 (C11), 116.1 (C12), 134.1 (C13), 118.5 (C14), 165.1 (C15), 53.0 (C16).

Kaempferol (**4**): yellow needles. ^{1}H-NMR (500 MHz, DMSO-d_6): 12.48 (1H, s, 5-OH), 10.79 (1H, s, 7-OH), 10.11 (1H, s, 4'-OH), 9.39 (1H, s, 3-OH), 8.05 (2H, d, *J* = 9.0 Hz, H2' and H6'), 6.93 (2H, d, *J* = 9.0 Hz, H3' and H5'), 6.44 (1H, d, *J* = 2.0 Hz, H8), 6.20 (1H, d, *J* = 2.0 Hz, H6). ^{13}C-NMR (125 MHz, DMSO-d_6): 146.8 (C2), 135.7 (C3), 175.9 (C4), 160.7 (C5), 98.2 (C6), 163.9 (C7), 93.5 (C8), 156.2 (C9), 103.1 (C10), 121.7 (C1'), 129.5 (C2' and C6'), 135.7 (C3' and C5'), 159.2 (C4').

4-hydroxybenzoic acid (**5**): white needles. ^{1}H-NMR (500 MHz, acetone-d_6): 7.91 (2H, d, *J* = 8.5 Hz, H2 and H6), 6.91 (2H, d, *J* = 9.0 Hz, H3 and H5. ^{13}C-NMR (125 MHz, acetone-d_6): 121.8 (C1), 131.8 (C2, C6), 115.1 (C3, C5), 161.7 (C4), 166.7 (-COOH).

Phytol (**6**): white powder. ^{1}H-NMR (500MHz, $CDCl_3$): 5.41 (1H, t, *J* = 7.0, H2), 4.15 (2H, d, *J* = 6.5 Hz, H1), 2.0 – 0.7 (protons of CH, CH_2, CH_3). ^{13}C-NMR (125 MHz, $CDCl_3$): 59.61 (C1), 123.34 (C2), 140.45 (C3), 40.04 (C4), 25.33 (C5), 36.86 (C6), 32.87 (C7), 37.55 (C8), 24.64 (C9), 37.62 (C10), 32.97 (C11), 37.48 (C12), 24.95 (C13), 39.56 (C14), 28.14 (C15), 22.85 (C16), 22.77 (C17).

RESULTS AND DISCUSSION

Compound (1) was obtained as lightly orange needles. The ^{1}H-NMR (500MHz, DMSO-d_6) spectrum showed the presence of five aromatic protons. In ring A, there were three protons at δ 7.82 (1H, t, *J* = 8.0Hz, H6), 7.74 (1H, d, *J* = 7.5Hz, H5) and 7.40 (1H, d, *J* = 8.0Hz, H7). In ring C, two protons broad singlet were resonated at δ 7.71 (1H, brs, H4) and 7.31 (1H, brs, H2). A low field signals at δ 11.96 that integrated for two protons was attributed to two identical hydroxyl protons that were strongly chelated to a carbonyl group. A triplet at δ 5.62, integrated for two protons, was suggested the presence of a hydoxymethyl group. The ^{13}C-NMR spectrum showed the signals of 15 carbons, which included two carbonyl downfield signals at δ 191.6 and 181.5, indicative the presence of the chelated and nonchelated carbonyl respectively. Based on its spectral data and by comparison with data from the literature, 1 was assigned as aloe emodin [5].

Compound (2) was isolated as orange needles. Although NMR spectra of 2 were run in pyridine-d_5 (different from the solvent DMSO-d_6 of 1), the structure of 2 was nearly similar to 1 with five aromatic protons: 8.04 (1H, d, *J* = 8.0Hz, H5), 7.99 (1H, d, *J* = 8.0Hz, H7), 7.60 (1H, t, *J* = 8.0Hz, H6) for ring A and 8.11 (1H, brs, H4), 7.67 (1H, brs, H2) for ring C; two chelated and nonchelated carbonyl at δ 188.6 and 182.6; one hydoxymethyl group (δH 5.00, δC 63.2). The only different is that 2 has one chelated hydroxyl group and the second hydroxyl group was changed to *O*-glucoside. Based on HSQC and HMBC (table 1), this glucoside linked to C8 of the aglycon moiety. From these data, 2 was identified as aloe emodin-8-*O*-*β*-glucoside.

Compound (3) was obtained as yellow powder. The HR-ESI-MS displayed the pseudomolecular ion peak at *m/z* 299.0587 $[M+H]^+$ (calcd. for $[C_{16}H_{11}O_6+H]^+$ = 299.0550)

corressponding to the molecular formula $C_{16}H_{11}O_6$. The ^{1}H-NMR spectrum supported the chelated nature of two hydroxyl groups to the carbonyl by the characteristic downfield signals at δ 12.03 (1H, s, 1-OH) and 11.97 (1H, s, 8-OH). A monosubstituted ring A was indicated by the three coupled protons at δ 7.89 (1H, dd, *J* = 7.5Hz, *J* = 1.0Hz, H5), 7.74 (1H, t, *J* = 8.0, H6) and 7.37 (1H, dd, *J* = 8.5Hz, *J* = 1.0 Hz, H7). A disubstituted ring C was indicated by the two coupled protons at δ 8.43 (1H, d, *J* = 1.5Hz, H4); 7.95 (1H, d, *J* = 1,5Hz, H2).

Table 1. The ^{1}H-NMR (500 MHz) and ^{13}C-NMR (125 MHz) spectra for 1 and 2

N^o	Compound (**1**) (DMSO-d_6)		Compound (**2**) (pyridine-d_5)			
			HSQC		HMBC (^{1}H→^{13}C)	COSY (^{1}H↔^{1}H)
	δ_C	δ_H (*J*, in Hz)	δ_C	δ_H (*J*, in Hz)		
1	161.6		163.1			
2	120.7	7.31 brs	121.4	7.67 brs	4, 13	4
3	153.7		153.2			
4	117.1	7.71 brs	116.7	8.11 brs	2, 10, 13	2
5	119.3	7.74 d (7.5)	121.2	8.04 d (8.0)	10, 12	6, 7
6	137.3	7.82 t (8.0)	135.0	7.60 t (8.0)	7, 8	5, 7
7	124.4	7.40 d (8.0)	123.0	7.99 d (8.0)	12	6, 5
8	161.3		159.4			
9	191.6		188.6			
10	181.5		182.6			
11	133.4		133.1			
12	115.9		116.0			
13	114.5		116.3			
14	133.1		127.0			
15	62.0	5.62 t (6.0)	63.2	5.00 overlapped	2, 3, 4	
1-OH		11.96 br	-	13.34 br		
8-OH		11.96 br	-			
1′	-	-	102.7	5.81 d (7.5)	8	2’
2′	-	-	74.9	4.57 t (8.5)		1’, 3’
3′	-	-	78.4	4.38 t (8.5)		2’, 4’
4′	-	-	71.1	4.43 t (7.5)		3’, 5’
5′	-	-	79.4	4.25 m		4’, 6’a, 6’b
6′a 6′b	-	-	62.4	4.60 dd (12.0, 2.5) 4.42 dd (12.5, 5.5)		5’, 6’b 5’, 6’a

A three-proton singlet at δ 4,00 indicated the presence of a methoxy group. The ^{13}C NMR spectrum showed the signals of one methyl ester group (-$COOCH_3$) at δ 165,1 and 53,0; two chelated and nonchelated carbonyl carbons at δ 193,1 (C9) and 181,1 (C10); two oxygenated aromatic carbons at δ 163,0 (C1) and 162,6 (C8). The NMR spectral data of compound (3) closely resembled the known compound rhein [6] except the signals of one -OCH_3 moiety. Besides, the HMBC showed the correlation between the proton of this methoxyl group with carbonyl ester, so compound (3) was established as rhein methyl ester. This is the first time that rhein methyl ester (3) was isolated from the *Cassia* genus.

Comparison of ^{1}H-NMR and ^{13}C-NMR data and physical data with those reported in the literature confirmed that compounds 4 - 6 were kaempferol (4) [7], 4-hydroxybenzoic acid (5) and phytol (6) [8].

Table 2. The ^{1}H-NMR (500 MHz) and ^{13}C-NMR (125 MHz) spectra for 3

N°	Compound (**3**) ($CDCl_3$)		
	HSQC		HMBC ($^{1}H \rightarrow ^{13}C$)
	δ_C	δ_H (*J*, in Hz)	
1	163.0		
2	125.5	7.95 d (1.5)	1, 4, 15
3	138.1		
4	120.4	8.43 d (1.5)	2, 10, 14, 15
5	120.6	7.89 dd (7.5, 1.0)	7, 10, 12
6	137.9	7.74 t (8.0)	8, 11
7	125.1	7.37 dd (8.5, 1.0)	5, 8, 12
8	162.6		
9	193.1		
10	181.1		
11	133.7		
12	116.1		
13	134.1		
14	118.5		
15	165.1		
16	53.0	4.00 s	15
1-OH	-	12.03 s	1, 2
8-OH	-	11.97 s	7, 8,12

Góp phần tìm hiểu thành phần hóa học lá cây Muồng Trâu *Cassia alata* l., (Caesalpiniaceae)

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- **Thòng Sủi Din**
- **Trương Lê Hùng Phong**
- **Phan Nhật Phương**
- **Phạm Quốc Quỳnh**
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TÓM TẮT

Từ lá cây Muồng Trâu Cassia alata L. (Caesalpiniaceae), đã cô lập được sáu hợp chất, bao gồm: aloe emodin (1), aloe emodin-8-O-β-glucosid (2), rhein metyl ester (3), kaempferol (4), acid 4-hydroxybenzoic (5) và phytol (6). Trong đó, hợp chất rhein methyl ester (3) lần đầu tiên được cô lập từ chi Cassia. Cấu trúc của các hợp chất được xác định bằng các phương pháp phổ nghiệm.

Từ khóa: *Cassia alata, anthraquinone.*

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