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Flavonoids from *Anthemis stiparum Pomel.*

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ABSTRACT

Three flavonoid aglycones ,Hispidulin (4',5,7- trihydroxy-6-methoxyflavone), Jaceosidin (4',5,7-trihydroxy-3', 6-dimethoxyflavone) and Eupatilin (5,7- dihydroxy-3',4', 6-trimethoxyflavone) have been identified for the first time from the aerial parts of *AnthemisStiparumPomel* . The structures of compounds were determined using detailed spectroscopic analyses.

Keywords: Asteraceae, *Anthemis stiparum*, Flavonoids.

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INTRODUCTION

Flavonoids are natural products found as biochemical constituents of plants, Because of their potential therapeutic significance [1], the number of identified flavonoids is increasing rapidly and extensive screening of their actions is being carried out in many laboratories [2-4]. Anthemisis a genus of about 130 species of aromatics herbs in the Asteraceae [5], is grown in Algeria in about 10species.Specie*Stiparum* is an endemic plant in Algeria and Morocco [6]. Several members of this genus are used in folk medicine. Algerians use *Anthemis stiparum* as an Antispasmodic. The phytochemical studies of this plant have not been reported. We have now investigated the chemical constituents of this plant. The chromatographic separation of the ethyl acetate extracts of a concentrated ethanol from *Anthemis stiparum* afforded three known flavones aglycones, Hispidulin (**I**), Jaceosidin (**II**) and Eupatilin (**III**).

EXPERIMENTAL

a) Experimental procedure.

NMR spectra were obtained on a Bruker model AMX-400 spectrometer with standard pulse sequences, operating at 400 MHz for ^{1}H. $CDCl_3$ and $(CD_3)_2CO$ was used as solvent and TMS as internal standard. EIMS were taken on a Micromass model Autospec (70 eV) spectrometer. UV Spectra (MeOH): Shimadzu (190–3200 nm, UV-3101PC) spectrophotometer. Column chromatography (CC) was carried out with Silica gel 60 (Merck, 200-400 mesh). Flash column with Silica gel fine (Merck, Silica gel 60 PF254). TLC: Silica gel 60(Merck, F254) and the detection with a spraying reagent ($CH_3COOH/H_2O/H_2SO_4$; 80:16:4) followed by heating.

b) Plant material.

ANTHEMIS STIPARUM POMEL was collected from Stil area (El-oued), in south of Algeria in April 2005 and the plant was identified by Dr.YoucefHallis on the basis of Quezel and Santa[6] A voucher specimen has been deposited in the Herbarium of the laboratory of biochemistry, scientific and technical center for arid areas (CRSTRA), Touggourt, Algeria under the code number Astantmsti2005

c) Extraction and isolation.

Air dried aerial parts of *Anthemis stiparum* (3.42Kg)were extracted three times with EtOH at room temperature, the EtOHextracted was evaporated in vacuo to give dark brown syrup (324g).The syrup was suspended in water and extracted three times with EtOAc.The ethyl acetate layer was concentrated in vacuo to give a black syrup(158g) which was subjected to chromatography on silica gel and eluted with a gradient of n-hexane – EtOAc and EtOAc-MeOH to give 23 fractions(23**f**).300 mg of **f**15(n-hexane – EtOAc 1:1)was subjected to chromatography on silica gel and eluted with $CHCl_3$-Acetone to give **I**(11mg) ,**II**(12mg),**III** (21mg).

Hispidulin **(I):**

Pale yellow; EIMS and UV see tables(1,2); HNMR (400MHz, $(CD_3)_2CO$): δ= 3.74ppm (3H, s, 6-OMe),δ =6.49ppm (1H, s, H-8orH-3) δ =6.50ppm (1H, s, H-3or H-8) , δ=6,89ppm(2H,d,J=8.6Hz,H-3', 5'), δ=7,83ppm (2H,d,J=8.56;H-2', 6'), δ=9.1ppm (1H, 5OH).

Jaceosidin **(II):**

Pale yellow ; EIMS and UV see tables(1,2) ; HNMR(400MHz,$(CD_3)_2CO$) δ=3.74 and 3.86ppm (6H,s,two-OMe), δ=6.50ppm (1H, s, H- 3or H-8) and δ=6.57ppm (1H, s, H-3or H-8), δ =6.87ppm(1H,d,j=8.32Hz, H-5') ,δ= 7.48ppm(1H,dd,j=8.40,1.84Hz, H-6'), δ= 7.51ppm(1H,d,j=1.84Hz,H-2').

Eupatilin **(III):**

Yellow; EIMS and UV see tables(1,2) ; HNMR(400MHz,($CDCl_3$) δ =3.89,3.90,3.97ppm (9H,s,three-OMe), δ= 6.90ppm(1H,d,j=8.4Hz,H-5'), δ =7.19ppm (1H, s, H-2'), δ =7.44ppm (1H, d, J=8.4Hz, H-6').

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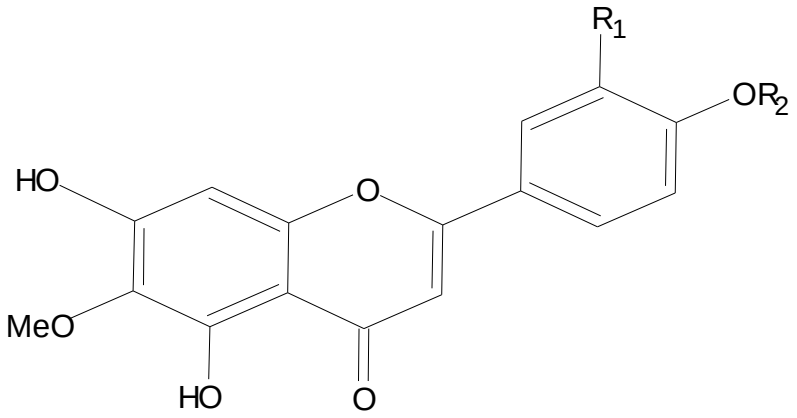
RESULTS AND DISCUSSION

Compound **I** was obtained as a pale yellow. The EIMS of **I** exhibited a molecular ion peak at *m/z* 300(100%) for $C_{16}H_{12}0_6$ in accord with a flavones containing three hydroxyl and one methoxyl group, the peak *m/z*=69is diagnostic of 6-oxygenated [7],and the peak (M-43) $^+$ as characteristic of 6-methoxy flavones and flavanols(table1)[8] .**I** appeared as purple fluorescent spot on a paper chromatogram in UV light, changing to yellow with ammonia indicating the presence of free 5-and 4'-hydroxyl groups .UV maxima in methanol at 336 and 276 and the shifts obtained with diagnostic reagents(table2) suggested the presence of a methoxyl group at C-6(Δ +25nm for band I in $AlCl_3$/HCl compared to band I in MeOH) and hydroxyl groups at positions 5,7,4'[9-12].The ^{1}HNMR spectrum of **I** exhibited one singlet at δ 3.74 for one methoxy group, singlets at δ 6.49 and6.50 assigned to H-3 and H-8 or H-8 and H-3respectively,and a two proton doublet appeared at δ6,89 which is acharacteristic of H-3' and H-5',and a two proton doublet appeared at δ7,83 which isa characteristic ofH-2' and H-6'and signal for one proton at 9.1 for 5-OH[13-14].these spectral data established the structure of **I** as 4',5,7-trihydroxy-6-methoxyflavone (hispidulin).

Compound **II** was obtained as a pale yellow. The EIMS of **II** exhibited a molecular ion peak at *m/z* 330(100%) for $C_{17}H_{14}0_7$ in accord with a flavones containing three hydroxyl and two methoxyl groups, the peak *m/z*=69is diagnostic of 6-oxygenated[7], and the peak (M-43) $^+$ as characteristic of 6-methoxy flavones and flavanols (table1) [8] **.II** appeared as purple fluorescent spot on a chromatogram paper in UV light, changing to yellow with ammonia indicating the presence of free 5-and 4'-hydroxyl groups .UV maxima in methanol at 344 and 275 and the shifts obtained with diagnostic reagents(table2) suggested the presence of a methoxyl group at C-6(Δ +14 nm for band I in $AlCl_3$/HCl compared to band I in MeOH) and hydroxyl groups at positions 5,7,4' [9-12].The ^{1}HNMR spectrum of **II** exhibited two singlets at δ 3.74 and 3.86 for two methoxy groups, singlets at δ 6.50 and 6.57 assigned to H-3 and H-8 or H-8 and H-3respectively,and doublet at δ 6.87assigned to H-5',and doublet-doublet at δ 7,48 assigned to H-6',and doublet at δ 7,51 assigned at H-2'[14]. these spectral data established the structure of **II** as 4',5,7-trihydroxy-3', 6-dimethoxyflavone(Jaceosidin).

Compound **III** was obtained as a yellow. The EIMS of **III** exhibited a molecular ion peak at *m/z* 344(100%) for $C_{18}H_{16}0_7$ in accord with a flavones containing two hydroxyl and three methoxyl groups, the peak *m/z*=69is diagnostic of 6-oxygenated [7], and the peak (M-43) $^+$ as characteristic of 6-methoxy flavones and flavanols (table1) [8] . The UV maxima in methanol at 342 and 276 and the shifts obtained with diagnostic reagents(table2) suggested the presence of a methoxyl groups at C-6(Δ +18 nm for band I in $AlCl_3$/HCl compared to band I in MeOH) and hydroxyl groups at positions 5,7 and the absent of 4'-OH[9-12]**.**The ^{1}HNMR spectrum of **III** exhibited three singlets δ 3.89,3.90,3.97 for Three methoxy groups, doublet at δ 6.90 assigned to H-5', and singlet at δ 7.19 assigned to H-2',and doublet at δ 7.44 assigned to H-6'[13-14].. These spectral data established the structure of **III** as 5, 7- dihydroxy-3', 4', 6-trimethoxyflavone (Eupatilin).



I:R_1=H,R_2=H; II:R_1=OMe,R_2=OME; III:R_1=OMe, R2=Me

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Table 1: MS data for flavonoids												
Flavonoid	**$[M]^+$**	**$[M-H]^+$**	**$[M-Me]^+$**	**$[M-H_2O]^+$**	**$[M-HCO]^+$**	**$[M-COMe]^+$**	**$[A_1-Me]^+$**	**$[A_1-MeCO]^+$**	**$[A_1-MeCO-CO]^+$**	**$[B_1]^+$**	**$[B_2]^+$**	$[(CH2)_3CH=CH_2]^+$
Hispidulin I	**300**	**299**	**285**	**282**	**271**	**257**	**167**	**139**	**111**	**118**	**121**	**69**
(%)	**(100)**	**(7)**	**(68)**	**(39)**	**(8)**	**(56)**	**(15)**	**(16)**	**(1)**	**(11)**	**(6)**	**(46)**
Jaceosidin II	**330**	**329**	**315**	**318**	**301**	**287**	**167**	**139**	**111**	**148**	**151**	**69**
(%)	**(100)**	**(7)**	**(66)**	**(40)**	**(6)**	**(40)**	**(10)**	**(9)**	**(1)**	**(4)**	**(4)**	**(28)**
Eupatilin III	**344**	**343**	**329**	**326**	**315**	**301**	**167**	**139**	**111**	**162**	**165**	**69**
(%)	**(100)**	**(6)**	**(66)**	**(48)**	**(7)**	**(39)**	**(7)**	**(8)**	**(~~0)**	**(4)**	**(6)**	**(13)**

MS were recorded at 70eV.Valuesare given in m/z and in parentheses the % abundance relative to the base peak. The A_1, B_1,and B_2 terminology for the fragments is given in [12].

Table 2: UV data for flavonoids						
Flavonoid	**MeOH(λmax, nm)**	**NaOH(λmax, nm)**	**AlCl3(λmax, nm)**	**AlCl3/HCl(λmax, nm)**	**NaOAc(λmax, nm)**	**NaOAc/H3BO3(λmax, nm)**
Hispidulin I	**336**	**396**	**362**	**361**	**380**	**348**
	276	**328**	**303**	**292**	**277**	**276**
		276	**282**	**260**		
Jaceosidin II	**344**	**408**	**374**	**360**	**399**	**361**
	275	**337**	**282**	**281**	**322**	**277**
		275	**262**	**260**	**276**	
Eupatilin III	**342**	**370**	**368**	**359**	**369**	**351**
	276	**309**	**287**	**290**	**314**	**276**
		275	**268**	**257**	**276**	

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