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CHEMICAL AND BIOLOGICAL STUDIES OF *SINAPIS ARVENSIS* GROWING IN EGYPT

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نبات *السينابيس أرفينسيس* ل. التابع للعائلة الصليبية ينمو فى مصر منتشرا في حقول القمح والذرة. وفي دراسة كيمياتية لمكونات هذا النبات تم فصل أربعة مركبات فلافونيدية من زهور النبات وهي: أيزورامنيتين-٧،٣-أ-ثناني الجلوكوز ، أيزورامنيتين-٣-أ-جلوكوز-٧-أ-رامنوز وكامفيرول-٣-أ-جلوكوز بالإضافة إلى مركب جديد هو: (٢،٢'-ثناني خلات-٣،٣'-ثناني هيدروكسيل)-١،١'-ثناني بروبيل ايثر. وكذلك تم فصل أربعة مركبات من بذور النبات وهي: البيتا أميرين، البيتاسيتوستيرول ، البيتاسيتوستيرول-٣-أ-جلوكوز ، والسيرينجين. هذا وقد أوضحت دراسة الأحماض الدهنية المستخلصة من بذور النبات وجود حامض الايروسيك الضار بنسبة ١١,٥٦٪ مما يمنع الاستخدام الآدمي للزيت. وقد تم التعرف على المركبات السابق ذكرها بواسطة الطرق المختلفة للتحليل الكيماوي والطيفي.

وقد تم عمل در اسه بيولوجية للنبات تبين منها أن الخلاصة الكحولية للأوراق والمركبات الفلافونيدية المفصولة من الزهور لها تأثير مضاد للميكروبات كما أن الزيت المستخرج من البذور له نفس التأثير ولكن بنسبة أقل. كما أن المواد الفلافونيدية المفصولة من الزهور لها تأثير مضاد للالتهاب. بالإضافة إلى أن للزيت المستخرج من البذور تأثير طفيف كمدر للبول.

From the ethyl acetate fraction of the alcohol extract of the flowers of Sinapis arvensis L. a new compound given the name: (2,2`-diacetyloxy-3,3`-dihydroxy)-1,1`-dipropylether. In addition, three flavonoidal glycosides were also isolated and identified as: kaempferol-3-O-ß-glucopyranoside, isorhamnetin-3,7-O-ß-diglucopyranoside and isorhamnetin-3-O-ß-glucopyranoside-7-O-α-rhamnopyranoside. Meanwhile the chloroform fraction of the alcohol extract of the seeds afforded ß-amyrin, ß-sitosterol, ß-sitosterol-3-O-ß-glucopyranoside and syringin. The GC/MS analysis of the fixed oil of the seeds revealed the presence of 14 fatty acids including a high percent (11.56%) of harmful erucic acid.

The identification of the isolated compounds was based on different methods of physical, chemical and spectral analysis.

Moreover the biological screening showed that the alcoholic extract of the leaf, the flavonoids of the flower and to a lesser extent the oil of the seed possesses a significant antimicrobial activity, while the flavonoids of the flower showed a significant anti-inflammatory activity. In addition, the oil of the seed showed insignificant diuretic activity.

INTRODUCTION

The genus *Sinapis* is represented in Egypt by five species namely: *S. alba L, S. aucheri L, S. arvensis L, S. turgida L* and *S. alionii L.*[1,2] The plant *Sinapis arvensis L.* is synonemous to *Brassica sinapistrum*.[3] Moreover the plant has the following common names: Mustard and Charlock in Europe,[4] khardal, qirellah and kaber afrit in Egypt.[5] In some books the plant was mentioned as Wild mustard,[6] meanwhile here in Assiut it was called El-Nawar.[7]

Previous studies concerning *Sinapis arvensis L.* revealed the isolation of several flavonoids from the flower and leaf including: isorhamnetin-3-O-ß-glucopyranoside-7-O-α-rhamnopyranoside,[8] 3,7-diglucosides of kaempferol and isorhamnetin,[9] 3-glucoside-7-

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rhamnosides of kaempferol and quercetin (as inseparable mixture),[9] in addition to 3-sophoroside-7-glucosides of quercetin and isorhamnetin.[9] Also several glucosinolates have been reported in the seed including glucobrassicin, neoglucobrassicin and sinapin.[6] Moreover some sterols including: ß-sitosterol, campesterol,[10,11] 5-avenosterol[11] and cholesterol have been identified.[12]

EXPERIMENTAL

General

MPs and MMPs on Stuart Scientific (SMP 1, England), ^{1}H- and ^{13}C-NMR at 400 and 100 MHz respectively in DMSO-d_6 with TMS as internal standard on JEOL spectrometer. UV measurments on Perkin-Elmer model 550 spectrophotometer. Column chromatography with silica gel (70-230 mesh size, E-Merck).

GC/MS of fatty acid methyl esters on Varian-3400 with coiled column of 2 m length and 4 mm internal diameter and the column was packed with Db-wax supported on chromosorb P (60-80 mesh size), the column temperature was 255°, injector temperature was 220°, detector temperature was 255° and nitrogen was used as a carrier gas at a flow rate of 34 ml/min. For sterol acetates the GC/MS was carried out using coiled column of 2 m length and 4 mm internal diameter and the column was packed with Db-wax supported on chromosorb P (60-80 mesh size) Db-1 column at a temperature of 300°, injector temperature of 250°, detector temperature of 255° and nitrogen was used as a carrier gas at a rate of 46 ml/min.

TLC on precoated silica gel sheets (aluminum foil, GF-254, E-Merck) and the following systems were used: 1: Hex.-EtOAc (9:1), 2: Hex.-EtOAc (85:15), 3: $CHCl_3$-MeOH (85:15), 4: $CHCl_3$-MeOH (8:2), 5: $CHCl_3$-MeOH-H_2O (75:25:3), 6: $CHCl_3$-MeOH-H_2O (65:30:5), 7: BuOH-AcOH-H_2O (4:1:5) and 8: AcOH-H_2O (1:1).

Plant material

The seeds, leaves and flowers of *Sinapis arvensis L.* were collected from the cultivated plants obtained from the experimental station of Al-Azhar University, Assiut, Egypt from December (1997) till February (1998). The plant was kindly identified by Dr. Salah El-Nagar (Associate Professor of Botany, Department of Botany, Faculty of Science, Assiut University, Assiut, Egypt).

Extraction and isolation of the constituents of the seeds

Two kilograms of the air-dried powdered seeds were defatted with n-hexane to yield 300 ml of fixed oil, which kept for further studies. The marc left was extracted with 70% alcohol. The alcohol extract was evaporated till syrupy, diluted with water and extracted successively and exhaustively with chloroform and n-butanol. The chloroform fraction was then chromatographed on silica gel column using Hex.-EtOAc gradient. Elution with Hex.-EtOAc (97:3) afforded compound **1**, Hex.-EtOAc (95:5) afforded compound **2**, Hex.-EtOAc (83:17) afforded compound **3**, Hex.-EtOAc (80:20) afforded compound **4** and Hex.-EtOAc (70:30) afforded a mixture of compounds which are still under investigation. One kilogram of the powdered seeds was percolated with n-hexane till exhaustion, and then the solvent was distilled off to yield 150 ml of the fixed oil.

Extraction and isolation of the flavonoids of the flower

One kilogram of the air-dried powdered flowers was extracted with 70% alcohol. The concentrated alcoholic extract (270 g) was diluted with 500 ml distilled water then successively and exhaustively extracted with n-hexane, chloroform, ethyl acetate and finally with n-butanol. The ethyl acetate fraction was then chromatographed on silica gel column using $CHCl_3$-MeOH gradient, where elution with $CHCl_3$-MeOH (85:15) afforded compound **5**, $CHCl_3$-MeOH (75:25) afforded compound **6**, $CHCl_3$-MeOH (75:25) afforded compound **7**, $CHCl_3$-MeOH (70:30) afforded compound **8** and $CHCl_3$-MeOH (63:37) afforded a minor mixture of flavonoids, which are still under investigation.

Acid hydrolysis of the glycosides[13]

Each glycoside (25 mg) was separately dissolved in TFA (2 M) in a sealed tube and autoclaved at 120°/1 bar for 1 hr. TFA was then removed under vacuum. The residue in each case was extracted with 1 ml of distilled water and the receptive aqueous extract of each was analyzed for sugars by TLC alongside with authentic samples. The water insoluble residue was dissolved in methanol and analyzed for the aglycone.

Identification of the isolated compounds

Compound 1: White amorphous powder, 40 mg, R_f 0.36 using system 1, m.p 200-202°, not depressed by authentic sample of ß-amyrin. Co-chromatography with authentic ß-amyrin using system 1 and superimposable IR spectra showed their identity.

Compound 2: White fine needles, 100 mg, R_f 0.41 using system 2, m.p 134-136°. It was identified as ß-sitosterol by direct authentication (mmp and co-chromatography in system 2).

Compound 3: Spherical crystals, 300 mg, R_f 0.46 using system 3, m.p 275-277° which was not depressed by mixing with authentic ß-sitosterol glucoside. It was identified as ß-sitosterol glucoside by direct authentication (mmp, co-chromatography using system 3 and superimposable IR spectra).

Compound 4: Colourless crystals, 57 mg, R_f 0.53 using system 4, m.p 191-193°. ^{1}H-NMR (DMSO-d_6) at δ_H: 6.72 (2H, s, H-2, 6); 6.56 (1H, dt, J= 15.75, 5.86, H-2`); 6.35 (1H, d, J= 15.75, H-1`); 4.28 (2H, br d, J= 5.86, H-3`); 3.77 (6H, s, 2x CH_3O-) and 4.85 (1H, d, J= 7.3, H-1 sugar). ^{13}C-NMR (DMSO-d_6) at δ_C: 132.5 (C-1); 104.4 (C-2, 6); 152.6 (C-3,5); 133.8 (C-4); 130.1 (C-7); 128.3 (C-8); 61.4 (C-9); 56.2 (2x CH_3O-); 102.5 (C-1 glucose); 74.2 (C-2 glucose); 76.5 (C-3 glucose); 70.1 (C-4 glucose); 77.1 (C-5 glucose) and 61.0 (C-6 glucose).

Compound 5: Yellowish brown powder, 30 mg, R_f 0.49 using system 4. Except for a peak at m/z 43 (100%) (acetyl group); EIMS, did not show any significant peaks and similarly did FABMS. ^{1}H-NMR (DMSO-d_6) at δ_H: 1.66 (6H, s, 2x $\underline{CH_3}$-O); 3.28 (4H, d, J= 4.88, H-1,1`); 3.34 (4H, d, J= 5.62, H-3,3`) and 3.41 (2H, m, H-2,2`). $^{13}$C-NMR and DEPT measurement (DMSO-$d_6$) at $\delta_C$: 24.7 (2x $CH_3$); 62.8 (C-1,1`); 63.0 (C-3,3`); 72.5 (C-2,2`) and 176 (2x C=O).

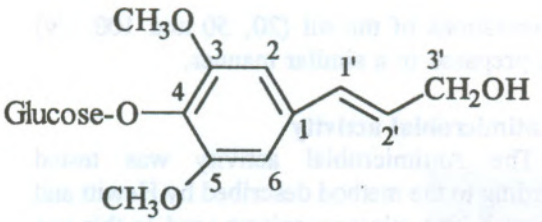
Compound 6: Yellowish powder, 80 mg, R_f 0.41 using system 4, m.p 243-244°. UV λ_{max} nm: 356, 272, 217 sh (MeOH); 410, 370 sh, 280 (NaOMe); 373, 280, 220 sh (NaOAc); 356, 272, 221 sh (NaOAc/H_3BO_3); 402, 350 sh, 278 ($AlCl_3$); 402, 356 sh, 278 ($AlCl_3$/HCl). ^{1}H-NMR (DMSO-d_6) at δ_H: 8.02 (2H, d, J= 8.79, H-2`,6`); 6.88 (2H, d, J= 8.79, H-3`,5`); 6.42 (1H, d, J= 1.7, H-8); 6.19 (1H, d, J= 1.7, H-6) and 5.42 (1H, d, J= 7.33, H-1 sugar). ^{13}C-NMR given in Table 1. Acid hydrolysis afforded D-glucose (co-chromatography with authentic sample using system 7) and kaempferol (co-chromatography with authentic sample using system 8 and superimposable UV spectra).

Compound 7: Yellowish powder, 245 mg, R_f 0.47 using system 5, m.p 221-222°. UV λ_{max} nm: 355, 316 sh, 268 (MeOH); 412, 322 sh, 272 (NaOMe); 355, 316 sh, 268 (NaOAc); 355, 268, 221 sh (NaOAc/H_3BO_3); 407, 356 sh, 278 ($AlCl_3$); 407, 367 sh, 278 ($AlCl_3$/HCl). ^{1}H-NMR (DMSO-d_6) at δ_H: 7.9 (1H, d, J= 1.81, H-2`); 7.55 (1H, dd, J= 1.81, 8.3, H-6`); 6.9 (1H, d, J= 8.3, H-5`); 6.8 (1H, d, J= 1.7, H-8); 6.4 (1H, d, J= 1.7, H-6); 5.6 (1H, d, J= 7.31, H-1 glucose); 5.4 (1H, s, H-1 rhamnose) and 3.83 (3H, s, -OCH_3). ^{13}C-NMR given in Table 1. Acid hydrolysis afforded D-glucose and L-rhamnose (co-chromatography with authentic sample using system 7) and isorhamnetin (co-chromatography with authentic sample using system 8 and superimposable UV spectra).

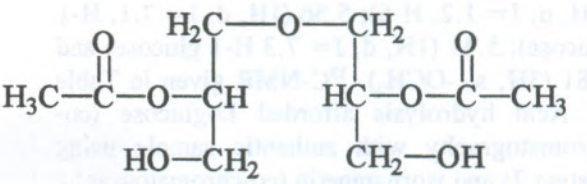
Compound 8: Yellowish powder, 65 mg, R_f 0.57 in system 6, m.p 209-210°. UV λ_{max} nm: 356, 305 sh, 254 (MeOH); 410, 328 sh, 264 (NaOMe); 356, 328 sh, 254 (NaOAc); 356, 314 sh, 254 (NaOAc/H_3BO_3); 405, 367 sh, 260 ($AlCl_3$); 405,350 sh, 260 ($AlCl_3$/HCl). ^{1}H-NMR (DMSO-d_6) at δ_H: 7.9 (1H, d, J= 1.7, H-2`); 7.53 (1H, dd, J= 1.7, 8.54, H-6`); 6.92 (1H, d,

Table 1: ^{13}C-NMR of compounds **6-8** (100 MHz, DMSO-d^6).

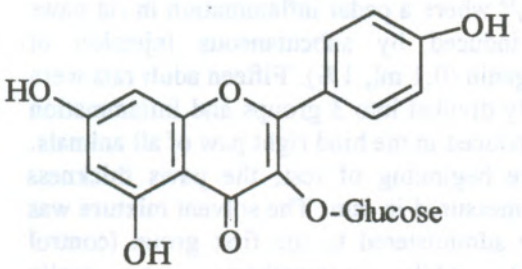
Carbon no.		Comp. 6	Comp. 7	Comp.8
Aglycone:	2	156.5	156.2	156.1
	3	133.5	133.5	133.4
	4	177.7	177.8	177.6
	5	161.5	161.1	160.9
	6	99.0	98.6	99.4
	7	164.4	161.7	162.9
	8	94.0	94.8	94.6
	9	156.7	157.1	156.0
	10	104.3	105.9	105.7
	1'	121.2	121.2	121.0
	2'	131.2	113.7	115.3
	3'	115.4	149.8	149.6
	4'	160.2	147.2	147.0
	5'	115.4	115.4	113.6
	6'	131.2	122.6	122.2
	OCH_3	--	55.9	55.7
3-O-glucosyl:	1"	101.2	101.0	100.7
	2"	74.5	74.3	74.4
	3"	76.6	77.3	77.2
	4"	70.1	69.8	69.9
	5"	77.6	76.4	77.5
	6"	61.0	60.6	60.7
7-O-rhamnosyl:	1'''	--	98.8	--
	2'''	--	71.6	--
	3'''	--	69.9	--
	4'''	--	69.8	--
	5'''	--	70.2	--
	6'''	--	17.8	--
7-O-glycosyl:	1'''	--	--	99.8
	2'''	--	--	73.1
	3'''	--	--	76.5
	4'''	--	--	69.7
	5'''	--	--	77.5
	6'''	--	--	60.7



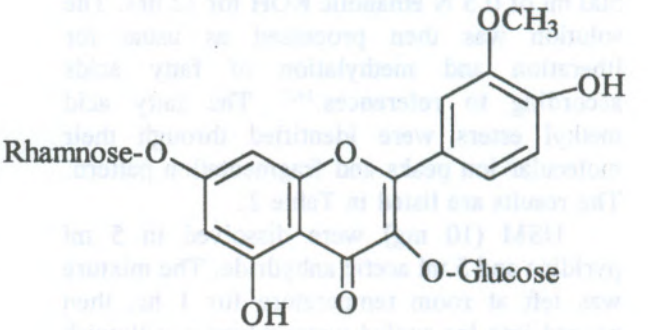
Compound 4



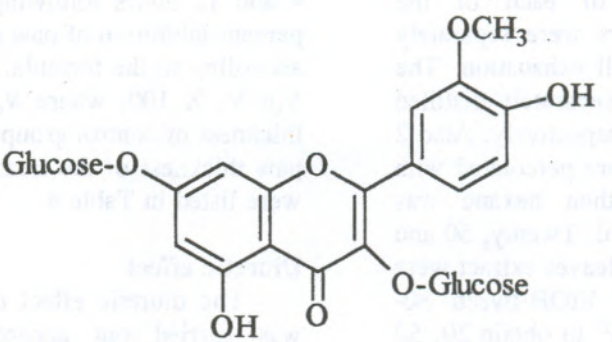
Compound 5



Compound 6



Compound 7



Compound 8

Figure 1

J= 8.54, H-5`);6.79 (1H, d, J= 1.2, H-8); 6.4 (1H, d, J= 1.2, H-6); 5.56 (1H, d, J= 7.1, H-1 glucose); 5.41 (1H, d, J= 7.3 H-1 glucose) and 3.81 (3H, s, $-OCH_3$). ^{13}C-NMR given in Table 1. Acid hydrolysis afforded D-glucose (co-chromatography with authentic sample using system 7) and isorhamnetin (co-chromatography with authentic sample using system 8 and superimposable UV spectra).

Investigation of the seed fixed oil

One hundred ml of the oil prepared with n-hexane extraction of the seeds was refluxed with 500 ml of 0.5 N ethanolic KOH for 12 hrs. The solution was then processed as usual for liberation and methylation of fatty acids according to references.[14,15] The fatty acid methyl esters were identified through their molecular ion peaks and fragmentation pattern. The results are listed in Table 2.

USM (10 mg) were dissolved in 5 ml pyridine and 5 ml acetic anhydride. The mixture was left at room temperature for 1 hr, then poured into ice-cooled water where a yellowish precipitate was formed. The solution was filtered and the precipitate was washed with cold distilled water, then dried.[16] The acetate derivatives were analyzed by GC/MS.

Biological screening

Preparation of the extracts

Five hundred grams of each of the powdered leaves and flowers were separately extracted with 70% EtOH till exhaustion. The alcohol extract of each was separately distilled off yielding 54 g and 58 g respectively. Also 2 kg of the powdered seeds were percolated with n-hexane till exhaustion, then hexane was distilled yielding 320 ml of oil. Twenty, 50 and 100 mg of the residue of the leaves extract were dissolved in a mixture of EtOH-tween 80-distilled water (2:2:20 v/v/v)[17] to obtain 20, 50 and 100 mg% concentration. In addition 40 g of the residue of the alcoholic extract of the flower were chromatographed on silica gel to get 685 mg of enriched flavonoids mixture. The same concentrations of the mixture of flavonoids (20, 50 and 100 mg%) were prepared similarly. The concentrations of the oil (20, 50 and 100 v/v) were prepared in a similar manner.

1- Antimicrobial activity

The Antimicrobial activity was tested according to the method described by Hewitt and Vincent.[18] The microorganisms used in this test were kindly supplied and identified by Dr. T. El-Khamisy, Lecturer of Microbiology, Faculty of Pharmacy, Al-Azhar University, Assiut. The results were listed in Table 3.

2- Anti-inflammatory effect

The anti-inflammatory effect was done according to the method described by Winter *et al.*,[19] where a pedal inflammation in rat paws was induced by subcutaneous injection of carragenin (0.1 ml, 1%). Fifteen adult rats were equally divided into 3 groups and inflammation was induced in the hind right paw of all animals. At the beginning of test, the paws thickness were measured in mm. The solvent mixture was orally administered to the first group (control group), while indomethacin was orally administered to the second group at a dose of 8 mg/kg of body weight one hour after induction of inflammation. The mixture of flavonoids was orally administered to the third group at a dose of 100 mg/kg of body weight one hour after induction of inflammation and finally the paw thickness of all groups were measured at 1, 2, 3, 4 and 12 hours following administration. The percent inhibition of paw oedema was estimated according to the formula: % inhibition = $(V_0 - V_t)/ V_0 \times 100$, where V_0: is the average paw thickness of control group and V_t: is the average paw thickness of the treated group. The results were listed in Table 4.

Diuretic effect

The diuretic effect of the oil of the seed was carried out according to the method described by Babini[20] using 3 groups of white male rabbits of 4 animals each. The first group received 2 ml of Furosemide orally, while the second group received 5 ml of the seed oil and the third group was used as control (untreated). Urine was collected after 1, 2, 4, 8 and 12 hours

Table 2: The GC/MS of fatty acid methyl esters.

Fatty acid methyl ester	M/Z	Rel.int.*	d %
	130(M^+)	8 %	
Caproic	88	18 %	4.6 %
t_R = 7:27	74	100 %	
	270 (M^+)	24 %	
	255	20 %	
Palmitic	199	16 %	13.4 %
t_R = 27:38	143	32 %	
	87	72 %	
	74	100 %	
	326(M^+)	52 %	
	283	24 %	
	227	8 %	
Arachidic	199	12 %	18.4 %
t_R= 37:10	143	32 %	
	87	68 %	
	74	100 %	
	298(M^+)	46 %	
	255	20 %	
Stearic	199	16 %	13 %
t_R= 32:07	143	32 %	
	101	8 %	
	74	100 %	
	294 (M^+)	28 %	
	263	18 %	7.4 %
	234	6 %	
	178	8 %	
	164	10 %	
Linoleic	122	16 %	
t_R= 31:55	108	10 %	7.4 %
	80	46 %	
	47	100 %	
	66	40 %	
	292 (M^+)	18 %	
	264	12 %	
	236	10 %	
Linolenic	162	8 %	6.2 %
t_R= 31:48	136	16 %	
	108	46 %	
	74	100 %	
	322 (M^+)	18 %	
	291	24 %	
	249	6 %	
Unknown	207	6 %	6.4
t_R= 36:22	150	24 %	
	94	100 %	
	80	60 %	
	66	46 %	

Fatty acid methyl ester	M/Z	Rel.int*	Estimated %
	352(M^+)	16 %	
	320	72 %	
	278	14 %	
	236	18 %	
	208	7 %	
Erucic	152	10 %	11.56 %
t_R= 41:10	110	24 %	
	96	52 %	
	68	64 %	
	54	100 %	
	296 (M^+)	18 %	
	265	20 %	
	222	16 %	
Oleic	166	12 %	6.2 %
t_R= 32:00	110	22 %	
	82	40 %	
	74	45 %	
	54	100 %	
	382 (M^+)	50 %	
	339	20 %	
	283	10 %	
Lignoceric	199	18 %	
t_R= 45:13	143	42 %	3.6 %
	87	76 %	
	74	100 %	
	314 (M^+)	2 %	
	296	20 %	
	283	6 %	
Dihydroric-	227	40 %	
inoleic	170	16 %	7.3 %
	142	34 %	
t_R= 34:37	86	40 %	
	74	100 %	
	58	26 %	
	338 (M^+)	12 %	
	292	36 %	
Unknown	250	14 %	
t_R = 37:58	208	16 %	5.8 %
	166	6 %	
	110	20 %	
	54	100 %	
	281 (M^+)	6 %	
	171	4 %	
	126	24 %	
Unknown	111	28 %	2.94 %
t_R= 29:15	97	46 %	
	70	100 %	

Rel. int*: Relative intensity. t_R: Retention time.

following administration and results were listed in Table 5.

RESULTS AND DISCUSSIONS

I- Identification of compounds 1-4 of the chloroform fraction of the seed

The chloroform fraction of the seed was found to contain: ß-amyrin (**1**), ß-sitosterol (**2**), ß-sitosterol-3-glucoside (**3**) and syringin (**4**). The first three compounds were identified by direct authentication (mmp and co-chromatography), meanwhile compound **4** was identified by comparing its physical and spectral data with literature.[21]

II- Identification of compounds 5-8 of the ethyl acetate fraction of the flower

The ^{1}H-NMR of **5** revealed the presence of 2 equivalent methyl groups at δ_H 1.66 (6 H, s), a multiplet signal centered at δ_H 3.41 (2 H, m) was assigned for 2 equivalent oxygenated methine groups. In addition to two doublet signals at δ_H 3.28 (2 H, d, J= 4.88) and 3.34 (2 H, d, J= 5.62) were assigned for 2 unequivalent methylene groups. This was confirmed by ^{13}C-NMR with DEPT measurement, which showed the presence of two equivalent acetyloxy groups at δ_C 24.7 (2$\underline{CH_3}$–CO groups) and 176 (2CH_3–$\underline{C=O}$ groups), a signal at δ_C 72.5 was assigned for two equivalent oxygenated methine groups (2$\underline{CH}$–O). The ^{1}H- and ^{13}C-NMR indicated a dimeric glycerol structure linked through terminal ethereal oxygen. The acyl moiety is located at C-2 due to the downfield shift of C-2 (+3 ppm) and upfield shift of C-1 and C-3 (-4 and -3.5 ppm respectively) in comparison with unsubstituted glycerol.[22] Accordingly **5** was assigned the structure (2,2`-diacetyloxy-3,3`-dihydroxy)-1,1`-dipropyl ether, which is a new compound as shown in Figure 1.

Identification of the flavonoids of the flower

The acid hydrolysis of **6**, yielded D-glucose and the aglycone was identified as kaempferol from its UV data with different ionizing and complexing agents[23] and co-chromatography with authentic kaempferol. The sugar attachment was assigned to C-3 by comparing the UV data before and after hydrolysis.[24] The ^{1}H- and ^{13}C-NMR spectra were also identical to those reported for kaempferol-3-O-ß-glucopyranoside.[25]

The acid hydrolysis of **7** yielded glucose and rhamnose, the aglycone was identified as isorhamnetin from its UV data with different ionizing and complexing agents[26] and co-chromatography with authentic isorhamnetin. **7** was found to have 3-O-ß-glucopyranosyl and 7-O-α-rhamnopyranosyl moieties based on ^{13}C-NMR since the existed upfield shift of C-3 was identical to 3-O-glucosylation rather than 3-O-rhamnosylation.[27]

The acid hydrolysis of **8** yielded glucose and the aglycone was identified as isorhamnetin in the same way as described in **7.** On comparing the UV data before and after hydrolysis together with the ^{13}C-NMR evidences **8** was identified as isorhamnetin-3,7-O-ß-diglucopyranoside. Both **7** and **8** were previously isolated from *Sinapis arvensis*[8,9] but this represents their first isolation from the plant growing in Egypt.

Identification of the fatty acids and unsaponifiable matter

The oil of the seeds was investigated for its fatty acids and USM composition as shown in the experimental part. The results of GC/MS analysis of fatty acid methyl esters (Table 2) showed the presence of caproic 4.6%, palmitic 13.4%, stearic 13%, arachidic 18.4%, lignoceric 3.6%, linoleic 7.4%, linolenic 6.2%, erucic 11.56%, oleic 6.2% and dihydroricinoleic acid 7.3%. Although the total amount quoted for unsaturated fatty acids was 52.4%; the presence of harmful erucic acid[28,29] in such a high percent (11.56%) prevents the human consumption of the oil. The USM was found to contain ß-sitosterol 56%, campesterol 17.3% and stigmasterol 4.6% in addition to other components which could not be identified.

Table 3: The antimicrobial activity.

Sample	Conc. mg/ml	Diameter of inhibition zone (mm)		
		E-coli	Bacillus	Klebsiella
Alcoholic extract of the leaf	50	14.3 ±0.65	16.2 ±0.48	--
	100	18.2 ± 0.55	21.3 ±0.69	--
Mixture of flavonoids	20	12.5 ± 0.57	14.3 ± 0.56	13.1 ±0.61
	50	15 ± 0.63	17.2 ± 0.87	15.4 ±0.64
	100	19.6 ± 0.69	22 ± 0.96	19.6 ± 0.73
Oil of the seed	50	12.2 ± 0.42	--	--
	100	14.8 ± 0.52	--	--

Table 4: The anti-inflammatory effect of the mixture of flavonoids.

Animal gp.	The tested compound	% increase in paw volume (mean ± S.E) after time				
		1 hr	2 hr	3 hr	4 hr	12 hr
Gp. I (control)	Solvent mix.	9.6 (±0.049)	15.14 (±0.051)	20.17 (±0.056)	21.73 (±0.057)	14.87 (±0.041)
Gp. II	Indomethacin (8mg/kg)	7.41 (±0.032) 25.5[a]	9.63 (±0.043) 36.4[a]	8.34 (±0.052) 58.6[a]	6.52 (±0.032) 70.0[a]	3.78 (±0.038) 74.5[a]
Gp. III	Mixture of flavonoids (100mg/kg)	5.93 (±0.021) 40.4[a]	11.91 (±0.027) 26.0[a]	16.81 (±0.042) 16.7[a]	17.68 (±0.028) 18.6[a]	12.81 (0.027) 13.8[a]

a: Percent of oedema inhibition.

Table 5: The diuretic effect of the oil of the seeds.

Sample	1 hr	2 hrs	4 hrs	8 hrs	12 hrs	Total vol.
Control	0 ±0.0	0±0.0	0±0.0	4±2.9	0±0.0	4
Furosemide	4±2.5	5±3.8	2±2.4	7±1.8	11±2.4	29
The oil	0±0.0	3±3.1	3±2.8	4.5±2.1	2±2.2	16.5

Results of biological screening

1- Antimicrobial activity: (Table 3)

The antimicrobial activities of the alcoholic extract of the leaf, the flavonoids of the flower and the oil of the seed were done as prementioned in the experimental part. The inhibition zone diameters produced by the leaf extract, the flavonoids and the oil were compared with these reported for reference antibiotics under the same conditions according to the NCCLS (1994).[30] It was noteworthy to mention that the activity of the 50% concentration of the flavonoids mixture was nearly similar to the inhibition zone produced by 10 μg of streptomycin (17 mm), ampicillin (14 mm) and tobramycin (15 mm).

2- Anti-inflammatory effect: (Table 4)

The anti-inflammatory effect of the mixture of flavonoids was done as prementioned in the experimental part. From the data listed in Table 4 one can conclude that:

1- The mixture of flavonoids showed a significant anti-inflammatory effect at P= 0.05, where a marked reduction of the paw oedema was observed.

2- The anti-inflammatory effect of the mixture of flavonoids was most prominent and more potent than indomethacin during the first hour, and then declines to 71.4% of indomethacin action after 2 hours and to 28.5% after 3hours, 26.6% after 4 hours and finally reaches the lowest effect after 12 hours regarding the indomethacin effect.

3-Diuretic effect: (Table 5)

The diuretic effect of the oil of the seeds was done as prementioned in the experimental part. From the data listed in Table 5, the oil has an insignificant diuretic effect starting within 2 hours after administration, and reaching the maximum effect after 8 hours. On comparing the effect of the oil with that of furosemide, the oil was found to have 57% of furosemide activity.

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