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Flavonoids glycosides from leaves of *Catha edulis* (Celasteraceae)

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ABSTRACT

*Five flavonoid glycosides having kaempferol**3**, quercetin**4** and myricetin**2**, **5** and **6** skeleton were isolated from leaves of Catha edulis species together with quercetin. The structure of **ampelopsin(2)**, was assigned by combination on 1D and 2D NMR spectroscopy for the first time as well as MSESI. While the structures of**kaempferol 3-O-β-Glucopyranoside (3)**, **Quercetin 3-O-β- Galactopyranoside(4)**, **Myricetin 3-O-β-Glucopyranoside (5)** and**Myricetin 3-O-β-Xylofuranoside(6)** were identified by ^{1}H and ^{13}C NMR, as well as UV spectroscopy and also confirmed by the spectral data of aglycones and TLC of the sugars obtained after acid hydrolysis. Compounds **3**, **5** and **6**were reported for the first time from this plant.*

Key words: Celasteraceae,*Catha edulis*, flavonoid glycosides.

INTRODUCTION

Khat belongs to natural drugs known as *Catha edulis*Forsk and has other names related to its growing regions [1]. It is a tree always green, growing in the highest hill expanding from East to South Africa, Afganistan and Yemen [2].It is customarily chewed in these areas to attain a state of euphoria and stimulation. The World Health Organization (WHO) has also classified Khat as a drug of abuse which can produce mild to moderate psychic dependence [3].

Several phytochemical studies showed that the chemical constituents of *Catha edulis*leaves include (-) cathinone, (+)-norpseudoephedrine, ethereal oils, sterols, triterpenes, tannins, ascorbic acid, etc… [4-8]. Flavonoids and their biological activities havebeen widely studied in earlier years and many flavonoids and flavonoids glycosides have been reported from *Catha edulis*[9-10].In continuation of our study on this species [11-12],and in the aim to find other secondary metabolites not described until now, we report here the isolation and the structure elucidation of flavonolglycosides from EtOAc and n-butanolfractions of the soluble parts of the aqueous EtOH extract of the leaves of this species. The structures of the isolated compounds were identified on the basis of spectroscopic studies, acid hydrolysis and comparison with literature data.

MATERIALS AND METHODS

Plant material

The leaves of *Catha edulis*have been collected from Hajjahregion in the north –west of sana'a in mid-September 2008.A voucher specimen of the plant material has been deposited in the department of biology (Sana'a University).

Extraction

Leaves of *Catha edulis*were air-dried and macerated with EtOH/H2O (7/3; v/v) for 24 hours four times. The crude extract was concentrated at room temp. and diluted with distillate water. After precipitation of chlorophyll with $Pb(OAc)_4$ and filtration, the remaining aqueous solution was extracted successively with $CHCl_3$, EtOAc and*n*-BuOH. The organic layers were dried with Na_2SO_4 giving after removal of solvents under red. pressure, $CHCl_3$, EtOAc and *n*-BuOH extracts respectively.

Separation and isolation

A part of the EtOAc extract (3g) was fractionated by CC on silica gel using chloroform with increasing percentages of methanol to yield 10 fractions (1–10) obtained by combining the eluates on the basis of TLC analysis. Fraction 5 which contained a major component was resubmitted to preparative TLC ($CHCl_3$/MeOH; 9:1) to afford compound **1**. The fraction 7was subjected to preparative TLC ($CHCl_3$/MeOH; 9:1) to afford 4 subfractions(7-1; 7-4). The subfraction 7-3 contained a pure compound **2**.

The *n*-BuOH extract (15 g) was subjected to a silica gel column chromatography being eluted with a gradient system of $CHCl_3$/MeOH with increasing polarity to afford 21 fractions. Three main fractions (7, 17 and 21) were collected. Fraction 7 was further resubmitted to preparative TLC ($CHCl_3$/MeOH; 8.5:1.5) to afford 7 subfractions. The subfraction 7-6 contained an impure compound which was purified on Sephadex LH-20 column eluting with MeOH to give compound **3**.Fraction 17 was subjected to preparative TLC ($CHCl_3$/MeOH; 8.5:1.5) to afford 4 subfractions. The subfraction 17-3 was purified over silica gel column using CHCl3/MeOH; 8.5:1.5 and afforded pure compound **4**. The fractionation of the important fraction 21was carried out on Whatman N°3 paper with the system AcOH 15%to lead 4 subfractions. The subfractions 21-1 and 21-3 were purified on Sephadex LH-20 column eluting with MeOH to give pure compounds **5** and **6**respectively.

Acid hydrolysis

Solutions of compounds **3**, **4**, **5** and **6** in 2 ml (HCl 4N) were heated for 2 h and left to cool. The mixture was extracted with EtOAc and the EtOAc fractions were used for detection of the aglycone. The aqueous fractions were concentrated and using for identification of sugars. The sugars were identified by TLC using solvent system (acetone–water; 90:10)by comparison with authentic samples.

RESULTS AND DISCUSSION

From the EtOAc and *n*-butanol extracts obtained from the hydroalcoholic solution of *Catha edulis* leaves, six flavonoid compounds (**1-6**) (figure 1), were obtained by different chromatographic methods. The compounds were identified on the basis of their spectral data and of consistency with literature data for similar structures. Among the earlier flavonoid compounds **3, 5** and **6** were isolated for the first time from the leaves of this plant.

Compound 1 : yellow powder soluble in methanol, UV λmax (nm); MeOH: 257, 371; + NaOH: 246, 416, 329; +$AlCl_3$: 269, 439; + $AlCl_3$/HCl: 269, 430; +NaOAc: 259, 397; + NaOAc/H_3BO_3: 259nm, 386nm.^{1}H NMR (MeOH-d_4, 250MHz,:δppm, *J*:Hz): 7.76 (1H, *d*, *J*=2.1, H-2'), 7.65 (1H, *dd*, *J*=8.5; 2.1, H-6'), 6.90 (1H, *d*, *J*=8.5, H-5'), 6.41 (1H, *d*, *J*=2.1, H-8), 6.20 (1H, *d*, *J*=2.1, H-6). ^{13}C NMR (MeOH-d_4, 62.9MHz, δ:ppm) 146.5 (C-2), 155.8 (C-3), 175.9 (C-4), 161.5 (C-5), 97.8 (C-6), 164.2 (C-7), 92.9 (C-8), 156.7 (C-9), 103.5 (C-10), 122.7 (C-1'), 114.5 (C-2'), 144.8 (C-3'), 147.3 (C-4'), 114.8 (C-5'), 120.2 (C-6'). This compound was identified as quercetin [13].

Compound 2: white crystals soluble in methanol,The molecular formula of this compound**,** was determined as [M+2H+Na] corresponding to $C_{21}H_{24}O_{12}Na$ on the basis of TOF MS ES+ spectrum at *m/z* 491.1156 (100%) according to the $C_{21}H_{22}O_{12}$ (calculated 491.1156) indicating a compound containing 11 unsaturations.Its ^{1}H NMR spectrum of this compound exhibited a typical AX system due to H-2 and H-3 of a dihydroflavonol [1**4**]at 5.00 (*d*, *J* 10.6 Hz) and δ H 4.55 (*d*, *J* 10.6 Hz) respectively. These assignments were confirmed by the ^{13}C NMR, HSQC and HMBC spectra which showed three C-ring carbon signals at δC86.6 (C-2), 81.0 (C-3) and 198.5 (C-4)**[15]**. The trans-configuration of the upper dihydroflavonol unit could be deduced from the coupling constant between H2 and H3 ($^3J_{H2\text{-}H3}$=10.6 Hz) in the ^{1}H NMR spectrum. Besides, the ^{1}H NMR (MeOH-d_4, 400MHz,δ:ppm, *J*:Hz) exhibited : 6.54 (2H, *s*, H-2'&H-6'), 5.93 (1H, *d*, *J*=1.9, H-8), 5.91(1H, *d*, *J*=1.9, H-6), 4.12 (1H, brs, H-1" Rhamnose), 3.62 (1H, br*d*, *J*=3.2, H-2"), 3.70 (1H, *dd*, *J*=9.5;3.2, H-3"), 3.33 (H-4"obscured by the signal of the water of the solvent), 4.27 (1H,*dq*,*J*=9.5; 6.2, H-5") all these signals were deduced from COSY and finally at δ1.21(3H, *d*, *J*=6.2, -$CH_3$of rhamnose). ^{13}C NMR (CD_3OD, 100MHz, δppm): 198.5 (C-4), 171.1 (C-7), 168.0 (C-5), 166.6 C-9, deduced from HMBC spectrum), 149.6 (C-4'), 137.5 (C-3' & C-5'), 130.8 (C-1'), 110.2 (C-6', deduced from HSQC spectrum), 110.1 (C-2', deduced from HSQC spectrum), 105.0 (C-10, deduced from HMBC spectrum), 99.9 (C-6), 98.8 (C-8), 104.6 (C-1"), 74.3 (C-2"), 74.7 (C-3"), 76.3 (C-4"), 73.0 (C-5"), 20.4 (C-6"). On the basis of all these results, this compound was characterized asDihydromyricetin 3-*O*-α-rhamnopyranoside, named Ampelopsin. This compound

has been isolated from this plant in 1981 [16], while this study has permitted the establishment of structure on the basis of combining all spectral data (^{1}H, ^{13}C NMR, DEPT 135, HSQC, HMBC as well as MS-ESI).

Compound 3: yellow powder soluble in methanol, UV λ_{max} (nm):MeOH: 263, 351; + NaOH: 269, 391, 331. ^{1}H NMR (MeOH-d_4, 250 MHz, δ:ppm, *J*:Hz): 8.12 (2H, *d*, *J* = 8.24, H-2'&H-6'), 6.87 (2H, *d*, *J* = 8.24, H-3'&H-5'), 6.26 (1H, brs, H-8), 6.11 (1H, brs, H-6), (3.5- 4.2 sugar protons).Acid hydrolysis of compound 3 produced Kaempferol and Glucose. This compound was characterized as Kaempferol-3-*O*-*β*-glucopyranoside, named Astragalin [17].

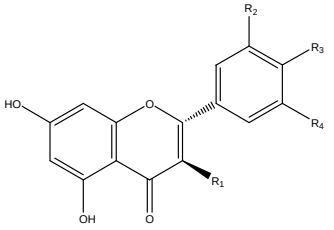
Compound 4: yellow powder soluble in methanol, UV λ_{max} (nm):MeOH: 257, 356; + NaOH: 269, 403, 329; +$AlCl_3$: 269, 431; + $AlCl_3$/HCl: 269, 403;+NaOAc: 273, 381, 323;+NaOAc/H_3BO_3: 263, 381. ^{1}H NMR (MeOH-d_4, 250MHz, δ:ppm, J:*Hz*): 7.88 (1H, *d*, *J* = 2.1, H-2'), 7.61 (1H, *dd*, *J* = 8.5, 2.1, H-6'), 6.82 (1H, *d*, *J* = 8.5, H-5'), 6.42 (1H, *d*, *J* = 1.9, H-8), 6.22 (1H, *d*, *J* = 1.9, H-6), 5.20 (1H, *d*, *J* =7.7, H-1" Galactose), (3.40- 4.00 sugar protons). Acid hydrolysis of compound 4 produced Quercetin and Galactose while the configuration of anomeric sugar was deduced by its

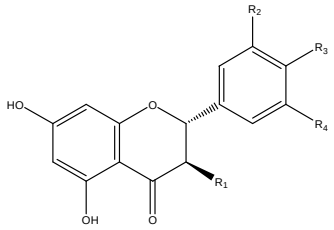
J_{H-H}coupling constant. This compound was characterized as Quercetin-3-*O*-*β*-galactopyranoside [18].

Compound 5: pale yellow powder soluble in methanol, UV λ_{max}(nm); MeOH: 257, 306, 307; + NaOH: 268, 393, 328; +$AlCl_3$: 265, 432, 302; + $AlCl_3$/HCl: 270, 406, 371,309; +NaOAc: 274, 386, 323; + NaOAc/H_3BO_3: 261, 389. ^{1}H NMR(MeOH-d_4, 250MHz, δ: ppm, *J*:Hz): 7.31 (2H,*s*,H-6'&H-2'), 6.37 (1H,*d*,*J* = 2.1, H-8), 6.18 (1H,*d*,*J* = 2.1, H-6), 5.18 (1H,*d*,*J* = 6.8, H-1"Glucose), (3.50- 4.00 sugar protons). Acid hydrolysis of compound 5 produced Myricetin and Glucose while the configuration of anomeric sugar was deduced by its J_{H-H} coupling constant. This compound was characterized as Myricetin-3-*O*-*β*-glucopyranoside, named isomyricetin [19].

Compound 6: yellow powder soluble in methanol, UV λ_{max}(nm); MeOH: 275, 364; + NaOH: 268, 386; +$AlCl_3$: 269, 440; + $AlCl_3$/HCl: 271, 412,309; +NaOAc: 274, 381, 325; + NaOAc/H_3BO_3: 261, 386. ^{1}H NMR(MeOH-d_4, 250MHz, δ:ppm, *J*:Hz): 7.31 (2H,*s*,H-6'&H-2'), 6.37 (1H,*d*,*J*=2.1, H-6), 6.18 (1H,*d*,*J* =2.1, H-8), 5.18 (1H,*d*,*J* = 6.5, H-1" Xylose), (3.30- 3.80sugar protons).^{13}CNMR +JMOD (MeOH-d_4, 62.9MHz, δ:ppm):156.9 (C-2), 134.3 (C-3), 177.9 (C-4), 161.5 (C-5), 98.4 (C-6), 164.7 (C-7), 93.2 (C-8), 157.2 (C-9), 104.1 (C-10), 120.2 (C-1'), 108.3 (C-2'), 145.0 (C-3'), 136.6 (C-4'), 145.0 (C-5'), 108.3 (C-6'),103.7 (C-1''),71.53 (C-2''), 67.86 (C-3''), 72.80 (C-4''), 65.78 (C-5''). Acid hydrolysis of compound 6 produced Myricetin and Xylose while the configuration of anomeric sugar was deduced by its J_{H-H} coupling constant. This compound was characterized as Myricetin-3-*O*-*β*-Xylofuranoside[20].

The structures of these two flavonoids were elucidated by extensive UV analyses and NMR spectroscopic analyses as well as by comparing their spectroscopic data with those reported in the literature [21-23].





1. R_1= OH, R_2= OH, R_3= OH, R_4= H
3. R_1= OGlu-Pyr, R_2= H, R_3= OH, R_4= H
4. R_1= OGala-Pyr, R_2= OH, R_3= OH, R_4= H
5. R_1= OGlu-Pyr, R_2= OH, R_3= OH, R_4= OH
6. R_1= OXylo-Fur, R_2= OH, R_3= OH, R_4= OH

2. R_1= OGlu-Rha, R_2= OH, R_2= OH, R_4= OH

Figure 1. Structures of Compounds **1- 6**

The present studyallowed the isolation and structural determination of five flavonoid glycosides together with oneflavonol named:Quercetin**1**, (2R, 3R) Dihydromyricetin-3-O-α-rhamnoside (Ampelopsin)**2**, Kaempferol-3-*O*-*β*-glucopyranoside**3**, Quercetin-3-*O*-*β*-galactopyranoside**4**, Myricetin-3-*O*-*β*-glucopyranoside**5**andMyricetin-3-*O*-*β*-

Xylofuranoside**6**. In our Knowledge, this is the first report describing the isolation of compounds**3**, **5** and **6**from *Catnaedulis*.

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