Asian Journal of Chemistry; Vol. 28, No. 11 (2016), 2479-2481

ASIAN JOURNAL OF CHEMISTRY

http://dx.doi.org/10.14233/ajchem.2016.20036

Phytochemical Investigation of *Abutilon hirtum* (Lam.) Sweet

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Received: 18 April 2016; *Accepted*: 29 July 2016; *Published online*: 10 August 2016; AJC-18022

Two flavones namely 5,3'-dihydroxy-3,7,4'-trimethoxy flavones (**1**), 5,3'-dihydroxy-3,6,7,4'-tetramethoxy flavone (**2**) with known compound stigmasterol have been isolated from the ethyl acetate extract of *Abutilon hirtum*. Their structures were established on the basis of spectroscopic analysis.

Keywords: *A. hirtum*, IR, NMR, EI-Mass.

INTRODUCTION

Abutilon hirtum is an herb belonging to the family Malvaceae. It grows to a height of 3 to 5' and is used as a common herbal drug in the Indian subcontinent. There has been a number of reports on the pharmacological actions of various parts of the plants. Chopra *et al*. [1] and Asha and Banerjee [2] studied the roots, leaves, stem and seeds of *S. cordifolia* are used in traditional medicine against chronic dysentery, asthma and gonorrhoea. The plant of *A. manihot* is used as anti-inflammatory activity [3]. The whole plant of *U. sinuata* shows antibacterial activity [4]. In continuation of our studies on medicinal plants, we have isolated two flavons, along with a known compound stigmasterol from the ethyl acetate extract of *A. hirtum*. In the present paper we report the isolation and characterization of two flavones (**1-2**). The compounds are characterized by spectral techniques using IR, NMR and Mass spectroscopy.

EXPERIMENTAL

The IR spectra were recorded on a Shimadzu FT IR model, 460, respectively. The ^{1}H NMR and ^{13}C NMR spectra were recorded on a Brucker AM 400 FT spectrometer operating at 400 MHz for ^{1}H NMR and 100 MHz for ^{13}C NMR spectra. The shift values are reported as parts per million (ppm) relative to tetra methyl silane. The mass spectra were obtained on a Shimadzu QP-2010 instrument. Column chromatography was performed on silica gel (60-120 mesh).

The flowering plants of *A. hirtum* were collected from Coimbatore district during the month of October 2011. The identification of the plant was verified by the Taxonomists of Botanical Survey of India, Coimbatore.

Extraction and isolation of compounds: The plant materials were chopped into small pieces, dried at room temperature and grind into powder. The dried powder (5 kg) of *A. hirtum* was extracted with ethyl acetate (15 days) on removal of the solvent under reduced pressure at < 40 °C the ethyl acetate extract gave a green mass (700 g). The concentrated residue was subjected to chromatographic separation over a column of silica gel. The column was eluted with (i) petroleum ether (ii) petroleum ether: ethyl acetate with increasing polarity. The eluents were monitored by TLC on silica gel plates.

5,3'-Dihydroxy-3,7,4'-trimethoxy flavone (1): Yellow amorphous powder, m.p. 164-168 °C. IR (KBr, ν_{max}, cm^{-1}): 3422, 2928, 1657, 1586, 1209. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 12.8 (1H brs OH-5), 5.9 (1H brs OH-3'), 7.7 (merged H-2'),7.05 (d 8.4 Hz H-5'), 7.62 (d merged H-6'), 6.44 (d 2.0 Hz H-8), 6.36 (d 2.0 Hz H-6), 3.86 (OCH_3-3), 3.87 (O-CH_3-7), 3.98 (O-CH_3-4'); ^{13}C NMR (100 MHz $CDCl_3$) ppm: 154 (C-2),138 (C-3), 179 (C-4), 161 (C-5), 97 (C-6), 165 (C-7), 92 (C-8), 156 (C-9), 105 (C-10), 121 (C-1'), 110 (C-2'), 146 (C-3'), 148 (C-4'), 114 (C-5'), 121 (C-6') ; M S *m/z* % : 344 (M^+,100); 343 ([M-1]$^+$ 60); 329 ([M-15]$^+$ 55); 313 ([M-31]$^+$ 15); 301 ([M-43]$^+$ 55);

5,3'-Dihydroxy-3,6,7,4'-tetramethoxy flavone (2): Yellow amorphous powder, m.p. 187-188 °C. IR (KBr, ν_{max}, cm^{-1}): 3352, 2939, 1649, 1562, 1213. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 6.4 (s H-8), 7.8 (d 7.2 Hz H-2'), 7.0 (d 8.8 Hz H-5'), 7.8 (d 7.2 Hz H-6'), 12.4 (5-OH), 5.9 (3'-OH); ^{13}C NMR (100 MHz $CDCl_3$) ppm: 156 (C-2), 138 (C-3), 179 (C-4), 152 (C-5), 131 (C-6), 157 (C-7), 97 (C-8), 158 (C-9), 165 (C-10), 122 (C-1'), 111 (C-2'), 146 (C-3'), 149 (C-4'), 115 (C-5'), 122 (C-6'), 111

(C-2'), 146 (C-3'), 149 (C-4'), 115 (C-5'), 122 (C-6'); M S *m/z* % : 374 (M^+,50); 359 ([M-15]$^+$ 100); 344 ([359-15]$^+$ 10); 331 ([359-28]$^+$ 5).

RESULTS AND DISCUSSION

The dried powder (5 kg) of the plant material of *A. hirtum* was extracted with ethyl acetate for 15 days. Removal of the solvents from the extract gave a product of mass (700 g). Purification of ethyl acetate extract results into the isolation of two new compounds **1** (70 mg) and **2** (85 mg) along with stigmasterol.

Compound **1** (70 mg, 0.01 %) was isolated as a yellow amorphous powder, m.p. 164-168 °C. It was dissolved in alcohol, treated with magnesium powder and conc. HCl, results in yellow colour product indicates the presence of flavanoids. The IR spectrum of compound **1** indicates the presence of a conjugated carbonyl group chelated to a phenolic hydroxyl group. A band at 1586 cm^{-1} referred to C=C stretching of the conjugated carbonyl system. The molecular formula of compound **1** was deduced as $C_{18}H_{16}O_7$ from its mass (*m/z* 344, M^+) and ^{13}C NMR spectra.

The ^1H NMR spectrum of compound **1** shown two broad singlets at δ 12.63 and 5.98 each integrating for a proton, which were exchanged with D_2O are characteristic of phenolic hydroxyl groups. Beside were hydroxyl protons three methoxyl protons were observed as singlets at δ 3.98, 3.87 and 3.86 (O-CH_3-3). An ABX pattern at δ 7.70 (merged d and dd, 2H, H-2 and H-6) and δ 7.05 (d, 1H-8.4 Hz, H-5') were assigned to H-6', H-2' and H-5' of ring B respectively which means the ring B is 3',4' substituted. The outstanding two aromatic protons of ring A also appeared as a pair of doublets at [δ 6.36 and δ 6.44 (d, J = 2.0 Hz)] corresponding to two *meta* coupled protons, which resembled those of H-6 and H-8, thus confirming 5,7-disubstituted ring A.

The ^{13}C NMR and DEPT-135 in $CDCl_3$ showed the presence of three methoxy carbons, five methane carbons, nine quaternary carbons and one carbonyl carbon. The presence of a C-3 methoxy carbon was supported by the ^{13}C NMR in which the methoxy signal appeared downfield shift at δ 60.19 methoxy carbons usually resonance at δ 55-56.5. However a downfield shift to the range of δ 59-60.3 is observed when the methoxy group having substituents in both *ortho* position and suggested that one of the methoxyl group is located on C-3 [5].

Flavones have following characteristic chemical shift of carbon of C-ring C-2 (155-165), C-3 (136-139) and C-4 (176-184). HSQC NMR was used to finalize the assignment of proton and carbon. Through the HMBC NMR spectrum signals the three methoxy signals showed correlation with C-4' (148 ppm), C-7 (165 ppm) C-3 (138 ppm). Two *meta* coupling protons of ring A (H-6 & H-8) exhibited long range correlations to C-10 (105 ppm), C-9 (156 ppm), C-7 (165 ppm) and C-5 (161 ppm). On the basis of HSQC and HMBC the full assignments of compound **1** were shown in the Table-1 and Fig. 1. Based on the above spectral data and comparing the ^{13}C NMR signals on its nucleus with those of published data [6]. Compound **1** is characterized as 5,3'-dihydroxy-3,7,4'-trimethoxy flavones (Fig. 2).

TABLE-1
^1H NMR, ^{13}C NMR AND HMBC DATA OF COMPOUND **1**

Position	^1H NMR	^{13}C NMR	HMBC
2	–	154	–
3	–	138	–
4	–	179	–
5	–	161	–
6	6.36 (d, 2.0 Hz)	97	C-8, C-10, C-5
7	–	165	–
8	6.44 (d, 2.0 Hz)	92	C-6, C-9, C-7, C-10
9	–	156	–
10	–	105	–
1'	–	121	–
2'	7.70 (merged)	110	C-6', C-3'
3'	–	146	–
4'	–	148	–
5'	7.05 (d, 8.4 Hz)	114	C-1', C-6', C-3', C-4'
6'	7.62 (d, merged)	122	C-1', C-4', C-2'
OCH_3-3	3.86	60	C-3
OCH_3-7	3.87	55	C-7
OCH_3-4'	3.98	56	C-4'
5-OH	12.8	–	C-5, C-4
3'-OH	5.9	–	C-5', C-4'

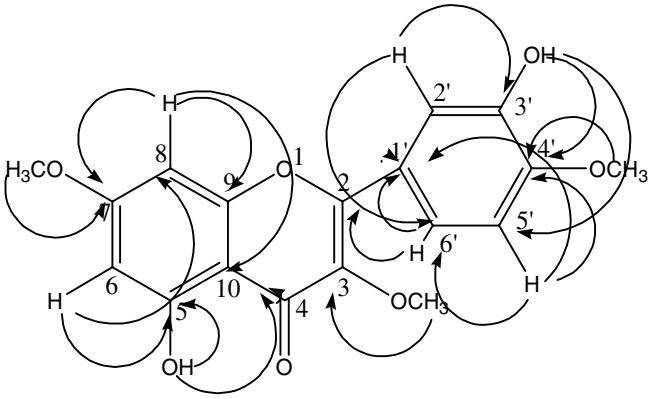

Fig. 1. HMBC NMR correlations of compound **1**

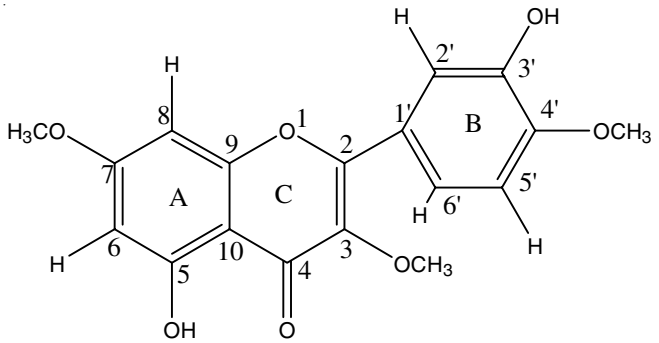

Fig. 2. 5,3'-Dihydroxy-3,7,4'-trimethoxy flavones (compound **1**)

Compound **2** (85 mg, 0.0121 %) was isolated as a yellow powder, m.p.: 187-188 °C. It also gave qualitative tests for flavones. The IR spectrum of compound **2** showed absorptions at 3352 (OH) and 1649 (CO, unsaturated) cm^{-1}. The mass spectrum of **2** showed the molecular formula $C_{19}H_{18}O_8$. ^{13}C NMR and ^1H NMR spectra are also consistent with this molecular formula. Compound **2** showed ^1H NMR and ^{13}C NMR spectra, which are very similar to Compound **1**. Compound **2** differed from **1** by mass *m/z* 30 corresponds to the presence of additional methoxy group. The basic structural similarity of the B

& C ring for compound **2** and **3** was suggested by the mutual comparison of proton NMR and HSQC spectral data with the only noticeable difference being presence of a new methoxyl resonance in **2** and disappearance of signal of one of *meta* coupled ring. A proton (H-6) shows singlet at δ 6.4 integrating for one proton was ascribed to H-8 and the additional methoxy group was fixed at C-6 position. The interpretation of HSQC and HMBC experiments allowed all protons and carbon assignments were tabulated in Table-2 and Fig. 3. Compound **2** is thus characterized as castacin or 5,3'-dihydroxy-3,6,7,4'-tetramethoxy flavones (Fig. 4), which is very similar to the structure **1** and it is also being reported for the first time through the present study from the plant *A. hirtum*. The data was compared with literature [7]. A known natural product stigmasterol were also isolated from ethyl acetate extract.

TABLE-2
^{1}H NMR, ^{13}C NMR AND HMBC DATA OF COMPOUND **2**

Position	^{1}H NMR	^{13}C NMR	HMBC
2	–	156	–
3	–	138	–
4	–	179	–
5	–	152	–
6	–	131	–
7	–	157	–
8	6.4 (s)	97	C-6, C-4, C-7, C-10
9	–	158	–
10	–	105	–
1'	–	122	–
2'	7.8 (d, 7.2 Hz)	111	C-1'
3'	–	146	–
4'	–	149	–
5'	7.0 (d, 8.8 Hz)	115	C-2', C-3', C-4'
6'	7.8 (d, 7.2 Hz)	122	C-1', C-3', C-4', C-2
OCH_3-3	–	59	–
OCH_3-6	–	60	–
OCH_3-7	–	55	–
OCH_3-4'	–	55	–
5-OH	12.4	–	C-8, C-10, C-9
3'-OH	5.9	–	–

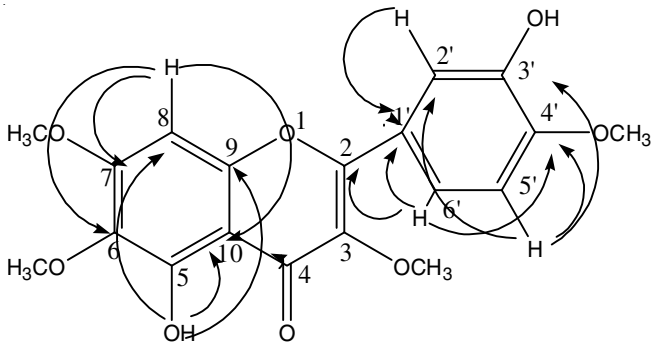

Fig. 3. HMBC NMR correlations of compound **2**

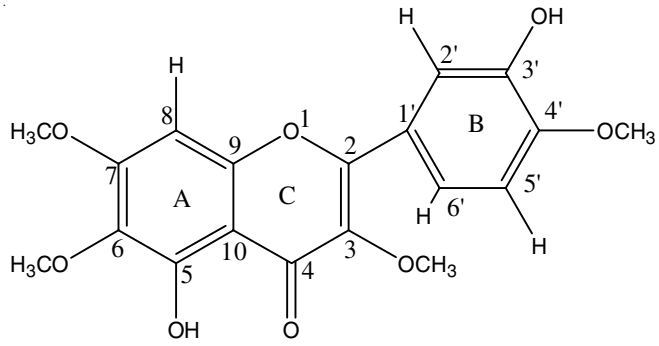

Fig. 4. 5,3'-Dihydroxy-3,6,7,4'-tetramethoxy flavones (compound **2**)

Conclusion

The precipitates obtained from ethyl acetate extract of the plant *Abutilon hirtum* yielded three compounds. They were identified as 5,3'-dihydroxy-3,7,4'-trimethoxy flavones, 5,3'-dihydroxy-3,6,7,4'-tetramethoxy flavones and a known compound stigmasterol.

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