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Research Article

The Flavonoids and Anticomplement Activity of two Cruciferous Plants Growing in Egypt

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ABSTRACT
The study of the flavonoids of the aerial parts of *Carrichtera annua* and *Farsetia aegyptia* (family Crucifereae) resulted in isolationof quercetin(**1**), quercetin-3-O-arabinoside(**2**), quercetin-3-O-glucoside(**3**), quercetin-3-O-(6-feruloyl- - glucopyranosyl -1 →2 - -arabinopyranoside)-7-O -glucopyranoside (**4**) and quercetin-7-O-arabinosyl-3-O-glucoside(**5**) from *C. annua*. Three flavonoids were isolated from *F. aegyptia* and identified as isorhamnetin (**6**), isorhamnetin-3-O-rhamnosyl-7-O-glucoside (**7**) and isorhamnetin-3-O-(feruolyl-sophroside)-7-O-rutinoside (**8**). The aqueous alcoholic extract ,chloroform , ethyl acetate ,butanol fractions of *C. annua* in addition to the new compound (4) were tested for their influence on the classical(CP) and alternative (AP)pathways of complement mediated hemolysis. All the extracts showed anti-complement activity but compound (4) has the strongest effect on both CO and AP pathways.

Key words: Crucifereae, *Carrichtera annua, Farsetia aegyptia*, flavonoids and anti-complement activity.

INTRODUCTION

Egypt is very rich in it's medicinal plants belonging to many families, one of the largest families is Crucifereae which include vegetable crops, garden with flowers and weeds[1]. The genus *Carrichtera* and *Farsetia* genus (family Crucifereae) is represented in Egypt by only one species known as *C. annua* (L) DC growing in Sinai peninsula specially at Elarish region, while *Farsetia* genus is represented by three species in which *F. aegyptia* Turra. is the most common one growing at Seuz-Cairo Deseret road (WadiHagool)[2]. The two plants are known to be used by the native Bedounis as antidiabetic and antispasmodic. Moreover, *F. aegyptia* is used for the relieve of rheumatic pains and taken as a cooling medicine after pounding[3].

Many of the cruciferous plants serve as a source of food and condiment such as cabbage, turnip, radish and mustard. All of them contain unusual flavonol glycosides like (kampferol-8-C-7-*O*-β-diglucopyranoside) and glucosinolates. The latter upon hydrolysis provides many of the culinary flavors characteristic to these plants[4]. Flavonoids are a major group of constituents and are assumed to be among the beneficial components. Recently, they have also received considerable interest as components of foodstuffs and nutraceuticals because of their antioxidant and anticancer properties[5]. The flavonoidal fraction of the seeds of *Carrichtera annua*, was investigated using LC/ESI-MS and nano-ESI-MS/CID/MS. The flavonoidal fraction was found to contain 12 flavonol *O*-glycosides, which were structurally related and of which 11 were acylated with one or more benzoyl, feruloyl or sinapoyl groups[6]. In previous study different known and new flavonoids compounds were isolated from the seeds and the herb and investigated by LC/MS and or NMR[7]. Marzook et al[8] isolated kaempferol-3-*O*-(2″-α-L-arabinopyranosyl)-α-L-rhamnopyranoside--*O*-α-L-rhamnopyranoside, kaempferol-3-*O*-(2″-β-D-gluco-pyranosyl)-α-L-rhamnopyrano-side-7-*O*-α-L-rhamnopyranoside,kaempferol-3,7-di-O-α-L-rhamnopyranoside, kaempferol, isorhamnetin-3-*O*-β-L-arabinopyrano-side-7-*O*-(2‴-β-D-glucopyrano-syl)-α-L-rhamnopyranoside,isorhamnetin-3-*O*-α-L-rhamno-pyranosyl-7-*O*-β-D-gluco-pyranoside, isorhamnetin, apigenin-7-O-β-D-glucopyrano-side and apigenin .

The complement system plays an important role in the host defense system, inflammation and allergic reactions. Hence, the activators and inhibitors of the complement system are suggested to be modulators of the immune system. Activation of the complement system can proceed via two different pathways. The classical pathway (CP) is activated by immune complexes containing IgG od 1gM antibodies, while the alternative one (AP) is activated by a variety of substances, for instance polysaccharides, glycosylated flavonoids,….etc.[9].

here, this study represents, the isolation and structure elucidation of some flavonoids from both *Carrichteraannua* and *Farsetiaaegyptia.*

MATERIALS AND METHODS

Plant materials

The herbs ofboth plants (*Carrichteraannua* and *Farsetia*) were collected from their growing localities at the end of winter season (15-march). the plants werekindly, identified

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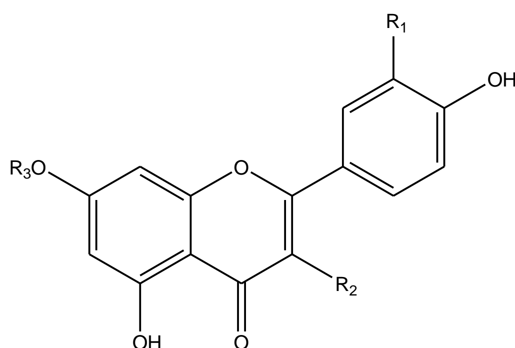

R_1, R_2= OH, R_3= H . **2-** R_1 = OH, R_2= *O*- arabinose , R_3= H.
3-R_1 = OH, R_2= *O*- glucose , R_3= H .
4- R_1 = OH, R_2= *O*- (6- feruloyl-glucose- arabinose) , R_3= glucose.
5- R_1 = OH, R_2= *O*- glucose , R_3= *O*- arabinose.
6- R_1 = OCH_3, R_2= OH , R_3= H
7- R_1 = OCH_3, R_2= *O-rhamnose*, R_3= glucose
8- R_1 = OCH_3, R_2= *O*-(feruloyl-sophroside), R_3= rutinose

by prof. Dr. K. Elbatanony, plant dept., fac. Of sci., Cairo univ., and voucher specimens were deposited at NRC herbarium.

Instruments

UV-Vis spectrophotometer pc. 2401Schimadzu.

Bruker NMR spectrometer operating at 500 MHz for ^1H and 125 MHz for C^{13}–NMR.

The FAB Mass spectra were recorded on a VG 70 SEDQ instrument using glycerol as the liquid matrix.

Extraction and isolation of flavonoids

About 1.5 g of air dried powdered herb of both*C. annua* and *F. aegyptia*were extracted, separately, with petroleum ether (br. 40 –60 °C) in a soxhlet. The defatted powders were extracted with methanol (80%, 4x2L). the combined methanol extract of each plant was evaporated *in vacuo*at 45ºC and the residues were dissolved in hot distilled water (500 ml), left in the refrigerator overnight and filtered the precipitated matters. The aqueous filtrates were partitioned with chloroform (500 ml x 3) then ethyl acetate(400mlx4) and finally with n-butanol (600 ml x 4). Each combined solvents were dried over anhydrous sodium sulfate and evaporated till dryness.

About 2.9 g of the ethyl acetate fraction of *C. annua* were subjected to preparative paper chromatography (PPC, Whatmann 3MM) using 15% acetic acid as a solvent system. Three main zones I,II and III (R_f 0.03, 0.35, 0.39 ,respectively) were eluted and further purified over SephadexLH-20 column eluted with 95% methanol to afford compounds **1**((mg), **2**(13 mg) and **3**(10 mg).

About 6.5 g of butanol fraction of *C. annua* were fractionated over a poly amide column (6S Riedel-deHaen) eluted with water (100%) followed by water/methanol gradient up to methanol (100%) and fraction 1Leach were collected. The fraction eluted with 30%methanol was further purified on a small polyamide column eluted with 40% methanol and the pure compound **(4)** was obtained by PPC using butanol: acetic acid: water (4:1:5) as developing solvent. The fraction eluted with 60 % methanol was further purified using PTLC (Silica gel plate, ethyl acetate: acetic acid: formic acid: water 100:10:10:20) the pure compound (**5**) was eluted and passed through Sephadex LH-20 column eluted with 85 % methanol.

About 1.5 g of the ethyl acetate fraction of *F.aegyptia* were subjected to PPC using B.A.W.(3:1:1) as an irrigating solvent. Two main bands I' and II' were eluted with 85%methanol and further purified over Sephadex LH-20 column which gave compounds **6** and **7** in pure form as a yellowish powder (12mg and 15mg respectively).

About 13 g of the butanol fraction of *F.aegyptia* were subjected to CC on polyamide, elution started with 100% water followed by increasing the methanol percentage till 100 % methanol. The fraction eluted with 30 % methanol was subjected for further purification using PTLC (silica gel plates, ethyl acetate: acetic acid: formic acid: water 100:10:10:20). The main compound (**8**) was passed over sephadex LH-20 column eluted with 80 % methanol to afford the compound pure as an amorphous poder (22 mg).

Bioassay: modulation of the complement system[9]

The complement modulation test applied was based on the assay models as described by Mayer and Platts-Mills[9] for the CP and AP respectively. The inhibition of the complement activity was determined as described by Klerx *et al*[9]. The test was performed in V-well microtiter plates. Human pooled serum (HPS) was used as a source of complement. Briefly, the tested samples were dissolved with DMSO in appropriate buffer (DMSO Conc.1 %). Then they diluted 1/2 or 1/3 in the plate with barbital buffer solution (BBS)(CP) or µl/10ml) (CP) or with AP-CFTD(AP) (final volume 50 µl/ weel for CP and 100 µl/weel for AP). thereafter, 50µl of the dilution of HPS IN BBS (140µL/10ml) (CP) or 25 µl of a dilution of HPS in AP: CFTD (1/1) (AP) were added per well, after a standard incubation at 37 °C for 60 min. for CP or for 30 min. for AP. Subsequently the plates were centrifuged for 10 min. at 2000 rpm, to quantify the haemolysis, about 50□□l of the supernatant were mixed with 200□□l water in a flat bottom microtiter plates, the absorption at 414 nm was measured with a Labsystems Multiscan Mcc/340. Controls in this assay consistes of erythrocytes incubated in water (100% heamolysis) in buffer supplemented with the appropriate HPS dilution (0% inhibition or +50% heamolysis). The anticomplement activity was expressed as the influence of the tested samples upon the CH_{50} and AH_{50} (respectively for classical and alternative pathways) which means the amount of the complement necessary to lyses half of a given quantity of erythrocytes in optimal conditions. The data were conducted as the mean ± SE of triplicate samples as shown in table (2).

RESULTS AND DISCUSSION

The data about both compounds **1** and **4** were published before by the authors and they identified as quercetin(**1**) and quercetin-3-O-(6-feruloyl- □ - glucopyranosyl -1 →2 -□□ -arabinopyranoside)-7-O□□ -glucopyranoside(**4**)[10]. The data of both compound **2**(*quercetin-3-O-arabinoside*), compound **6** (*isorhamnetin*) and compound **3** (*quercetin-3-O-glucoside*) were found the same as literature data[11,12]. While compound **5** (*quercetin-3-O- arabinosyl-7-O-*

Table 1:[13]Cnmr data of compound 7 and 8

Carbon no.	□□in ppm Compd.7	Compd.8	Carbon no.	□□in ppm Compd.8
2	157.90	156.10	Ferulic acid	
3	114.35	133.60	1''''	125.01
4	179.60	177.55	2''''	110.60
5	160.58	157.3	3''''	150.00
6	99.68	99.51	4''''	147.81
7	163.50	161.14	5'''''	115.42
8	95.65	94.5	6''''	123.10
9	157.90	156.82	7''''	145.71
10	107.29	105.63	8''''	113.00
1'	124.22	127.74	9''''	166.59
2'	132.26	115.20	OCH_3	55,72
3'	130.06	147.31	7-*O*-glucose	
4'	160.58	145.29	1'''''	97.90
5'	116.65	113.70	2'''''	73.62
6'	132.38	123.51	3'''''	71.10
3'-OCH_3	56.91	55.42	4'''''	69.23
3-*O*-glucose.			5'''''	75.64
1"	100.67	99.31	6'''''	65.94
2"	71.22	81.20	7-*O*-rhaminose	
3"	73.44	76.10	1''''''	101.69
4"	71.88	69.60	2''''''	70.51
5"	72.59	76.09	3''''''	70.21
6"	67.37	63.06	4''''''	72.80
7-*O*-rhaminose		3-*O*-glucose	5''''''	69.32
1'''	104.22	105.2	6''''''	17.83
2'''	69.12	73.10		
3'''	73.93	75.62		
4'''	71.51	79.59		
5'''	68.90	73.80		
6'''	18.01	66.22		

glucoside) was obtained as a yellowish powder and it's chromatographic behavior on pc in different solvents proved that, it is a diglycosidic compound also it's uv spectra in methanol and different shift reagents substantiated that , it is a flavonol type structure having a free OH groups at C5, 3',4', an *ortho* dihydroxy system and glycosylated at C3 and C7[13].

The +ve FABMS displayed a molecular ion peak M^+ at m/z = 597(M^+ + 1) which corresponds to the molecular formula $C_{26}H_{28}O_{16}$. The presence of an ion peak at m/z =303 (M^+ - 294) indicates the presence of two sugar moieties bonded to the aglycone. These two sugars are hexose and pentose which proved by acid hydrolysis to be glucose and arabinose in addition to quercetine as an aglycone.

To know the position of attachment of glucose with the aglycone, compound **5** was subjected to an enzymatic hydrolysis using □ - glucosidase enzyme, which proved the presence of glucose and a glycosidic compound. The UV data of this glycosidic compound revealed the presence of a free OH group at C7 which means the presence of the glucose at C7 and so arabinose at C3.

compound **7** (*isorhmentin-3-O-glucosyl-7-O-rhamnoside*): this compound was found to be a brownish powder, changed from brown to yellow under UV light with ammonia. it's uv spectra in methanol and different shift reagents substantiated that, it is a flavonol type structure having a free OH groups at C5 and C4', with no *ortho* dihydroxy system and it is glycosylated at C3 and C7[13], where it displayed band –I at □max(MeOH) = 352 nm. The compound exhibited a molecular ion peak M^+ at m/z= 623(M^+- 1) which corresponds to the molecular formula $C_{28}H_{23}O_{16}$ in the -ve FABMS. displayed other fragments at m/z =593 (M^+ - OCH_3), m/z =477 [M^+ - 146(i.e. deoxy hexose)] and m/z =315 correspond to the aglycone [M^+ - 308(i.e. deoxy hexose+hexose)] indicates the presence of two sugar moieties attached to the aglycone. These two sugars are hexose and deoxy hexose.

The ^{1}H-nmr (DMSO) showed signals at □ in ppm 7.72(1H, d, H-2'), 7.6(1H, d, H-6'), 6.87 (1H, d, H-5'), 6.7(1H, d, H-8), 6.65(1H, d, H-6), the anomeric protons of the two sugars at 5.5(1H, s) and 5.26(1H, d) for glucose and rhaminose respectively. The methoxy group proton appeared at 3,82(s, 3H, OCH_3at C-3') and the methyl group protons of the rhaminose moiety at 1.1 (s, 3H, CH_3)[14].

The^{13} C-nmr data showed two anomeric carbon signals at 100.76 and 104.22 ppm for glucose at C-3 and rhamnose at C-7 respectively. The presence of signal at 114.35 is characteristic for substituted C-3 in a flavonol type structure, which confirmed through the signal of C-4 at 177.5ppm. The signal at 56.9ppm confirmed the presence of a methoxy group at C-3'. The other data were shown in table (1).

Table 2: IC_{50} (g/ml) values of tested fractions on AP and CP pathways of the complement system

Tested fraction	IC_{50} (g/ml)	
	AP	CP
Alc. Extract *C. annua*	282.5	80.99
Butanol fraction *C. annua*	243.16	49.88
Compound 4 from *C. annua*	61.39	8.21
Alc. Extract of *F. aegyptia*	245.2	100.6
Butanol fraction of *F. aegyptia*	259.1	55.4
Compound 8 from *F. aegyptia*	89.6	15.5
Control(rosmarinic acid)	168.27	168.27

The acid hydrolysis of the compound revealed the presence of isorhmnetin as an aglycone, in addition to glucose and rhamnose as sugars. The enzymatic hydrolysis using -glucosidase enzyme, which proved the presence of glucose and isorhamnetin -7-*O*-rhamnoside compound, so, compound **7**could be identified as: *isorhmentin-3-O-glucosyl-7-O-rhamnoside*

The flavonoidal compound **8** (isorhamnetin-3-*O*-(feruolyl-sophroside)-7-*O*-rutinoside) was obtained from the n-butanol soluble fraction of the defatted aqueous methanol of *F. aegyptia* as a brownish powder. Acid hydrolysis of this compound, yielded and isorhamnetin as an aglycone, rhamnose and glucose as sugars in addition to ferulic acid. the UV spectra in methanol displayed band-I at $_{max}$=334 which proved the flavone or highly substituted flavonol at position C-3[(10)]. The ^{1}H-nmr (DMSO) showed signals at in ppm 8.01(1H, d, H-6'), 7.82(1H, d, H-2'), 6.7 (1H, d, H-5'), 6.4(1H, d, H-8), 6.3(1H, d, H-6), while the protons of ferulic acid appeared at 7.6(1H, d, H-7''''), 7.12(1H, d, H-6''''), 6.9(1H, d, H-5''''), 6.62(1H, d, H-8''''). The four anomeric protons of the sugar moieties displayed at 5.5(1H, s) 5.3(1H, d) 4.5(1H, d) and 4.4(1H, d). The two methoxy groups rotons appeared as sharp singlets at 3.85(s, 3H, $OCH_3$of isorhamnetine) and 3.75(s, 3H, $OCH_3$of ferulic acid) the protons of methyl group for rhamnose moiety at 1.1 (s, 3H , CH_3)[(14)].

The most important carbon signals in ^{13}C-nmr spectrum were two methoxy groups at 55.41 and 55.71. the anomeric carbon signals at 99.3, 105.2, 97.9 and 101.69 ppm for diglucose at C-3, glucose at C-7and rhamnose at C-7 respectively. The presence of signal at 114.35 is characteristic for substituted C-3 in a flavonol type structure, which confirmed through the signal of C-4 at 177.5ppm. The signal at 56.9ppm confirmed the presence of a methoxy group at C-3'. The other data were shown in table (1). The DEPT 135 expermint showed three signals in the negative half at 65.9, 63.5 and 63.0 due to the presence of three methylene groups of the three sugar moieties in addition to one signal at 17.8ppm in the positive half due to the methyl group of the rhamnose unit. The +ve FABMS showed a molecular ion peak M^+ at m/z = 1125(M^+ + 1) which corresponds to the molecular formula $C_{50}H_{60}O_{29}$ confirming the presence of three glucose units, one rhamnose unit, ferulic acid and isorhamnetine.

The acid hydrolysis of the compound revealed the presence of isorhmnetin as an aglycone, glucose and rhamnose as sugars in addition to ferulic acid. so, all the above data we can think compound **8** may be a new flavonoidal compound isolated for the first time from *F. aegyptia* and could be identified as: isorhamnetin-3-*O*-(feruolyl-sophroside)-7-*O*-rutinoside.

The study of the anti-complement activity (table 2) of different fractions of both plants as well as two of the isolated compounds (**4** and **8**) proved that, both the alcoholic and butanol fractions of both plants are less active than the control, while compound 4 and 8 are more active than the control on both AP and CP pathways, where IC_{50} are 61.39, 61.39, 89.6 and 15.5**g/ml** respectively. These results demonstrate the role played by substitution pattern and the nature of the substituents (sugars or methoxy groups) for increasing the complement modulating activity. Although, the complement system plays an important role in the host'sdefense against invading of microorganisms, so, it's activation may contribute to/or even evoke pathological reactions in a variety of inflammatory or degredative diseases (rheumatoid, arthritis, microbial infection, gout,…etc. the drugs that inhibiting the complement system could be valuable as therapeutic agents[15-16]. Accordingly, both compounds 4 and 8 may be useful in the treatment of these diseases.

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