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FLAVONOIDS AND CHALCONOID ISOLATED FROM FLOWERS OF COSMOS BIPINNATUS CAV. (ASTERACEAE)

FLAVONOID VÀ CHALCONOID CÔ LẬP TỪ HOA CỦA CÂY COSMOS BIPINNATUS CAV. (HỌ CÚC)

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ABSTRACT

*Leaves of the genus Cosmos have been studied in chemical constituents and biological activities in Vietnam. However, chemical constituents of Cosmos flowers have not been extensively investigated. The dried purple and white flower powders of Cosmos bipinnatus Cav. were exhautively macerated with ethanol solvent. The extract was concentrated under reduced pressure to give the ethanolic extract. The extract was subjected to column chromatography (CC) with gradient elution (petroleum ether → chloroform → ethyl acetate) to give fractions. Thin layer chromatography (TLC) was used to analyze the fractions. The chloroform and ethyl acetate column factions were then further purified and analyzed by CC and TLC to obtain pure flavonoids and chalconoid (**1-3**) named 7, 3', 4'-trihydroxy-6-methoxyaurone (**1**), 2'-hydroxy-4, 4'-dimethoxychalcone (**2**), and a mixture of 7, 2', 5'-trihydroxyflavone (**3a**) and 5, 7, 3', 4'-tetrahydroxyflavone (**3b**). Their chemical structure elucidation was accomplished by using 1D and 2D-NMR spectroscopy in combination with MS data.*

Keywords: *Asteraceae; cosmos; flavonoid; chalconoid; aurone.*

TÓM TẮT

*Thành phần hóa học và hoạt tính sinh học của lá chi Cosmos đã được nghiên cứu ở Việt Nam. Tuy nhiên, thành phần hóa học của hoa Cosmos chưa được nghiên cứu đầy đủ. Bột hoa khô màu tím và trắng của cây Cosmos bipinnatus Cav. được tận trích với dung môi etanol. Dịch trích sau đó được loại dung môi dưới áp suất thấp thu được cao etanol. Tiến hành sắc ký cột (CC) cao etanol với các dung môi có độ phân cực tăng dần (eter dầu hỏa → cloroform → etyl acetat) thu được các phân đoạn. Các phân đoạn cao cloroform và etyl acetat được tinh sạch và phân tích bằng CC và sắc ký lớp mỏng (TLC) thu được các hợp chất flavonoid và chalconoid tinh khiết (các hợp chất **1-3**): 7, 3', 4'-trihydroxy-6-methoxyaurone (**1**), 2'-hydroxy-4, 4'-dimethoxychalcone (**2**), và hỗn hợp 7, 2', 5'-trihydroxyflavone (**3a**) và 5, 7, 3', 4'-tetrahydroxyflavone (**3b**). Cấu trúc hóa học của các hợp chất được xác định bằng phổ cộng hưởng từ hạt nhân một chiều, hai chiều (1D, 2D-NMR) và khối phổ (MS).*

Từ khóa: *Họ cúc, chi cosmos; flavonoid; chalconoid; aurone.*

1. INTRODUCTION

Some plants of the genus *Cosmos* have been known in Vietnam [1] and their chemical constituents and biological activities have been studied [2-9]. *Cosmos bipinnatus* Cav. commonly called the garden *Cosmos*, has been chemically investigated [10-15]. Steroids and triterpenes were isolated from its leaves [2, 7, 10]. Koshi isolated flavone glycosides in the ray flowers of *Cosmos bipinnatus* Cav. [11]. Flowers of *Cosmos bipinnatus* Cav. have five colours as the Vietnamese name of the plant "five primary colours daisy". However, only few publications have reported the chemical constituents of *cosmos* flowers in Vietnam. In previous work, flavone glycosides have been isolated from the purple flowers of *Cosmos bipinnatus* Cav. [10]. Going on this research, we report the isolation and structural determination of 7, 3', 4'-trihydroxy-6-methoxyaurone (**1**), isolated from purple flowers and the compounds of 2'-hydroxy-4, 4'-dimethoxychalcone (**2**), and a mixture of 7, 2', 5'-trihydroxyflavone (**3a**) and 5, 7, 3', 4'-tetrahydroxyflavone (**3b)** isolated from white flowers of *Cosmos bipnnatus* Cav. (Figure 1).

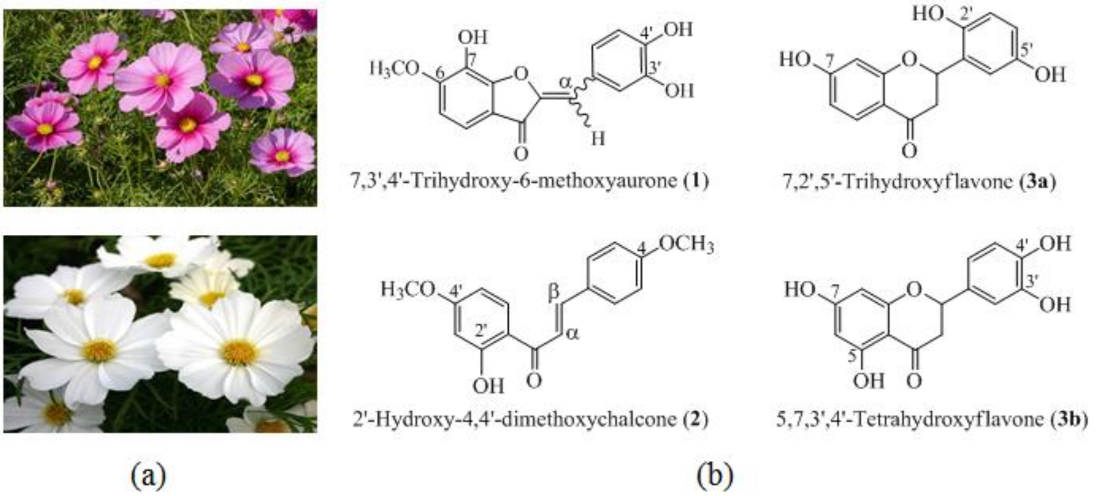

Figure 1. *(a)-The purple and white flowers of Cosmos bipinnatus Cav. and (b)-Chemical structures of compounds isolated from the purple and white flowers.*

2. RESULTS AND DISCUSSION

From the ethanol extract of the purple flowers, compound **1** was isolated. It was an orange powder with the melting point of 144-146 °C. It gave a dark blue spot on TLC with R_f = 0.27 eluted with chloroform: methanol (9:1) when revealed by solution of $FeCl_3$. The MS spectrum showed a pseudomolecular ion peak at *m/z* 300.9 $[M+H]^+$, indicating the molecular formula to be $C_{16}H_{12}O_6$.

The region δppm 6.50 ÷ 7.50 of the ^{1}H-NMR spectrum in DMSO-d_6 showed the presence of five aromatic protons and a singlet, signal for one proton at δ 6.66 (1H, s, H_α). A set of *ortho* coupled doublets at δ 7.33 (1H, d, *J* = 8.5 Hz) and 6.78 (1H, d, *J* = 8.5 Hz) indicated that the two aromatic protons were adjacent to each other for ring A. The ABX system was demonstrated by three sigmals at δ 7.44 (1H, d, *J* = 2.0 Hz); 6.86 (1H, d, *J* = 8.5 Hz) and 7.26 (1H, dd, *J* = 8.5; 2.0 Hz) for 1,3,4-trisubstituted benzene for ring C. The ^{13}C-NMR spectrum showed 16 carbons including 14 unsaturated carbons in the region of δ 90-160, a conjugated carbonyl group at 180.30 and a methoxy group ($-OCH_3$) at 60.70. The HMBC spectrum confirmed the location of methoxy group at C-6 of ring A through two-and three-bond correlations. The HMBC revealed the location of hydroxyl groups at C-7, C-3', C-4' of ring A and C. The proton H_α showed correlations with C-2, C-3, C-2', C-6'. From the above all data, we suggested the structure of compound **1** to be 7, 3', 4'-trihydroxy-6-methoxyaurone [12].

Compound **2** was isolated from the white flowers, it was an orange powder. The ^{13}C-NMR showed 15 carbons in downfield of δ 101-191 for a flavonoid skeleton [12-13]. The peak at δ 191.9 was a specific characteristic of carbonyl of chalcone. In the ^{1}H-NMR spectrum, most of resonances ranged in the δ 6.46-7.87 for a flavonoid, two singlets at δ 3.85 and 3.86 referred to two methoxy groups and a singlet at δ 13.53 denoted a chelated hydroxyl. This observation was supported by the MS spectrum with molecular ion peak at *m/z* = 284.9 $[M+H]^+$. This was suited to the formula of $C_{17}H_{16}O_4$ of a chalcone bearing two methoxy groups and one hydroxyl group. The ^{1}H-NMR spectrum showed two doublets, each one proton, at δ 7.46 and 7.87 with *J* = 15.5 Hz. That coupling constant revealed the *E* configuration of the C=C group corresponding for H_α and H_β of a chalcone. The spectrum also gave two other doublets, each two protons, at δ 6.94 and 7.61 with *J* = 9.0 Hz revealing a 1,4-disubstituted benzene ring. The HMBC spectrum gave the correlation between the ^{1}H signal at δ 3.85 ($-OC\mathbf{H}_3$) and the ^{13}C one at δ 161.80 (C4). These confirmed that the ring B should bear a methoxy group at C4. The signal of one proton at δ 7.83 (1H, d, *J* = 9.0 Hz) showed that this proton coupled with another proton in *ortho* position. Furthermore, following HMBC spectrum, this proton showed correlation with ^{13}C signal at δ 191.9 (>C=O). This revealed that one of three substituted groups on benzene ring is the carbonyl group (>C=O). All these evidences confirmed that the compound was a chalcone bonded to two methoxy groups at C-4, C-4’ and named to be 2’-hydroxy-4,4’-dimethoxychalcone [12].

From the white flowers of *Cosmos bipnnatus* Cav., a mixture of **3a** and **3b** with the ratio of 2:1 was isolated. The ^{13}C-NMR spectrum showed 30 carbons. Among them, the intensity of 15 carbons is twice higher than the remaining 15 carbons. LC-MS spectrum of the product revealed two peaks in which the peak with the retention time of 13.2 was doubled to the one with the retention time of 13.7. The mass spectrum positive mode showed that these peaks corresponded to the molecular ion of *m/z* 270.9 $[M+H]^+$ and 286.9 $[M+H]^+$, in agreement with the molecular formula of $C_{15}H_{10}O_5$ and $C_{15}H_{10}O_6$, respectively. These results suggested that the product was a mixture of two flavones with the ratio of 2:1 [12-13]. Unfortunately, the extensive efforts to separate these two compounds by silica gel CC, and preparative TLC with variety of solvent mixtures were not successful. Therefore, the structural elucidation and full ^{1}H and ^{13}C-NMR assignments of compounds **3a** and 3**b** were determined exactly based on the 2D-NMR spectra: HSQC, HMBC and COSY correlations.

Compound **3a** had molecular formula of $C_{15}H_{10}O_5$ with the presence of six aromatic protons and one proton at δ 6.16 (2H, s). In ring A, three protons at δ 6.69 (2H, dd, *J* = 1.5; 8.5 Hz); 6.72 (2H, brs) and 7.59 (2H, d, *J* = 8.5 Hz) indicated that they belonged to trisubstituted benzene ring. In ring B, an ABX system including δ 6.84 (2H, d, *J* = 8.5 Hz); 7.24 (2H, dd, *J* = 2.0; 8.5 Hz) and 7.44 (2H, d, *J* = 2.0 Hz) also indicated the presence of a trisubstituted benzene ring. There were 15 carbons in ^{13}C-NMR spetrum, including one carbonyl group at δ 181.0. The HSQC and HMBC spectra confirmed the location of hydroxyl group at C-7 in ring A and two hydroxyl groups at C-2’ and C-5’ in ring B. These evident spectra supported the structure of compound **3a** to be 7, 2’, 5’-trihydroxyflavone [12].

Compound **3b** had molecular formula of $C_{15}H_{10}O_6$. The ^{1}H-NMR spectrum showed the characteristic downfield signal for the chelated hydroxyl group at δ 12.96 (1H, s). In ring A, a set of *meta* coupled doublets at δppm 6.18 (1H, d, *J* = 1.5 Hz) and 6.44 (1H, d, *J* = 1.5 Hz) showed a tetrasubstituted benzen ring. The peaks at δ 7.41 (1H, dd, *J*=8.0, 2.9 Hz); 7.39 (1H, brs) and 6.89 (1H, d, *J*=8.0 Hz) were the signals of protons on trisubstituted benzene ring B. There were 15 carbons in ^{13}C-NMR spectrum, including one carbonyl group at δ 181.0. The HSQC and HMBC spectra confirmed the location of two hydroxyl groups at C-5 and C-7 in ring A and two hydroxyl groups at C-3' and C-4' in ring B. These evident spectra supported the structure of compound **3b** to be 5, 7, 3', 4'-tetrahydroxyflavone [12].

3. EXPERIMENTS

3.1 General experimental procedures

^{1}H and ^{13}C-NMR spectra were recorded on a Bruker (^{1}H at 500 MHz and ^{13}C at 125 MHz). LC-ESI-MS experiment was recorded on a LC- MSD- Trap- SL. Melting points were determined on a Krüss melting point meter model: M5000. Chromatography column (CC) was carried out on Merck Kieselgel 60 (63-200 mesh). Thin layer chromatography (TLC) was performed on precoated silica gel 60 F_{254} plates (Merck) and detection was carried out with 50% H_2SO_4 and $FeCl_3$ solution.

3.2 Plant material

Plant was collected in Da Lat City in April 2009 and was identified by pharmacist Phan Duc Binh, Department of Pharmacy, University of Medicine Ho Chi Minh City. Purple and white flowers had been separated from the plant to research the chemical constituents.

3.3 Extraction and isolation

The air-dried purple flower powder (64 g) was exhaustively macerated with ethanol solvent. After evaporating, the ethanolic extract was subjected to CC, eluting with chloroform and then chloroform- ethyl acetate with increasing ethyl acetate content to afford five fractions (PF1-PF5). The PF3 was rechromatographied on CC, purified by TLC to afford compound **1**.

Applying the same procedure on the air-dried white flower powder (140 g) to obtain 11.7 g of crude ethanol extract. This extract was subjected to flash CC and separated into four different fractions: petroleum ether (3.4g), chloroform (2.3g), ethyl acetate (3.8 g) and methanol (1.8g) extracts. The ethyl acetate extract (3.8 g) was rechromatographied on CC and purified by preparative TLC to yield mixture of compounds **3a** and **3b** with the ratio of 2:1. The same work for chloroform extract (2.3 g), compound **2** was isolated.

3.4 7, 3', 4'-Trihydroxy-6-methoxyaurone (1):

13.5 mg, orange powder. Mp = 144-146 °C. R_f = 0.27 eluted with chloroform: methanol (9:1). LC-ESI-MS (positive mode) *m/z*: 300.9 $[M+H]^+$ (Calc. for $C_{16}H_{12}O_6$). ^{1}H-NMR (DMSO-*d*$_6$), δppm: 7.44 (1H, d, *J*=2.0 Hz, H-2'); 7.33 (1H, d, *J*=8.5 Hz, H-4); 7.26 (1H, dd, *J*=8.5, 2.0 Hz, H-6'); 6.86 (1H, d, *J*=8.5 Hz, H-5'); 6.78 (1H, d, *J*=8.5, H-5); 6.66 (1H, s, H_α); 4.03 (3H, s, $-OCH_3$). ^{13}C-NMR (DMSO-*d*$_6$), δppm: 145.6 (C2); 181.3 (C3); 119.3 (C4); 113.4 (C5); 132.3 (C6); 157.8 (C7); 158.0 (C8); 114.7 (C9); 112.3 (C_α); 132.34 (C1'); 117.9 (C2'); 145.5 (C3'); 148.2 (C4'); 116.0 (C5'); 124.6 (C6'); 60.70 ($-OCH_3$)

3.5 2'-Hydroxy-4, 4'-dimethoxychalcone (2):

15 mg, amorphous orange powder. LC-ESI-MS *m/z*: 284 (Calc. for $C_{17}H_{16}O_4$).

^{1}H-NMR ($CDCl_3$), δppm: 13.53 (1H, s, -O**H**); 7.87 (1H, d, *J*=15.5 Hz, H_β); 7.83 (1H, d, *J*=9.0 Hz, H-6'); 7.61 (2H, d, *J*=9.0 Hz, H-2, H-6); 7.46 (1H, d, *J*=15.5 Hz, H_α); 6.95 (2H, d, *J*=9.0 Hz, H-3, H-5); 6.49 (1H, dd, *J*=8.5, 2.5 Hz, H-5'); 6.47 (1H, d, *J*=2.5 Hz, H-3'); 3.85 (6H, s, 2-OCH_3). ^{13}C-NMR ($CDCl_3$), δppm: 127.5 (C1); 130.3 (C2); 114.5 (C3); 161.8 (C4); 114.5 (C5); 130.3 (C6); 117.8 (Cα); 144.2 (Cβ); 114.2 (C1'); 166.6 (C2'); 101.1 (C3'); 166.0 (C4'; 107.6 (C5'); 131.1 (C6'); 55.4 (-O**C**H_3); 55.5 (-O**C**H_3); 191.9 (>**C**=O).

3.6 7, 2', 5'-Trihydroxyflavone (3a):

14 mg, orange powder. LC-ESI-MS (positive mode) *m/z*: 270.9 $[M+H]^+$ (Calc. for $C_{15}H_{10}O_5$). ^{1}H-NMR (DMSO-d_6), δppm: 7.59 (2H, d, *J*=8.5 Hz, H-5a); 7.44 (2H, d, *J*=2.0 Hz, H-6'a); 7.24 (2H, dd, *J*=8.5, 2.0 Hz, H-4'a); 6.84 (2H, d, *J*=8.5 Hz, H-3'a); 6.72 (2H, brs, H-8a); 6.69 (2H, dd, *J*=8.5, 1.5 Hz, H-6a); 6.61 (2H, s, H-3a). ^{13}C-NMR (DMSO-d_6), δppm: 145.7 (C2a); 111.7 (C3a); 181.0 (C4a); 125.6 (C5a); 112.9 (C6a); 167.4 (C7a); 98.3 (C8a); 166.6 (C9a); 113.0 (C10a); 123.3 (C1'a); 147.9 (C2'a); 116.0 (C3'a); 124.4 (C4'a); 145.5 (C5'a); 117.9 (C6'a).

3.7 5, 7, 3', 4'-Tetrahydroxyflavone (3b):

14 mg, orange powder. LC-ESI-MS (positive mode) *m/z*: 286.9 $[M+H]^+$ (Calc. for $C_{15}H_{10}O_6$). ^{1}H-NMR (DMSO-d_6), δppm: 7.41 (1H, dd, *J*=8.0, 2.9 Hz, H-6'b); 7.39 (1H, brs, H-2'b); 6.89 (1H, d, *J*=8.0 Hz, H-5'b); 6.65 (1H, s, H-3b); 6.44 (1H, d, *J*=1.5 Hz, H-8b); 6.18 (1H, d, *J*=1.5 Hz, H-6b). ^{13}C-NMR (DMSO-d_6), δppm: 163.8 (C2b); 102.8 (C3b); 181.0 (C4b); 161.4 (C5b); 98.8 (C6b); 164.2 (C7b); 93.8 (C8b); 156.3 (C9b); 103.6 (C10b); 121.4 (C1'b); 113.3 (C2'b); 145.7 (C3'b); 149.7 (C4'b); 116.0 (C5'b); 118.9 (C6'b).

4 CONCLUSIONS

By using CC, TLC techniques and stepwise gradient elution with different solvents in polarity, four compounds, namely 7, 3', 4'-trihydroxy-6-methoxyaurone (**1**), 2'-hydroxy-4, 4'-dimethoxychalcone (**2**), and a mixture of 7, 2', 5'-trihydroxyflavone (**3a**) and 5, 7, 3', 4'-tetrahydroxyflavone (**3b)** were isolated from purple and white flowers of *Cosmos bipinnatus* Cav. The chemical structures of isolated compounds were determined by 1D, 2D-NMR and MS spectroscopies. Because the compounds isolated from the geneus *Cosmos* had antimutagen and antifungal activities, the isolated compounds should be tested for their antimutagen and antifungal activities.

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