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FLAVONOIDS FROM PULICARIA UNDULATA (L.) KOSTEL GROWN IN EGYPT

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From the ethanolic extract of the total herb of Pulicaria Undulata(L.) Kostel; five flavonoids were isolated. Their structures were established by physical, chemical and spectral methods (UV, IR? , NMR and MS) and proved to be: 7-methoxy kaempferel (Rhamnocitrin), 3,7 dimethoxy quercetion, 7-methoxy quercetin (Rhamnetin), dihydrokaempferol and quercetin 3-O-glucoside (Isoquercetrin).

A chromatospectrophotometric method was adopted for quantitative estimation of these flavonoids.

Pulicaria Undulata (L.) Kostel is much branched woolly procumbent herb belonging to family compositae.

It is a common plant in sandy and calcanous place[1].

Schulte et al reported the isolation of 5, 6, 3', trihydroxy 3,7,4' trimethoxyflavone and kaempferol 3-glucoside from petroleum ether extract of Pulicaria dystenterica blossoms [2]. Sarg investigated Pulicaria crispa growing in Saudia Arabia and mentioned that quercetin aglycone was detected in the plant material[3]. Khafagy et al[4]; reported the isolation of a dihydroflavonol from Pulicaria Undulata (L.) Kostel. This was proved to contain two hydroxyl groups in the B-ring and one methoxyl group. However the structure of the isolated compound was not elucidated. The detailed study of the plant and its flavonoid contents were not completely tackled. The present work was planned to study the flavonoid constituents of Pulicaria Undulata (L.)

EXPERIMENTAL

Material:

The overground portions of Pulicaria Undulata (L.) were collected from the sandy area on the road of Edfu to Marsa Alam in Upper Egyp'. The plant was identified by Prof. Dr. N-El-Hadidy, Prof. of Botany, Cairo University.

TLC:

20 g. of the overground portions were successively extracted with petroleum ether, chloroform and ethanol (70 %) The chloroform extract was examined by TLC on silica gel G using system I: chloroform-methanol (9 : 1) and revealed the presence of 6 flavonoidal spots. The ethanolic extract was examined by TLC on cellulose plates using system (II) chloroform-methanol-water (200 : 53 : 4) and revealed the presence of 2 spots.

Extraction and Fractionation:

3.5 kg of the defatted overground portion of Pulicaria undulata (L.) Kostel was extracted with ethanol and concentrated. The extract was fractionated with ether, chloroform and ethyl acetate. Chromatographing the ether chloroformic extracts over silica gel column, yielded 4 aglycones designated A_1-A_4, while chromatographing the ethyl acetate extract over silica gel column resulted in the isolation of one glycoside (A_5).

Mild Acid Hydrolysis for A_5:

2 mg of compound A_5 was refluxed with 20 ml of 1% aqueous HCl for 2 hours. Samples of the hydrolysate were withdrawn every 5 minutes, spotted on PC and developed in 15% acetic acid in water. The compound was hydrolysed to its aglycone on one step.

Acid Hydrolysis of A_5:

10 mg. of compound A_5 was refluxed with 50 ml of 8% aqueous HCl for 3 hours. The aglycone was extracted with ether, while the suger moiety in the hydrolysed was examined by PC using system n-butanol-pyridin-water(6:3:4).

A_1 7 methoxy Kaempferol(Rhamnocitrin):

m.p. 224-225°C (Lit. (6) m.p. 224-225°C); TLC. silica gel; G, system I: chloroform-methanol (9:1), R_f = 0.72.; M^+ 300; UV (MeOH), 266 , 370 nm; + $AlCl_3$, 274 , 356 nm; + $AlCl_3$/ HCl , 272, 358, 422 nm ; + CH_3 COONa, 268, 376 nm ; + CH_3- COONa/H_3BO_3 266, 366 nm; + Na Ome 274, 430 nm; + Zr OCl_2 430 nm.

NMR (D -pyridine) δ: 7.5 { broad signal, 3 OH,(disappeared by D_2O)}; 6.6-8.6 (2 H , d , J = 9 H_z , H-2`, H-6`); 5.7 - 6.1 (2 H, dd , J = 9 H_z , H - 3` ; H - 5`), 5.2 - 5.4 (2 H , q , J = 2.5 H_z , H - 8 H - 6); 3.1(3H, S, OCH_3).

A_2 (3,7 dimethoxyquercetin):

m.p. 238°C ; TLC, silica gel G, system I, R_f = 0.61 ; M^+ 330 ; UV (MeOH), 258, 360 nm; + $AlCl_3$, 278, 440 nm ; + $AlCl_3$/HCl 270, 364, 408 nm; + CH_3 COONa 262, 370 nm ; + CH_3- COONa/H_3BO_3 262 , 380 nm ; + NaOMe , 268, 394 nm ; + $ZrOCl_2$ 420 nm; NMR (D -pyridine) δ : 7.9 {broad signal-3 OH, (dissappeared by D_2O) }; 6.2-6.5 (2 H , T , J = 9 Hz , H - 2' , H - 6'); 6.0 - 6.2 = 1 H, d , J = 9 H , H - 5'); 5.7 - 6.0 (2 H , q , J - 2.5 Hz., H-8 , H-6) ; 2.9 3.1(6H, d , J = 12 Hz , 2 - OCH_3).

A_3 (7methoxyquercetin (Rhamnetin):

m.p., 290 - 292°C (Lit (7) 290 - 296°C); TLC silica gel G, system I, R_f = 0.54; M^+ 316 ; UV (MeOH), 256, 372 nm; + $AlCl_3$, 276 , 448 ; + $AlCl_3$/HCl 266 , 422 nm; CH_3 COONa.

262, 386 ; + CH_3 COONa/ H_3BO_3 262 , 390 nm; + NaOMe 420 nm ; + $ZrOCl_2$ 440 nm.
NMR (D -pyridine) δ : 6.4 - 6.8 (2 H . T , J = 9 H_z , H-2' , H - 6'); 6.0 - 6.4 (1 H , d , J = 9 H_z , H - 5'); 5.4 - 6.0 (2 H dd . J = 2.5 H_z , H - 8 , H 1 6) ; 3.2(3 H, S , - OCH_3).

A_4 (Dihydrokaempferol):

m.p., 225 - 2..°C (Lit. (7) m.p. 225 - 226°C) TLC, silica gel G, system I , R_f = 0,48.
M^+ 288 ; UV (MeOH), 293 , 328 nm ; + $AlCl_3$ 273 , 317 ; 366 nm ; + $AlCl_3$/HCl, 278 , 314 , 364 nm; + CH_3 COONa , 255, 279 , 329 nm; + CH_3 COONa/H_3BO_3 295 , 330 nm; + NaOMe 245 , 326 nm.
NMR (DMSO) δ : 7.45 (2 H, d , J = 9 H_z , H-2' , H-6'); 6.85 (2 H , d, J = 9 H_z , H-3' , H-5' ; 5.75 (2 H, q , J =2.5 H_z, H-8 , H-6): 5.0 (1 H, d , J = 11 H_z , H-2); 4.5(1H, d, J = 11 H_z, H- 3)

A_5 quercetin 3-O glucoside (isoquercetrin)

m.p., 217 - 218°C (Lit. (6) m.p., 217 - 219°C) ; TLC, cellulose system II: chloroform - methanol-water(200 : 53:4) R_f = 0.36.
UV (MeOH) 256 , 300 , 360 nm; + $AlCl_3$ 276, 306, 434 nm; + $AlCl_3$/HCl 270, 408 nm; + CH_3 COONa, 274, 386 nm; CH_3 COONa/ H_3BO_3 264 , 380 nm; NaOMe 276, 416 nm; + $ZrOCl_2$ 400 nm
IR (cm) : 3500 - 3200 (OH) ; 1660 (γ - pyrone), 1620, 1565 , 1520 (aromatic system) 1080, 1060, 1030, (pyranose from sugar).
NMR (DMSO) δ : 7.6 - 745(2 H , d, J = 9 H_z, H-2', H-6'); 6.75 - 6.95(1 H, d, J = 9 H; H-5'); 5.8-5.95(2 H, q, J = 2.5 H_z , H-8, H-6); 4-5.2 (6 H, m. protons of glucose).

Mild acid hydrolysis one step. Acid hydrolysis, yielded sugar glucose and aglycone quercetin.

Aglycone (quercetin) : yellow needles ; m.p. 316-318°C (Lit. (7) 316-318°C); UV $\lambda_{max}^{methanol}$ 258, 268 sh., 374 nm; + $AlCl_3$ 272, 444 nm; + $AlCl_3$/HCl , 268, 430 nm; + CH_3COOMa, 274, 384 nm ; + CH_3COONa/H_3BO_3 260, 386 nm; + NaOMe, 280, 424 nm; + $ZrOCl_2$ 448 nm.

Quantitative Estimation:

A chromatospectrophotometric method was adopted according to El-moghazy et al (5).

RESULTS AND DISCUSSION

Preliminary phytochemical study of the overground portion of Pulicaria undulata (L.) kostel revealed the present of free and combined flavonoids. TLC screening for the successive extracts(ether, chloroform and ethyl acetate) proved the presence of at least 6 flavonoidal aglycones in ether and chloroform extracts and 2 flavonoidal glycosides in the ethyl acetate extract. Chromatographing the ether and choroformic extracts over silica gel column succeeded in the isolation of 4 flavonoidal aglycones, while fractionation of ethyl acetate extract yielded one flavonoidal glycoside,. By extensive physical, chemical spectral analysis(6-12) the structures of the isolated flavonoids were proved to be:
7-methoxy kaempferol (rhamocitrin), 3,7 dimethoxy quercetin, 7methoxy quercetin (rhamnetin), dihydrokaempferol and quercetin 3-O-glucoside (Isoquercetrin).

A chromatospectrophotometric method was adopted to estimate flavonoids in the air-dried overground portion of Pulicaria undulata L. (kostel) and the percentages were found to be (gm%) w/w (1.56, 0.91, 0.22, 3.75) for 7-methoxy kaempferol(rhamnocitrin); 3,7 dimethoxy quercetin, 7-methoxy-quercetin(rhamnetin) and quercetin-3-O- glucoside (isoquercetrin) respectively.

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فلافونيدات الشاى الجبلى (بوليكاريا اند يولاتا)
الذى ينمو فى مصر

داود ونيس بشاى ـ كاميليا سعيد جمعه ـ محمود حافظ عساف
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فى هذا البحث تم اجراء المسح الاولى لنبات الشاى الجبلى (بوليكاريا انديولاتا) من العائلة المركبة ـ وقد اثبت ذلك وجود الفلافونيدات الحرة والجليكوزيدية .

وقد تمكن الباحثون من فصل اربعة من الفلافونيدات الحرة واحد الجليكوزيدات وذلك بواسطة كروماتوجرافيا العمود باستخدام هيلام السيلكا والسليولوز .

وقد تم التعرف على التركيب الكيماوى لهذه المركبات الفلافونيدية باستخدام طرق التحليل الطيفى المختلفة والرنين النووى المغناطيسى وطيف الكتلة .

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