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SOME BIOLOGICALLY ACTIVE FLAVONOIDS FROM EGYPTIAN MEMBERS OF THE COMPOSITAE

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ABSTRACT. A total of thirty flavonoids were isolated and identified from the aerial parts of *Centaurea alexandrina*, *Onopordum ambiguum* and *Tanacetum santolinoides*. Bioassay of major methylated flavonoids using the brine shrimp test was carried out. The results indicate that a structural activity relationship exists as accentuated by the enhancement of the cytotoxic activity in the presence of 6-methoxylation of the flavone nucleus.

INTRODUCTION

Several North African members of the family Compositae are known to contain biologically active components, and have been used in folk medicine for years [1]. In continuation of our phytochemical studies of Egyptian plants we have now investigated the flavonoids of three members of the Compositae, namely, *Centaurea alexandrina* Del., *Onopordum ambiguum* Fres. and *Tanacetum santolinoides* (DC.) Feinbrun & Fertig. A total of thirty flavonoids were isolated and identified. The biological activities of twelve flavonoids, mainly methylated derivatives, were tested using the brine shrimp bioassay method [2].

RESULTS AND DISCUSSION

Centaurea alexandrina proved to contain hispidulin (5,7,4'-trihydroxy-6-methoxyflavone), pectolinarigenin (5,7-dihydroxy-6,4'-dimethoxyflavone), 5,4'-dihydroxy-6,7-dimethoxyflavone, nepetin (5,7,3',4'-tetrahydroxy-6-methoxyflavone), 5,7,4'-trihydroxy-3,6-dimethoxyflavone, vitexin (apigenin-8-C-glucoside) and isovitexin (apigenin 6-C-glucoside). The following compounds were isolated and characterized from *Onopordum ambiguum*: apigenin, luteolin, chrysoeriol, hispidulin, nepetin and the 7-glucosides and 7-rutinosides of apigenin, luteolin and chrysoeriol. *Tanacetum santolinoides* was found to contain 16 flavonoids which were identified as apigenin-7-glucuronide, luteolin-7-glucuronide, -7-diglucoside, chrysoeriol-5-glucoside, -7-glucoside -7-glucuronide, jaceosidin-7-glucoside (5,7,4'-trihydroxy-6,3'-dimethoxy-7-glucoside), jaceosidin-7- glucuronide, apigenin-6,8-di-*C*-glucoside, chrysoeriol, cirsimaritin (5,4'-dihydroxy-6,7- dimethoxyflavone), cirsilineol (5,4'-dihydroxy- 6,7,3'-trimethoxyflavone), 5-hydroxy- 6,7,3',4'- tetramethoxyflavone, jaceidin (5,7,4'-trihydroxy-3,6,3'-

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trimethoxyflavone), chrysosplenetin (5,4'-dihydroxy-3,6,7,3'-tetramethoxyflavone) and artemetin (5-hydroxy- 3,6,7,3',4'-pentamethoxyflavone). Cirsilineol, 5-hydroxy-6,7,3',4'-tetramethoxyflavone and artemetin were previously isolated from *T. santolinoides* [3].

In the present study, a number of methylated flavonoids isolated from the three plants were tested for their biological activity using the brine shrimp test [2]. Several reference flavonoids were also tested for comparison purposes (Table 1). The results indicate that a structural activity relationship exists. Methylation appears in general to play a role in increasing the activity of flavonoids towards brine shrimp. Furthermore, the presence of a 6-methoxyl group clearly enhanced the activity of the flavonoids, this is shown by the activity of the two pairs artemetin-quercetin and patuletin-3-rutinoside-rutin. Several methylated flavonoids have been demonstrated to have cytotoxic activities [4,5]. The existence of 6- and/or 8-methoxyl groups on a flavone nucleus has been reported to inhibit the aldose reductase activity [6,7]. Of the compounds tested in the present study, hispidulin, cirsilineol and jaceosidin have been reported elsewhere to be biologically active. Hispidulin showed significant cytotoxic activity against L-strain fibroblast [8], cirsilinol (3'-demthoxy-cirsilineol) was found to be a potent inhibitor of 5-hipoxygenase (IC_{50} being 10 times lower than that of quercetin) [9], while jaceosidin suppressed the growth of *Trichophyton gypseum* at a concentration of 6.1 μg/ml [10].

Table 1. Results of the brine shrimp bioassay (percent deaths at 24 hr)

Flavonoids	500 μg	250 μg	125 μg	62.5 μg	31.2 μg	15.6 μg	7.8 μg	3.9 μg	LD_{50} μg
1. Jaceidin (5,7,4'-OH,3,6,3'-OMe)	--	--	100	100	100	95	95	80	< 3.9
2. Eupafolin (5,7,3',4'-OH,6-OMe	--	--	100	100	80	80	75	45	~ 3.9
3. Artemetin (5-OH,3,6,7,3,'4'-OMe)	--	--	95	90	75	55	50	40	7.8
4. Nobiletin (5,6,7,8,3,'4'-OMe)	--	100	80	75	65	60	45	30	> 7.8
5. Jaceosidin (5,7,4'-OH,6,3'-OMe)	--	100	100	95	80	70	35	--	> 7.8
6. Hispidulin (5,7,4'-OH,6-OMe)	100	85	75	73	65	60	--	--	15.6
7. Cirsimaritin (5,4',-OH,6,7-OMe)	--	95	85	75	55	50	40	--	< 15.6
8. Cirsilineol (5,4'-OH,6,7,3'-OMe)	--	100	100	100	55	--	--	--	< 31.2
9. 5-OH,3,6,7,4'-OMe	--	100	100	100	50	45	--	--	31.2
10. Patuletin-3-rutinoside	--	100	100	65	50	35	15	10	31.2
11. Pectolinarigenin (5,7-OH,6,4'-OMe)	100	85	65	55	45	40	--	--	< 62.5
12. Quercetin	--	75	50	50	40	35	35	--	62.5
13. Apigenin-6,8-di-*C*-glucoside	100	35	30	20	10	--	--	--	> 250
14. Rutin	60	40	30	25	10	10	--	--	250-500

* based on data from the Table

EXPERIMENTAL

Plant material. *Centaurea alexandrina* Del. and *Onopordum ambiguum* Fres. were both collected about 25 km West of Alexandria in July. *Tanacetum santolinoides* (DC.)

Feinbrun & Fertig (= *T. sinaicum* Del. ex DC. = *Pyrethrum santolinoides* DC.) was collected from St. Catherine mountain in August. Voucher specimen are deposited at the Herbarium, National Research Centre, Cairo.

Isolation and identification. Plant material was extracted with 70% ethanol and the extracts were subjected to column chromatography using polyamide and sephadex LH-20. Polymethylated flavonoids were further purified by prep. TLC on silica gel. Glycosides were further purified by paper chromatography. Identification was carried out using chemical methods which included acid hydrolysis and demethylation [11], as well as physical methods which included UV, MS and NMR [11-13]. The following 18 flavonoids were identified using common standard methods: apigenin, luteolin, apigenin-7-glucoside, -7-glucuronide, -7-rutinoside, luteolin-7-glucoside, -7-glucuronide, -7-diglucoside, -7-rutinoside, chrysoeriol-5-glucoside,-7-glucoside, -7-glucuronide, -7-rutinoside, jaceosidin-7-glucoside,-7-glucuronide, vitexin, isovitexin and apigenin 6,8-di-*C*-glucoside. The twelve remaining methylated flavonoids were identified as follows:

5,7,4'-Trihydroxy-3'-methoxyflavone (chrysoeriol). UV λ_{max} (MeOH) nm: 250, 267, 344; +NaOMe: 265, 275sh, 325, 400; +$AlCl_3$: 262, 272, 298, 363, 390sh; +$AlCl_3$/HCl: 260, 275, 295, 358, 390sh; +NaOAc: 273, 320, 360; +NaOAc/H_3OB_3: 250sh, 268, 345; MS *m/z* (rel. int.): 300 $[M]^+$ (100), 299 $[M-H]^+$ (1), 272 $[M-CO]^+$ (3), 271 $[M-HCO]^+$ (4), 257 $[M-COMe]^+$ (10), 152 $[A-Me]^+$ (3), 124 $[A^1-MeCO]^+$ (6), 148 $[B^1]^+$ (9), 133 $[B^1-15]^+$ (11). Demethylation gave luteolin.

5,4'-Dihydroxy-6,7-dimethoxyflavone (cirsimaritin). UV λ_{max} (MeOH) nm: 275, 333; +NaOMe: 277, 305, 380; +$AlCl_3$: 263sh, 285sh, 300, 362; +$AlCl_3$/HCl: 262sh, 285sh, 299, 354; +NaOAc: 273, 366, 388sh; +NaOAc/H_3OB_3: 274, 344. MS *m/z* (rel. int.): 314 $[M]^+$ (100), 313 $[M-H]^+$ (22), 299 $[M-Me]^+$ (89), 296 $[M-18]^+$ (4), 286 $[M-CO]^+$ (5), 285 $[M-HCO]^+$ (24), 271 $[M-COMe]^+$ (31), 181 $[A^1-Me]^+$ (27), 153 $[A^1-MeCO]^+$ (55), $[B^1]^+$ 118 (14); ^1H NMR (acetone-d_6): δ 6.85 (1H, *s*, H-3), 6.67 (1H, *s*, H-8), 7.96 (2H, *dd*, H-2',6'), 7.04 (2H, *dd*, H-3',5'), 3.80 (3H, *s*, OMe), 3.99 (3H, *s*, OMe).

5,4'-Dihydroxy-6,7,3'-trimethoxyflavone (cirsilineol). UV λ_{max} (MeOH) nm: 250sh, 272, 344; +NaOMe: 267sh, 279, 330, 403; +$AlCl_3$: 255sh, 284sh, 300sh, 360; +$AlCl_3$/HCl: 255sh, 284sh, 300, 357; +NaOAc: 274, 317sh, 390; +NaOAc/H_3OB_3: 272, 344; MS *m/z* (rel. int.): 344 $[M]^+$ (100), 343 $[M-H]^+$ (12), 329 $[M-Me]^+$ (67), 326 $[M-18]^+$ (37), 315 $[M-HCO]^+$ (13), 301 $[M-COMe]^+$ (33), 181 $[A^1-Me]^+$ (5), 153 $[A^1-COMe]^+$ (10), 148 $[B^1]^+$ (10), 151 $[B^2]^+$ (10); ^1H NMR (acetone-d_6): 6.81 (1H, *s*, H-3), 6.69 (1H, *s*, H-8), 7.61 (2H, *d*, H-2',6'), 6.97 (1H, *d*, H-5'), 3.75 (3H, *s*, OMe), 3.93 (3H, *s*, OMe), 3.95 (3H, *s*, OMe).

5-Hydroxy-6,7,3',4'-tetramethoxyflavone. UV λ_{max} (MeOH) nm: 353sh, 275, 388; +NaOMe: 292, 308, 280sh; +$AlCl_3$: 260, 297, 366; +$AlCl_3$/HCl: 257, 290, 358; +NaOAc: 253sh, 275, 338; +NaOAc/H_3OB_3: 253sh, 275, 338; MS *m/z* (rel. int.): 358 $[M]^+$ (100), 357 $[M-H]^+$ (18), 343 $[M-Me]^+$ (71), 340 $[M-18]^+$ (36), 330 $[M-CO]^+$ (5), 329 $[M-HCO]^+$ (21), 315 $[M-COMe]^+$ (22), 181 $[A^1-Me]^+$ (20), 153 $[A^1-MeCO]^+$ (44), 132 $[B^1]$ (6), 135 $[B^2]^+$ (37); ^1H NMR ($CDCl_3$): δ 6.56 (1H, *s*, H-3), 6.60 (1H, *s*, H-8), 7.53 (2H, *dd*, H-2',6'), 6.99 (1H, *d*, H-5'), 3.94 (3H, *s*, OMe), 3.98 (3H, *s*, OMe), 3.99 (3H, *s*, OMe), 4.00 (3H, *s*, OMe).

5,7,4'-Trihydroxy-3,6,3'-trimethoxyflavone (jaceidin). UV λ_{max} (MeOH) nm: 255, 271, 350; +NaOMe; 272, 355, 410; +$AlCl_3$: 266, 280sh, 302sh, 375, 410sh; +$AlCl_3$/HCl: 263, 281, 300sh, 367, 410sh; +NaOAc: 273, 320sh, 372; +NaOAc/H_3OB_3: 255, 371, 350; MS *m/z* (rel. int.): 360 $[M]^+$ (100), 359 $[M-H]^+$ (25), 345 $[M-Me]^+$ (65), 342 $[M-18]^+$ (15), 317 $[M-COMe]^+$ (28), 167 $[A^1-Me]^+$ (8), 148 $[B^1]^+$ (3), 133 $[B^1-Me]^+$ (5), 151 $[B^2]^+$ (22). ^1H NMR (acetone-d_6): δ 6.59 (1H, *s*, H-8), 7.78 (1H, *d*, H-2'), 7.00 (1H, *d*, H-5'), 7.69 (1H, *dd*, H-6'), 3.88 (3H, *s*, OMe), 3.89 (3H, *s*, OMe), 3.95 (3H, *s*, OMe). Demethylation gave quercetagetin.

5,4'-Dihydroxy-3,6,7,3'-tetramethoxyflavone (chrysosplenetin). UV λ_{max} (MeOH) nm: 272, 350; +NaOMe: 285, 405; +$AlCl_3$: 272, 278sh, 367, 405sh; +$AlCl_3$/HCl: 272, 278sh, 365, 410sh; +NaOAc: 272, 354; +NaOAc/H_3OB_3: 272, 350; MS *m/z* (rel. int.): 374 $[M]^+$ (100), 373 (28), 359 $[M-Me]^+$ (73), 356 $[M-18]^+$ (8), 181 $[A^1-Me]^+$ (11), 153 $[A^1-MeCO]^+$ (14), 133 $[B^1-CO]^+$ (13), 151 $[B^2]^+$ (15), 123 $[B^2-28]^+$ (24). Demethylation gave quercetagetin.

5-Hydroxy-3,6,7,3',4'-pentamethoxyflavone (artemetin). UV *λmax* (MeOH) nm: 254, 272, 345; +NaOMe: 250sh, 288, 328, 385; +$AlCl_3$: 265, 280sh, 300sh, 372; +$AlCl_3$/HCl: 262, 281, 300sh, 363, 410sh; +NaOAc: 255, 272, 345, 405sh; +NaOAc/H_3OB_3: 255, 272, 345; MS *m/z* (rel. int.): 388 $[M]^+$ (100), 387 $[M-H]^+$ (20), 373 $[M-Me]^+$ (69), 370 $[M-18]^+$ (8), 345 $[M-COMe]^+$ (14), 181 $[A^1-Me]^+$ (9), 153 $[A^1-MeCO]^+$ (10), 162 $[B^1]^+$ (3), 165 $[B^2]^+$ (24); ^1H NMR ($CDCl_3$): δ 6.52 (1H, *s*, H-8), 7.75 (2H, *dd*, H-2', H6'), 7.00 (1H, *d*, H-5'), 3.90 (3H, *s*, OMe), 3.96 (3H, *s*, OMe), 4.00 (3H, *s*, OMe). Demethylation gave rise to quercetagetin.

5,7,4'-Trihydroxy-6-methoxyflavone (hispidulin). UV λ_{max} (MeOH) nm: 272, 332; +NaOMe: 262, 268, 277, 326, 392; +$AlCl_3$: 262sh, 279sh, 302, 357; +$AlCl_3$/HCl: 259sh, 278sh, 300, 350; +NaOAc: 275, 300sh, 355; +NaOAc/H_3OB_3: 275, 335; MS *m/z* (rel. int.): 300 $[M]^+$ (100), 285 $[M-Me]^+$ (8), 282 $[M-18]^+$ (2), 272 $[M-CO]^+$ (18), 257 $[M-COMe]^+$ (3); ^1H NMR (acetone-d_6): δ 6.68 (1H, *s*, H-3), 6.55 (1H, *s*, H-8), 7.77 (2H, *d*, H-2',6'), 6.96 (2H, *d*, H3',5'), 3.92 (3H, *s*, OMe).

5,7,3',4'-Tetrahydroxy-6-methoxyflavone (nepetin) UV λ_{max} (MeOH) nm: 272, 345; +NaOMe: 272, 330, 405; +$AlCl_3$: 273, 365, 415; +$AlCl_3$/HCl: 260, 280, 295, 363; +NaOAc: 273, 320, 375; +NaOAc/H_3OB_3: 262, 373; MS *m/z* (rel. int.): 316 $[M]^+$ (52), 315 $[M-1]^+$ (9), 301 $[M-Me]^+$ (40), 298 $[M-18]^+$ (33), 273 $[M-COMe]^+$ (49), 167 $[A^1-Me]^+$ (32), 139 $[A^1-COMe]^+$ (38), 134 $[B^1]^+$ (36), 137 $[B^2]^+$ (30), 109 $[B^2-28]^+$ (11); ^1H NMR (acetone-d_6): δ 6.25 (2H, *d*, H-3, 8), 6.69 (1H, *d*, H-5'), 7.15, 7.20 (2H, H-2',6'), 3.40 (3H, *s*, OMe).

5,7,4'-Trihydroxy-3,6-dimethoxyflavone. UV λ_{max} (MeOH) nm: 270, 340; +NaOMe: 275, 325, 397; +$AlCl_3$: 278, 305, 360; +$AlCl_3$/HCl: 280, 305, 355; +NaOAc: 273, 300, 370; +NaOAc/H_3OB_3: 270, 345; MS *m/z* (rel. int.): 330 $[M]^+$ (100), 329 $[M-1]^+$ (48), 315 $[M-Me]^+$ (55), 312 $[M-18]^+$ (26), 287 $[M-MeCO]^+$ (43), 167 $[A^1-Me]^+$ (16), 139 $[A^1-COMe]^+$ (9), 121 $[B^2]^+$ (52), 93 $[B^2-28]^+$ (16).

5,7-Dihydroxy-6,4'-dimethoxyflavone (pectolinarigenin). UV λ_{max} (MeOH) nm: 273, 330; +NaOMe: 275, 292, 365; +$AlCl_3$: 280, 300, 353; +$AlCl_3$/HCl: 280, 300, 348; +NaOAc: 274, 360; +NaOAc/H_3OB_3: 273, 330; MS *m/z* (rel. int.): 314 $[M]^+$ (100), 313 $[M-1]^+$ (7),

299 $[M\text{-}Me]^+$ (73), 296 $[M\text{-}18]^+$ (61), 271 $[M\text{-}COMe]^+$ (65), 167 $[A^1\text{-}Me]^+$ (27), 139 $[A^1\text{-}COMe]^+$ (34), 132 $[B^1]^+$ (19), 135 $[B^2]^+$ (17), 107 $[B^2\text{-}28]^+$ (8).

5,4'-Dihydroxy-6,7-dimethoxyflavone. UV λ_{max} (MeOH) nm: 273, 333; +NaOMe: 275, 305sh, 380; +$AlCl_3$: 262sh, 282, 300, 358; +$AlCl_3$/HCl: 262sh, 282, 300, 353; +NaOAc: 273, 335; +NaOAc/H_3OB_3: 273, 335; MS *m/z* (rel. int.): 314 $[M]^+$ (88), 313 $[M\text{-}H]^+$ (32), 299 $[M\text{-}Me]^+$ (100), 296 $[M\text{-}18]^+$ (7), 271 $[M\text{-}COMe]^+$ (37), 181 $[A^1\text{-}Me]^+$ (31), 153 $[A^1\text{-}MeCO]^+$ (70), 181 $[B^1]^+$ (16), 121 $[B^2]^+$ (19).

Brine shrimp test. This was carried out according to the method described by Meyer *et al.* [2]. The eggs of brine shrimp, *Artemia salina*, were hatched in a shallow rectangular dish (10 x 25 cm) filled with artificial sea water (Instant Oceans) and double-distilled water. A plastic divider with several holes (2 mm) was used to make two compartments. The eggs were placed into one compartment which was darkened, while the second was illuminated. After 48 hr the phototropic nauplii were collected with a pipette from the lighted compartment. Ten shrimps were transferred to each sample vial using a disposable pipette and artificial sea water was added to make 5 ml. The vials were maintained under illumination. Survivors were counted with the aid of a magnifying glass after 24 hr. Flavonoid solutions were added to each vial and dried prior to adding the artificial sea water and shrimps. Controls and three tests were carried our for each concentration. The results of the tests are shown in Table 1.

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