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Flavone and flavone glucoronide from *Zillamacroptera*L.(Brassicaceae) Growing in Algerian Sahara

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ABSTRACT

The Phytochemical investigation of the water-ethanol extract of the aerial parts of Zillamacroptera (Brassicaceae), reported the isolation for the first time two flavonoids derivatives named: 8,4'-Diméthoxy,5,7- Dihydroxyflavoneand 7-(6''-methylglucuronyl) apigenin. The structures of these compounds were elucidated on the basis of spectral data.

Key words: *Zillamacroptera*, Brassicaceae, Flavonoids, Medicinal plants, Sahara

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INTRODUCTION

Natural products have served as an important source of drugs since ancient times and about half of the useful drugs today have been derived from plants. Recently, the search for drugs derived from plants has accelerated. South Algeria with its rich floral resources and ethnobotanical history is an ideal place to screen plants for biological activity and as a source of new pharmacological compounds [A. Cheriti, 2000; A.Gurib-Fakim, 2006, Cheriti et al, 2012].Brassicaceae is one of the ten most economically important plant families in the world, itcontain a number of nutrients and phytochemicals and have been used to treat a wide range of human diseases[Bellakhdar, 1997;Nostro*etal*., 2000;Berreghioua*etal*., 2009].Among the species belonging to this family,*Zillamacroptera*which is a herbaceous medicinal plant widely distributed in Algerian Sahara [Ozenda, 1991], locally known by the common name "Boukhlala".

Following our phytochemical works[Cheriti*etal*., 2004, Belboukhari and Cheriti, 2006 ;Belboukhari*etal*., 2015, Lahmar et al, 2015] on the secondary metabolitsand bioactives substances from medicinal plants growing in Algerian Sahara,

Now we are interested in isolating the natural compounds responsible of the antimicrobial activities [Berreghioua*etal*., 2014]from theethyl acetate fraction of the water-ethanol extractof the aerial parts of *Zillamacroptera*.

MATERIALS AND METHODS

General experimental procedure: UV spectra were obtained in MeOH solvent with Unicam UV 300 spectrophotometer and Specord 200 Plus spectrophotometer. IR spectra wereobtained with a Thermo Nicolet Avatar 320 FT-IR spectrophotometer. The NMR spectra were taken on a BrukerAvanceGP 250 (^{1}H :250 or 300 MHz; ^{13}C : 75 or 125 MHz) Spectrometer. TLC was carried out on silica gel 60 F254 plates (Merck, Germany). Column chromatography was performed over silica gel 60 (Merck, particle size 230-400 mesh).

Plant material: The whole plants of *Zillamacroptera*were collected during the period of (February-March 2011) from Becharand identified by PrA. Maarouf(Naama University). A voucher specimen is deposited in the herbarium of Phytochemistry and Organic Synthesis Laboratory (LPSO)of BecharUniversity under the number CA00/71.The plants parts were grounded into powder from using the grinder.

Extraction and isolation :170g of aerial parts of *Z. macroptera* are contacted with 1700 mL of a mixture of distilled water and ethanol (50/50 (v /v)) with stirring for 48 hours at room temperature. This extract solution is filtered and evaporated (50%). The operation of the maceration was repeated three times, by renewing the solvent, to increase the mass of the extract.The macerate in green color is fractioned successively by four Solvents :*n*-hexan (5x 50mL) for degreasing , chloroform (3 x 50mL) , ethyl acetate (3 x 50mL) and*n*-butanol (3 x 50mL)[Belboukhari and Cheriti, 2007].

To purify theconstituents of AcOEt fraction (2.3 g), liquid chromatography on column was achieved by usinga column in glass of type 20 mm/300 mm (29/39) filled with astationary phase of silica gel (0,20 mm) and the mobile phasechosen for this separation is chloroform /methanol (100 /0 , 80/20 , 50/50 , 20/80, 0/100).

RESULTS AND DISCUSSION

According to our previous works[Berreghioua*etal*., 2009 ;Belboukhari and Cheriti, 2009 ;Berreghioua and Cheriti, 2011], we summarized the information collected from the ethnomedical survey on the traditional uses *of Zillamacroptera*and the usage rate : gastric disorders and stomach pain (47%), diarrhea(21%),kidneys,liver and pancreas pain(11%), Nausea and colds(7%), Respiratory ailments(6%), Eczema(3%).

We summarized in table 1 the information collected from the phytochemical screening.The experimental results on of phytochemical screening revealed the presence of flavonoids, saponosids and tannins. The presence of tannins is confirmed by positive reaction with ferric chloride ($FeCl_3$). Flavonoids test results showed positive reaction in the presence of magnesium and HCl. In contrast, the study indicated that insatured sterols and cardinolids were absent in aerial part extracts of our studied plant. However, we observed less presence of steroids and alkaloids.

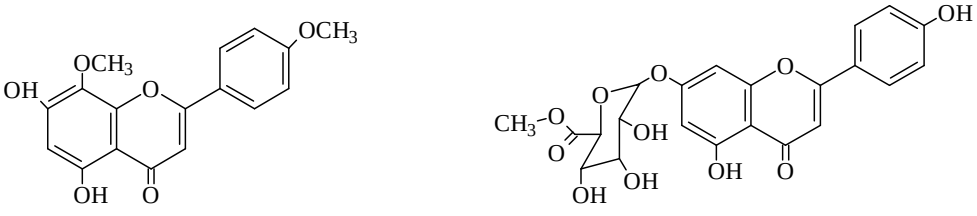
The separation has been done previously on a mass of (2.3 g) ofAcOEt extract. TLC analysis of the samples separated by liquid chromatography on column revealed the existence of two products to different Rf. We have successfully separated one product in the third column and one in the fourth column.We regrouped the results after several separations and analysischromatographic (Table 2).

Table 1 : Results of phytochemical screening

Alkaloïds	+
Cardinolids	-
Flavonoïds	++
Glycosidsflavonoïds	+
Insaturedsterols	-
Saponosids	++
Steroids	+
Tanins	++

Table 2 : Results of liquid column chromatography (C3,C4 third and fourth Column)

	R_f(Colorin TLC)				
Compounds	BAW (4/1/5)	$CHCl_3$/MeOH/H_2O (9/1/0.5)	$(CH_3)_2CO/H_2O$ (1/1)	Column	Fractions
1	0.56(blue)	0.15 (purple)	0.49 (purple)	C3	46-56
2	0.86 (red)	0.77 (purple)	0.40 (red)	C4	77-80



(1) **(2)**

Figure1:Structures of the molecules isolated from*Zillamacroptera*

***8,4' -diméthoxy,5,7- dihydroxyflavone* (1)**
Molecular formula: $C_{17}H_{14}O_6$, 64.96(C), 4.45(H), 30.57(O); UV_{max}(MeOH) : 320, 270 nm ;(MeOH+NaOH) :372,210, 326 nm ; (MeOH+$AlCl_3$) :360,282,310 nm ; (MeOH +$AlCl_3$ + HCl) : 360.5, 282, 308 nm ;(MeOH +NaOAc) : 346, 279,326 nm ; (MeOH +NaOAc+H_3BO_3) 321.5,276 nm. IR(KBr): 3410.02,2967.53, 2934.75, 2858.27, 1629.14, 1508.95, 1416.08, 1459.79, 1257.66, 1028.22, 1055.54, 995.45, 924.43, 667.68cm^{-1}. ^{1}H RMN(δ (ppm)) : 6.86(H-3, s), 12.62(OH-C5, s), 6.28(H-6, s, *J*=2.1Hz), 7,88(H-2',d, *J*=8.7Hz), 7.13(H-5',d, *J*=8.7Hz), 7,88(H-6',d, *J*=8.7Hz), 3.85(OMe-C8), 3.85(OMe-C-4').^{13}CRMN (DMSO-d6,δ(ppm)): 162.9(C-2),103.1(C-3),181.3(C-4),156.2(C-5),103.7(C-5a),98.1(C-6),157.1(C-7),127.8(C-8),148.8(C-8a),122.9(C-1'), 128.6(C-2'),114(C-3'), 162.3(C-4'), 114 (C-5'), 128.6 (C-6'), 58.3(OMe-C8), 53.4 (OMe-C-4').

***7-(6''-methylglucuronyl) apigenin* (2)**
Molecular formula : $C_{22}H_{20}O_{11}$, 57.39(C), 4.34(H), 38.26(O);UV_{max}(MeOH) : 3 32, 270 nm ;(MeOH+NaOH) : 384, 273nm ;(MeOH +$AlCl_3$) : 375, 271, 310nm ; (MeOH +$AlCl_3$ + HCl) : 375.5, 272, 308nm ; (MeOH +NaOAc) : 372, 278nm ; (MeOH+NaOAc+H_3BO_3) : 334, 278nm.IR(KBr): 3610.24, 2920.24, 2850 .25, 1699.55, 1640 .99, 1541 .92, 1523.59, 1457, 1098.65, 800.32 cm^{-1}.^{1}H RMN (δ (ppm)) : 6.72 (H-3, s), 13 (OH-5, s), 6.23(H-6, d, *J*=2.2Hz), 6.5(H-8,d, *J*=2.2Hz), 5.36 (H-1', d, *J*=7.4Hz) , 7.90(H-2', d, *J*=8.8Hz), 7.05(H-3', d, *J*=8.8Hz), 7.05(H-5', d, *J*=8.8Hz), 7.90(H-6', d, *J*=8.8Hz), 5.30 (H1''glucoronide, d, *J*=7.3), 3.58 (H-2'', *ddd*, *J*=7.2 Hz), 4,87 (OH-2'', *d*, *J*=4.0 Hz), 3.59 (H-3'', *td*,*J*=8.9 Hz),4.60(OH-3'', *d*,*J*=4.0Hz), 3.70(H-4'',*ddd*,*J*=4.8 Hz), 4,63 (OH-4'', *d*, *J*=4.8 Hz), 4.25(H-5'',d, *J*=9.4Hz), 3,69(-OCH_3, s). ^{13}C RMN (δ(ppm)): 165.6(C-2),104.4(C-3), 183.3(C-4),162.1(C-5), 106.9(C-5a), 100.4(C-6), 163.9(C-7), 95.6(C-8), 158.4(C-8a), 123.2(C-1'), 129.4(C-2') , 116.9(C-3'), 163.1(C-4'), 116.9(C-5'), 129.4(C-6'), 101.1(C-1''), 74.2(C-2''), 77.0(C-3''), 72.6(C-4''), 76.5(C-5''), 52.5(-OCH_3).

According to the results of IR analysis of compounds**(1)** and **(2)**, the absorption bands observed toward (3410-3610 cm^{-1}) correspond to the vibration of elongation O-H (valence vibration), the alipbatic links C-H is presented in the IR specter by fine and intense bands toward 2934 cm^{-1} . The frequency of vibrations situated toward (2850-2967cm^{-1}) corresponds to the valance vibration of CH_2, the bands of absorption toward (2850 -22858cm^{-1}) associate has vibrations of syrnmetrical valence of the CH_2[Belboukhari 2007; Belboukhari and Cheriti, 2007 ; Wagner *etal*.2011]. The frequency of vibration (924- 995cm^{-1}) corresponds to the distortion vibration out ofthe plan ofthe unsaturated hydrocarbons. The valence vibration ofthe cyclic ketones or aliphatic ketoncs (C = O) to be located toward (1629 -1699 cm^{-1})[Bitamet*al*., 2008]. The vibrations between (1028-1098 cm^{-1}) corresponds to the ether-oxyde group.We note the existence of the bands toward (1098-1258 cm^{-1}) in separated products, wich confirms the presence of an aromatic alcohol corresponds to the vibration (aromatic OH), and also the strips toward 1 028 to 1 098 cm^{-1} made us think about a primary, secondary alcohol or even tertiary[Bitam*et al*., 2008 ; Calderon, 2011].

The IR spectrum of these products indicates the presence of ansubstituted aromatic cycle confirmed by the vibrations ofelongation (C=C) situated toward 1508 cm^{-1} and 1629 cm^{-1}, the link (aromatic (=C-H)) is well identified by the distortions vibration toward 667 cm^{-1}.

The band of absorption toward1257 cm^{-1} characterizes an ether-oxide aliphatic. In IR, Theabsorption band situated to 924 – 995cm^{-1} characterizes a trans ethylic link, and the strip to 1629cm^{-1} can be probably assigned to the valence vibration of the (C=C)conjugated group. From these primary results, the compounds**(1)** and **(2)** can possess thegeneral structure of a flavanone, flavonol and flavone[Calderon, 2011].

The compound**(1)** was isolated as yellow crystals from the ethyl acetate extract. The chromatographic (TLC) profiles with the presence of two major peaks at 320 nm and 270 nm in UV-visible suggested that this compound belong to flavonoids class, type flavone (Mabry *etal*., 1970). The peak at 320 nm showed a bathochromic shift (52 nm) with NaOH indicating that the 4' position is substituted (Voirin, 1983). The same peak has a bathochromic shift (52 nm) with $AlCl_3$ establishing the presence of an OH in position 5(Rosler and Mabry, 1971). The Spectrum with(MeOH + $AlCl_3$ + HCl) for this peak does not present any hypsochromic effect with respect to the spectrum (MeOH + $AlCl_3$), this fact indicate the absence of orthodihydroxylated system for the two rings A and B.The fact that the second band at 270 nm is moved to (+ 9 nm) with (MeOH + NaOAc) accompanied with the appearance of a new band at 326 nm in favor of a free OH in position 7. The absence of ortho di OH in the position 4' is confirmed by the very small bathochromic shift (+1.5) of the 320 nm band after the addition of H_3BO_3 at (MeOH + NaOAc).

The ^{1}H NMR spectrum revealed a singlet signal at 12.62 ppm, whichconfirm the existence of an OH in position 5. A singlet at 6,28ppm (J = 2.1 Hz ; 1H) is attributable to H -6 ;in the other hand the singlet at 3.85 ppm with 6H integration is corresponding to the two methoxyl groups. The singlet signal at 6.86 ppm is attributable to H -3 and the signal at 7.13 ppm (J = 8.7 Hz ; 2H) as a doublet form is assigned to the protons H-3' and H - 5'.The same coupling constant that appears to 7.88 ppm with 2H integration, permits us to attribuate that signal to H -2 and H- 6 ' position.

The ^{13}C NMR spectrum confirmed the presence of two methoxyl located at 53.4 and 58.3 ppm. We can also distinguish the aromatic(CH) at98.1, 128.6 and 114 ppm and a carbonyl group at 181.3 ppm. The presence of peak at 103.1 ppm corresponding to carbon -3 which characterize a flavone type structure[Agrawal and Markham, 1989]. According to the chromatographic behavior (fluorescence under UV light (365 nm) and R_f) and the presence of one major peak at 332 nm this compound**(2)**is either a flavone or flavonol (mono-glycosylated)[Mabry *et al.*, 1970] . The bathochromic effect (52 nm) on this peak after the addition of NaOH indicates the presence of free OH in the position 4', another bathochromic shift (52 nm) was observed with $AlCl_3$ establishing the presence of an free OH in position 5. A small displacement of the peak is observed when we compare the spectrum ($AlCl_3$ + HCl) with that of $AlCl_3$;this fact suggests the absence of aorthodihydroxylated system on the cycle B.The second band exhibits a bathochromic shift (8 nm) with (MeOH + NaOAc) suggesting a free OH in position 7.However, the spectrum (MeOH + NaOAc + H_3BO_3) done a very small bathochromic shift (2nm) on this peak establishing the absence of the OH group in the di-ortho position C3'and C4 ' (Roslerand Mabry, 1971; Voirin, 1983).

The investigation of ^1H NMR spectrum shows the presence of two doublets with one integration (1H) at 6.5 and 6.23 ppm (J = 2.2 Hz) that characterizes the two protons of H-8 and H-6 respectively. The two doublets at 7.90 ppm and 7.05 ppm with 2Hintegration at each one (J = 8.8Hz) are due to (H- 2 ', H- 6') and (H- 3 ', H-5 ') respectively, this fact indicates also the oxygenation of B ring in the 4'position of a flavonoid. A singlet at 13 ppm is attributable to OH at C- 5 position. A singlet signal at 6.72 ppm with 1H integration is due to H-3,orientating towardsa flavonestructure. The singlet at 3.69 ppm is due to a methoxy group whereas the doublet at 5.30 ppm (J = 7.3 Hz) is attributable to the proton of a sugar (position 7) linked by a CO bond, since all other positions are occupied by protons. Based on the values of the coupling constants, the three doublets with 1H integrating to each one at 4.87 ppm (J = 4.0 Hz), 4.63 ppm (J = 4.8 Hz) and 4.60 ppm (J = 4.0 Hz).The triplet of doublets with 1H integration 3.59 ppm (J = 8,9Hz) and the two doublets of doublets split at 3.58 ppm (J = 7.2 Hz) and 3.70 ppm (J = 4 8 Hz) are due to the three protons of the three CH groups linked to the three hydroxyls of sugar. The doublet at 4, 25 ppm (J = 9.4 Hz) with 1H integration must also be part of CH pertining to sugar[Voirin, 1983;Rosler and Mabry, 1971]. The examination of ^{13}C NMR spectrum shows the presence of CH_2OH group at 59.2 ppm,whitch directing towards a glucosyl type substituent. All these data permits us to say that this compound **(2)**is 4 ', 5 -dihydroxy - 7 -O glucosylflavone known as 7 -O -glucosylapigenin or 7- (6 " - methylglucuronyl)apigenin[Ahmed *et al*., 1970].

CONCLUSION

In Saharan ethopharmacopea*Zillamacroptera*plant is used to treat rheumatism, gastric disorders, diarrhea, respiratory ailments and other diseases. From this study, the antimicrobial extract of the aerial parts from this Saharan medicinal plant was selected for phytochemical investigations. The chromatographic analysis of the Ethyl acetate fraction of the water-ethanol extract led to the isolationfor the first time two flavonoids derivatives: *,4'-Diméthoxy,5,7- Dihydroxyflavone***(1)**and *7-(6''-methylglucuronyl) apigenin***(2).**

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