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QUANTITATIVE DETERMINATION OF FLAVONOIDS PRESENT IN LIMONIUM SINUATUM MILL

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A chromatospectrophotometric method was carried out for quantitative estimation of flavonoids in the flowers and leaves of Limonium sinuatum Mill and proved that flowers contain the highest percentage of flavonoids. The percentage of flavonoids (gm %) in flowers and leaves was as follows respectively : total aglycones (calculated as apigenin), (0.09 , 0.24); apigenin, (0.048 , 0.17); luteolin (0.034 , 0.02) ; total glycosides (calculated as gossypin), (2.43 , 0.44); gossypin (0.96 , 0.14); 8-hydroxykaempferol-8-O-glucoside (1.01 , 0.03).

Limonium sinuatum Mill (=Statice sinuata L.) is a rough hairy plant belongs to family Plumbaginaceae[1]. From flowers and leaves of that plant a series of 8-hydroxyflavonoids were isolated[2,3]. It was proved that 8-hydroxyflavonoids are highly pharmacologically active[4] and since our previous studies showed that this plant is rich in such compounds, this paid our attention to estimate its flavonoidal content.

EXPERIMENTAL

Material :

The flowers and leaves of Limonium sinuatum Mill were collected from plants grown in the Medicinal Plant Experimental Station of the Faculty of Pharmacy, Assiut University, Assiut , Egypt .

Chromatographic Investigation

5 g. of each of the air dried powdered flowers and leaves were separately extracted with methanol and the extracts were concentrated under reduced pressure. PC screening using Whatman filter paper (3 mm)in system I : chloroform : methanol : water (150 : 45 : 5) revealed the presence of 9 flavonoidal spots in each of leaves and flowers (Table I).

Procedure For Assay of Flavonoids :

5 g. of each of air dried powdered flowers and leaves (P) were separately defatted with petroleum-ether (b.r. 40-60°)then extracted, till exhaustion, with methanol in soxhlet apparatus. The methanol extracts were separately concentrated and volume adjusted to 10 ml (V). 0.2 ml of each methanolic extract (V_1) was developed in system I.

After development, the paper chromatogram was dried and spots corresponding to apigenin , luteolin, 8-hydroxkaempferol-8-O-glucoside and gossypin (with R_f 0.92 , 0.46 and 0.29 respectively) were detected in UV-light. Each spot was cut and extracted with methanol 3 times (using electric shaker, 2 hours for each time) and the methanolic eluate for each spot was separately concentrated and volume adjusted to 10 ml (W). The absorbance for each eluate was determined at specific wave length (335 nm. for apigenin, 348 nm. for luteolin ; 375 nm. for 8-hydroxykaempferol-8-O-glucoside and 380 nm. for gossypin) and the concentration of each flavonoid in the corresponding eluate (C) was calculated from standard curves. The whole process was repeated 4 times and the mean of cocentration was calculated.

Accurate percentage of each flavonoid (X) in plant material was calculated from the following formula :

$$X = \frac{W.\ C.\ V.\ 100.\ 100}{P.\ V_1.\ E.\ 1000\ (100-M)}$$

W = volume of eluate in ml .

C = concentration of flavonoid in the eluate in mgm % (obtained from standard curve).

V = volume of methanol extract in ml. (obtained from plant material).

P = weight of plant material in g.

V_1 = volume of extract spotted on paper chromatogram in ml .

E = percentage of elution of each flavonoid from paper chromatogram.

M = moisture content in plant material.

Standard Curves :

A standard concentration absorption curves (Fig I) were prepared using pure apigenin, luteolin, gossypin and 8-hydroxykaempferol-8-O-glucoside.

Percentage of Elution of Each Flavonoid From Paper Chromatogram (E) :

0.1 ml. of 0.05% methanolic solution of each flavonoid was separately spotted on Whatman filter paper (3 mm) and the paper was developed in system I. After development, the spots corresponding to each flavonoid were detected by UV-light. Each spot was cut and extracted 3 times with methanol (using electric shaker, 2 hours for each time) and the eluates were concentrated and volume adjusted to 10 ml. The absorbance for each flavonoid was determined and the corresponding concentration was calculated from standard curves. The process was repeated 4 times and the average concentration in the eluate was calculated (A). The percentage of elution (E) was calculated from the following formula : $E = \frac{A}{B} \frac{100}{}$

A = average concentration in the eluate.

B = actual concentration spotted on PC.

The percentage of elution (E) was found to be : 81.73 (for apigenin); 82.03 (for luteolin); 83.32 (for 8-hydroxykaempferol-8-O-glucoside) and 85.12 (for gossypin).

Determination of Moisture Content in Plant Material (M)

Moisture content in flowers and leaves was carried out according to the E.P. (1963) and was found to be 3.75% (for flowes) and 4.37% (for leaves).

RESULTS AND DISCUSSION

Chromatographic investigation of the methanolic extracts of each of leaves and flowers revealed the presence of 9 flavonoidal spots (Table I). Spots 1,2,3 and 4 are aglycones (apigenin, luteolin, 8-hydroxykaempferol and gossypetin respectively) while spots 5,6,7,8 and 9 are glycosides (8-hydroxykaempferol-8-O-glucoside, gossypin, 8-hydroxykaempferol-3,8-O-diglucoside, gossypetin-3,8- diglucoside and an unknown glycoside respectively.

A chromatospectrophotometric method was carried out for quantitative estimation of flavonoids in the flowers and leaves. The percentage of total aglycones calculated as apigenin, total glycosides calculated as gossypin and individual major flavonoids are given in table II.

It was proved, from table II ,that percentage of total glycosides is much more higher and significant in flowers than in leaves. On the other hand, percentage of total aglycones is higher in leaves than in flowers . In flowers, the glycosides appear to be the major active constituents (2.43%)while aglycones appear to be minors (0.09%). In leaves, the percentage of total glycosides (0.44%) is nearly double the total aglycones (0.24%).

In flowers, the two glycosides gossypin and 8-hydroxykaempferol-8-O-glucoside are the major glycosides (0.96% and 1.01% respectively) while the percentage of total glycosides is 2.43%. In other words, these two glycosides constitute about 81% of the total glycosides in flowers.

From our previous studies, we can conclude that flowers can be used as an economic source for the preparation of 8-hydroxyflavonoid glycosides .

Table I. Chromatographic Properties of Flavonoids Present In Limonium Sinuatum Mill

No	h_{R_f} *system I*	*Organ*		*Colour*	
		Leaf	*Flower*	*UV*	*+ ammonia*
1	96	++	+	dark brown	dark yellow
2	92	+	+	dark brown	dark yellow
3	80	+	±	yellow	yellow
4	62	+	±	yellow	yellow
5	46	+	+++	yellow	yellow
6	29	++	+++	Orange	dark yellow
7	16	++	+	brown	yellow
8	12	++	+	dark brown	lemon yellow
9	5	±	+	lemon yellow	lemon yellow

Legend : +++ high concentration ; ++ medium concentration;
+ low concentration ; ± traces.
system I: chloroform : methanol : water (150:45:5)

Table II. Percentage Of Flavonoids In The Flowers And Leaves Of Limonium Sinuatum Mill Fam. Plumbaginaceae

Compound	*Concentration gm%*	
	Flowers	*leaves*
Apigenin	0.048	0.170
Luteolin	0.034	0.020
Gossypin	0.960	0.140
8-hydroxykaempferol-8-O-glucoside	1.010	0.030
Total aglycones calculated as apigenin	0.090	0.240
Total glycosides caluclated as gossypin	2.430	0.440

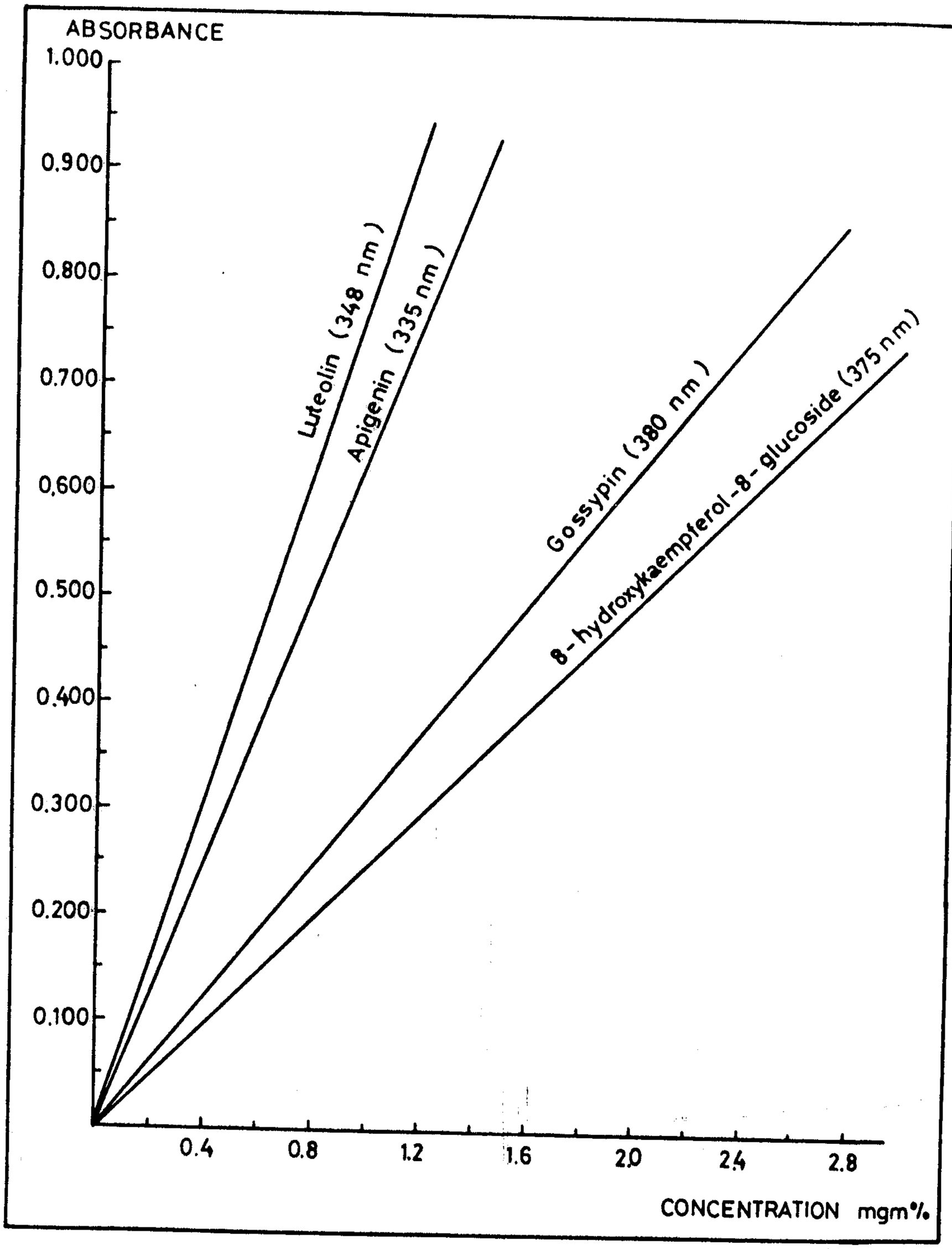

Fig.1. Standard curves for estimation of flavonoids in flowers and leaves of Limonium sinuatum Mill.

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التقدير الكمى للمركبات الفلافونية الموجـــودة فى نبـــات الليمونيـــم سنيـــواتم ميـــل الـــذى ينمو فى مصر

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أثبت الباحثون فى دراسات كيماوية سابقة ان اوراق وازهار نبات الليمونيم سنويواتاميل تحتوى على مجموعة كبيرة من المركبات الفلافونية وتم فصل ٨ مركبات من الاوراق ومركبين من الازهـــار وكذلك امكن التعرف على تركيبهم الكيميائى • نظرا لما ثبت حديثا من ان المركبات الفلافونيـــة التى تحتوى على مجموعة هيدروكسيل متصلة بذرة الكربون رقم ٨ فى المركب لها تاثير فارماكولوجـــى عالى وحيث ان معظم المركبات المفصولة من هذه المجموعة لذلك كان من الافضل تعيين النســـب المئوية لهذه المركبات فى اجزاء النبات المختلفة ووجدت كالاتى :

اولا : بالنسبة للمواد الفلافونية الحرة :

تبلغ نسبة المواد الفلافونية الحرة محسوبة على انها ابيجينين فى الازهار والاوراق ٠٩ر٢٤٥ر% على التوالى • تصل النسبة المئوية للابيجينين فى الازهـــار والاوراق ٠٤٨ر ١٢٥ر% بينما نسبـــة اللوتيولين ٠٣٤ر ٠٢٥ر% على التوالى •

ثانيا : بالنسبة للمواد الفلافونية على هيئة سكاكر :

تبلغ النسبة المئوية للمواد الفلافونية على هيئة سكاكر محسوبة على انها جوسيبين فى كل مـــن الازهار والاوراق ٤٣ر٢ ٤٤٥ر% على التوالى • تصل النسبة المئوية للجوسيبين فى الازهـــار والاوراق الى ٩٦ر ٥ ١٤ر% بينما النسبة المئوية لمركب الهربا ستين ـ ٨ ـ أ ـ جلوكوزيـــد الـــى ٠١ر١ ٠٣٥ر% على التوالى •

ومن هـــذا نجـــد الاتى :

١ـ النسبة المئوية الكلية للمواد الفلافونية على هيئة سكاكر اعلى بكثير من المواد الفلافونية الحرة

٢ـ يمثل جلوكوزيدى الجوسيبين والهربا ستين ـ ٨ ـ أ ـ جلوكوزيد حوالى ٨١ ٦٥ر٣٨% مـــن النسبة الكلية للمواد الفلافونية على هيئة سكاكر فى كل من الازهار والاوراق على التوالى •

٣ـ تحتوى الازهار على نسبة عالية من المواد الفلافونية على هيئة سكاكر (٤٣ر٢%) مما يشجـــع القول ان الازهار ممكن ان تستعمل كمصدر اقتصادى لتحضير مركبات فلافونية تحتوى عـــلى مجموعة الهيدروكسيل متصلة بذرة الكربون رقم ٨