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Two New Bio-active Flavones from *Grangea maderaspatana* (*Artemisia maderaspatana*)

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Two new 5-deoxyflavones, 6-hydroxy-2',4',5'-trimethoxyflavone (**1**), 6-hydroxy-3',4',5'-trimethoxyflavone (**2**) and a known flavone, 7,2',4'-trimethoxyflavone (**3**) have been isolated from the whole plant of *Grangea maderaspatana*. The isolated compounds were characterized by various spectral methods like UV, IR, Mass, ^{1}D and ^{2}D NMR including NOESY, COSY, HSQC and HMBC studies. The antioxidant and anti-fungal screening of the isolated compounds were performed *in vitro* by superoxide free radical scavenging activity method and agar cup method, respectively.

Key Words: *Grangea maderaspatana*, Compositae, 5-Deoxy-flavones, Antioxidant activity, Antifungal activity.

INTRODUCTION

Grangea maderaspatana (L.) Poir. (*Artemisia maderaspatana*) is an herb belongs to compositae family, found throughout the greater part of India[1,2]. The *Grangea* is a small genus containing only 6 species of which 2 occur in India. The leaves are regarded[1] as stomachie, antispasmodic, for irregular menses, in antiseptic, anodyne fomentation and the juice of leaves is employed as an instillation for ear-ache. The previous chemical investigation of *G. maderaspatana* resulted in the isolation of flavonoids[3] and clerodane derivative[4]. In present investigation on the whole plant of *G. maderaspatana*, the isolation, structural elucidation and bioactivity evaluation of two new 5-deoxyflavonoes,6-hydroxy-2',4',5'-trimethoxyflavone (**1**), 6-hydroxy-3',4',5'-trimethoxyflavone (**2**), together with a known flavone, 7,2',4'-trimethoxy-flavone (**3**) have been reported.

EXPERIMENTAL

Melting points were determined on a Kofler hot-stage apparatus and are uncorrected. IR spectra were recorded in KBr discs on a Bio-Rad win FT-IR spectrophotometer and UV spectra on a Shimadzu UV-240 spectrophotometer. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker AC 500 spectrometer operating at 500.032 and

125.73 MHz, respectively tetramethylsilane (TMS) as an internal standard. ^{1}H-^{1}H COSY, HSQC, HMBC and the phase-sensitive NOESY spectra were recorded using the standard pulse sequence. EI-MS were recorded at 70 eV (direct probe) on a Nermag R 10-10 mass spectrometer. Column chromatography was performed on Acme silica gel finer than 200 mesh (0.08 mm).

Plant material, extraction and isolation: The whole plant of *G. maderaspatana* was collected in August 2003 at Bapatla, India. The shade dried and powdered whole plant (2.5 Kg) of *G. maderaspatana* has been successively extracted with petroleum ether, acetone and methanol. The petroleum ether extract on purification over a silica gel column using petroleum ether and ethyl acetate, 8:2 yielded compound **3** (20 mg). The acetone extract on similar purification using petroleum ether and ethyl acetate, 6:4, 5:5 gave compounds **2** (20 mg) and **1** (35 mg), respectively.

6-Hydroxy-2',4',5'-trimethoxyflavone (1): Light yellow solid, m.p. 252-253 °C, UV: λ_{max} (MeOH) (log ε): 253 (4.17), 350 (4.13) nm; IR (KBr, ν_{max}, cm^{-1}): 3442, 3151, 2931, 2848, 1634 (>C=O), 1615, 1595, 1504, 1472, 1368; ^{1}H NMR: ($CDCl_3$ + DMSO-d_6) δ: 9.34 (1H, s, br, OH-6), 7.54 (1H, d, *J* = 2.5 Hz, H-5), 7.44 (1H, s, H-6'), 7.42 (1H, d, *J* = 9.0 Hz, H-8), 7.22 (1H, dd, *J* = 9.0, 2.5 Hz, H-7), 7.06 (1H, s, H-3), 6.64 (1H, s, H-3'), 3.98 (3H, s, OMe-2'), 3.94 (3H, s, OMe-4'), 3.93 (3H, s, OMe-5'); ^{13}C NMR: (Table-3); EIMS: m/z (rel. int. %) 328 $[M]^+$ (100), 297 (10), 285 (17), 192 (26), 177 (28), 149 (22), 137 (63), 108 (9), 69 (25), 44 (27); HRMS: (positive ion mode) m/z 329.1002 $[M+H]^+$ (Calcd. for $C_{18}H_{16}O_6$: 329.1025).

6-Hydroxy-3',4',5'-trimethoxyflavone(2): Pale cream powder, m.p. 223-225 °C, UV: λ_{max} (MeOH) (log ε): 248 (4.16), 336 (3.99) nm; IR (KBr, ν_{max}, cm^{-1}): 3448, 3119, 2949, 2832, 1640 (>C=O), 1616, 1593, 1504, 1457, 1358; ^{1}H NMR: ($CDCl_3$ + DMSO-d_6) δ: 9.90 (1H, s, br, OH-6), 7.53 (1H, d, *J* = 9.0 Hz, H-8), 7.46 (1H, d, *J* = 2.5 Hz, H-5), 7.26 (1H, dd, *J* = 9.0, 2.5 Hz, H-7), 7.19 (2H, s, H-2',6'), 6.77 (1H, s, H-3), 3.96 (6H, s, OMe-3',5'), 3.87 (3H, s, OMe-4'); ^{13}C NMR: (Table-1); EIMS: m/z (rel. int. %) 328 $[M]^+$ (100), 313 (39), 285 (10), 177 (9), 149 (8), 137 (15), 119 (6), 84 (11), 63 (16), 44 (36); HRMS: (positve ion mode) m/z 329.1011 $[M+H]^+$ (Calcd. for $C_{18}H_{16}O_6$: 329.1025).

7,2',4'-Trimethoxyflavone (3): Yellow amorphous powder, m.p. 129-130 °C, UV: λ_{max} (MeOH) (log ε): 238 (4.18), 339 (4.03) nm; IR (KBr, ν_{max}, cm^{-1}): 2966, 2883, 1640 (>C=O), 1600, 1509, 1440, 1376; ^{1}H NMR: ($CDCl_3$) δ: 8.07(1H, d, *J* = 8.8 Hz, H-5), 7.82 (1H, d, *J* = 8.7 Hz, H-6'), 7.03 (1H, s, H-3), 6.90 (1H, dd, *J* = 8.8, 2.3 Hz, H-6), 6.85 (1H, d, *J* = 2.3 Hz, H-8), 6.57(1H, dd, *J* = 8.7, 2.3 Hz, H-5'), 6.50 (1H, d, *J* = 2.3 Hz, H-3'), 3.95 (3H, s, OMe-7), 3.87(3H, s, OMe-7), 3.86 (3H, s, OMe-2'), 3.83 (3H, s, OMe-4'); ^{13}C NMR: (Table-1); EIMS: m/z (rel. int. %) 312 $[M]^+$ (78), 270 (10), 217 (40), 180 (22), 162 (50), 152 (100), 151 (22), 146 (10), 120 (40); HRMS: (Positive ion mode) m/z 313.1000 $[M+H]^+$ (Calcd. for $C_{18}H_{16}O_5$: 313.1076).

Antioxidant activity

Determination of superoxide free radical scavenging activity: The superoxide free radical scavenging activity of flavones was determined by the nitro blue tetrazolium chloride (NBT) method[5,6]. The reaction mixture contained EDTA (6.6 mM), NaCN (3 µg), riboflavin (2 µM), NBT (50 µM), various concentrations of the test drug in ethanol and a phosphate buffer (58 mM, pH 7.8) in a final volume of 3 mL. Optical density was measured at 560 nm. The test tubes were uniformly illuminated with an incandescent lamp for 15 min, after which the optical density was measured again at 560 nm. The per cent inhibition of superoxide radical generation was measured by comparing mean absorbance values of the control and those of the test substances. IC_{50} values were obtained from the plot drawn of concentration in µg *vs.* percentage inhibition and were converted into µM. All the tests were run in triplicate and averaged.

Antifungal activity

Determination by Agar cup method: The antifungal activity of flavones was studied by agar cup method[7,8]. Glass Petri dishes used were sterilized and potato dextrose agar was used as basal medium for test fungi. The saboroudes broth medium was prepared by taking peptone (1.0 g) and dextrose (4.0 g) in warm distilled water (100 mL). The selected fungal culture, single colony was inoculated in to broth medium and kept for incubation for overnight at 25 ºC. The saboroudes agar medium was prepared by taking peptone (1.0 g), dextrose (4.0 g) and agar (2.0 g) in warm distilled water (100 mL) and plated into Petri dishes, allowed to solidification. The overnight fungal culture was spread evenly over the entire surface and left undisturbed for few minutes to percolate the culture. Wells (4 mm) were created using a sterile borer into the solidified agar medium. The selected compounds were added to each well (10 and 20 µL) at peripheral and the reference compound (Fluconazole) was added at the centre. Thus the prepared plates were incubated at room temperature (at about 25 ºC) for about 3-5 d. After incubation period the plates were collected and record the inhibition zone in mm (from the margin of the well to surface of inhibition).

Dimethyl sulphoxide (DMSO) was used as solvent to prepare the stock solutions (5 mg in 0.5 mL) of the compounds initially and also to maintain proper control. A control well was also placed on the test plates to compare the effect of the test samples and to nullify the effect of solvent (DMSO), respectively.

RESULTS AND DISCUSSION

Compound **1** was obtained as light yellow solid. Its molecular formula $C_{18}H_{16}O_6$ was deduced from its HRMS (m/z 329.1002 $[M+H]^+$, 351.0845 $[M+Na]^+$) in conjunction with ^{13}C NMR and DEPT ^{13}C NMR which showed signals for all 18 carbon atoms of molecule. The UV absorption maxima at 253 and 350 nm suggested compound **1** to be a flavonoe[9]. The IR absorption bands at 3442, 2931, 2848,1634, 1615, 1595 and 1472 cm^{-1} and positive ferric chloride test indicated that the compound **1** to be phenolic flavone.

TABLE-1
^{13}C-NMR DATA (125 MHz, $CDCl_3$ + DMSO-d_6 AND 75 MHz, $CDCl_3$) FOR COMPOUNDS **1-3**

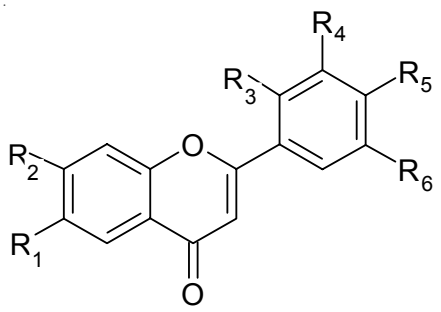
Carbon	**1**	**2**	**3**
2	158.6	161.7	160.4
3	108.5	104.9	111.1
4	176.4	176.9	178.3
4a	122.8	126.0	117.6
5	105.5	107.1	126.8
6	153.2	153.9	113.9
7	121.2	122.1	163.8
8	117.7	118.3	100.2
8a	148.3	148.8	158.0
1'	110.0	123.3	113.5
2'	152.2	102.7	159.4
3'	96.3	152.3	98.8
4'	151.4	139.7	163.0
5'	141.5	152.3	105.2
6'	110.7	102.7	130.2
7-OMe	–	–	55.7
2'-OMe	54.5	–	55.6
3'-OMe	–	55.2	–
4'-OMe	54.9	59.6	55.5
5'-OMe	55.1	55.2	–

TABLE-2
SUPEROXIDE RADICAL SCAVENGING ACTIVITY OF FLAVONES

Compound	IC_{50} µM
1	152
2	160
3	>200
Vitamin-C	852
Vitamin-E	726
BHA	966
BHT	381

TABLE-3
ANTIFUNGAL ACTIVITY OF FLAVONES

Compound	Concentration (µL)	Zone of inhibition (mm)	
		Aspergillus niger	*Penicillum chrysogenium*
1	10	4	3
	20	6	4
2	10	2	2
	20	3	2
3	10	6	3
	20	9	6
Fluconazole	10	9	8
	20	13	11



1: R_1=OH, R_2=R_4=H, R_3=R_5=R_6=OMe
2: R_1=OH, R_2=R_3=H, R_4=R_5=R_6=OMe
3: R_1=R_4=R_6=H, R_2=R_3=R_5=OMe

Fig. 1. Structure of compounds **1**, **2** and **3**

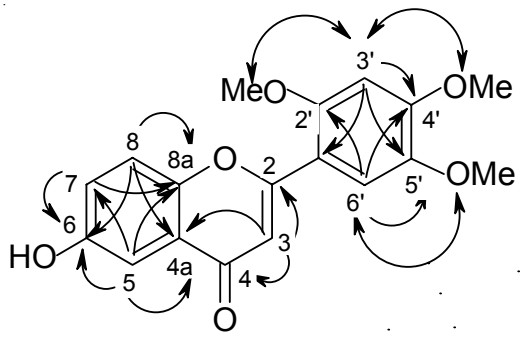


Fig. 2. Significant HMBC (→) and NOESY (⟷) correlations for **1**

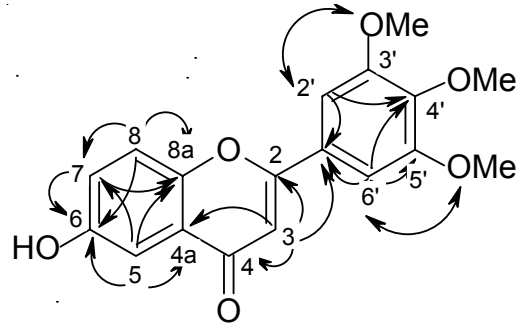


Fig. 3. Significant HMBC (→) and NOESY (⟷) correlations for **2**

The ^{1}H NMR spectrum of **1** showed 3 methoxyl singlets at δ 3.98, 3.94 and 3.93 and a sharp one-proton singlet at δ 7.06 ascribed to H-3. ^{1}H NMR spectrum of flavone (**1**) further showed a ABX spin coupled system at δ 7.54 (1H, d, *J* = 2.5 Hz), 7.22 (1H, dd, *J* = 9.0, 2.5 Hz) and 7.42 (1H, d, *J* = 9.0 Hz) was assigned to H-5, H-7

and H-8, respectively and 7.44 (1H, s) and 6.64 (1H, s) assigned to H-6' and H-3' conforming the presence of monosubstitution in ring-A and tri-substitution in ring-B. The electron impact mass spectrometry (EI-MS) of GM-3 showed 2 *retro*-Diels Alder fragments[10] at m/z 137 $[A1+H]^+$ and m/z 192 $[B1]^+$ indicating the presence of one hydroxyl group in ring-A and 3 methoxyl groups in ring-B, respectively. The three methoxyl groups in ring-B at δ 3.98, 3.94 and 3.87 were assigned to C-2', C-4' and C-5' positions as these three methoxyl protons showed NOE correlation with H-3' and H-6' protons in its NOESY spectrum. The NMR spectral assignments for **1** were further supported by its HSQC, HMBC and ^{1}H-^{1}H-COSY studies. Thus from the foregoing spectral studies the structure of compound **1** was characterized as 6-hydroxy-2',4',5'-trimethoxyflavone and reported first time as new compound.

Compound (**2**) was obtained as pale cream amorphous powder. Its molecular formula $C_{18}H_{16}O_6$ was deduced from its HRMS (m/z 329.1011 $[M+H]^+$, 351.0846 $[M+Na]^+$) and was corroborated by its ^{13}C NMR and DEPT ^{13}C NMR spectra which showed signals for all 18 carbon atoms of molecule. The UV absorption maxima at 248 and 336 nm suggested compound **2** to be a flavone[9]. The IR absorption bands at 3448, 2949, 2832,1640, 1616, 1593 and 1457 cm^{-1} and positive ferric chloride test indicated that the compound **2** to be phenolic flavone.

The ^{1}H NMR spectrum of **2** showed signals for 3 aromatic methoxyl groups at δ 3.96 and 3.87. In the HSQC spectrum, a sharp one-proton singlet at δ 6.77 correlating with C-3 (δ 104.9) was ascribed to H-3. The electron impact mass spectrometry (EI-MS) of **2** showed two *retro*-Diels-Alder fragments[10] at m/z 137 $[A1+H]^+$ and m/z 192 $[B1]^+$ indicating the presence of one hydroxyl group in ring-A and 3 methoxyl groups in ring-B, respectively. The 3 methoxyl groups in ring-B at δ 3.96 and 3.87 were assigned to C-3', C-5' and C-4' positions as these 3 methoxyl protons and H-2' and H-6' protons showed HMBC correlations with these carbons at 152.29 (C-3',5') and 139.69 (C-4'), respectively and by NOE correlation of 2 methoxyl groups at δ 3.96 with H-2' and 6' in the NOESY spectrum. The ^{1}H NMR spectrum of **2** further showed a ABX spin coupled system at δ 7.46 (1H, d, J = 2.5 Hz), 7.26 (1H, dd, J = 9.0, 2.5 Hz) and 7.53 (1H, d, J = 9.0 Hz) was assigned to H-5, H-7 and H-8 and 7.19 (2H, s) assigned to H-2' and H-6' conforming the presence of mono-substitution in ring-A and tri-substitution in ring-B. This was further substantiated by its 2D-NMR spectra. Thus from the foregoing spectral studies the structure of compound **2** was characterized as 6-hydroxy-3',4',5'-trimethoxyflavone and first time reported as natural compound.

The structure of the known compound as 7,2',4'-trimethoxyflavone (**3**) was conformed on comparison of its spectral data with authentic sample[11] and 2'-oxygenated flavone[12].

Antioxidant activity of flavones: The isolated flavones except non-phenolic, flavone have exhibited good antioxidant activity, than the known antioxidants, namely, vitamin C (IC_{50} = 852 µM), vitamin E (IC_{50} = 726 µM), BHA (IC_{50} = 966 µM) and BHT (IC_{50} = 381 µM) (Table-2). The study revealed that the antioxidant activity

of flavones (2-phenylchromones) increases with the increase in the number of phenolic hydroxyl groups (**1**, IC_{50} = 152 µM > **2**, IC_{50} = 160 µM > **3**, IC_{50} >200 µM). The isolated flavone having -OH group at 6th position and methoxyl groups at 2',4',5' positions exhibited little more activity than the compound containing methoxyl groups at 3',4',5' positions. The superior scavenging ability of these compounds (**1** and **2**) lends further support to the fact that the presence of only methoxyl groups did not show any significant effect on the activity of flavones. The results of inhibition concentration (IC_{50} in µM) of natural flavones have been incorporated in Table-2.

Antifungal activity of flavones: The antifungal activities of flavones (**1-3**) were studied *in vitro* at the concentration of 100 and 200 µg against two fungal stains. The screening results indicated that all the compounds exhibited antifungal activities to the tested fungi. It was noted that the flavone (**3**) with -OMe groups only showed a greater inhibitory activity against both fungi compared to the 6-hydroxy-flavones (**1** and **2**). It has also been observed that the flavone (**1**) with -OMe groups at 2',4' and 5' exhibited better fungicidal effect than the flavone (**2**) having -OMe groups at 3',4' and 5' positions.

From the results, it was concluded that the flavone ring system with methoxyl groups at 7, 2',4' positions were responsible for the greater antifungal effects.

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