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DETERMINATION OF IRIDOIDS, FLAVONOIDS AND MANNITOL IN JASMINS GROWING IN EGYPT

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ABSTRACT

A qualitative and quantitative study on the distribution of iridoids, flavonoids and mannitol in 5 of Jasmins has been carried out. The overground parts of the plants under investigation are free from flavonoidal aglycones. They contain flavonol glycosides but no flavones. Kaempferol glycosides are mainly restricted in J. azoricum, L. . On the other hand, quercetin-3-O-dirhamnoglucoside is present mainly in the 2 varieties of J. sambac, Ait. and absent in J. azoricum, L. . Iridoids (Jasminin and azoricin), flavonol glycosides (rutin and quercetrin) and mannitol are present in all samples in a significant concentrations and can be considered as finger prints for them.

INTRODUCTION

Jasminum is an important genus of Family Oleaceae. It includes about 200 species spread allover the tropical and subtropical regions around the world (1). In Egypt, 7 Jasmins are grown in different regions of the country (2-6):

J. mesnyi, Hance (J.primulinum, Hemsil); J.azoricum, L. ;
J. officinale Var. grandiflorum, Bailey ;
J. sambac, Ait. C.V. single flowers ;
J. sambac, Ait. C.V. double flowers ;
J. floribundum, R. Br. and J. fluminense Vell.

Determination of iridoids, flavonoids and mannitol in Jasmins growing in Egypt.

The **first** 5 plants are of common occurrence in Egypt and are cultivated as climbers and/or shrubs, for their fragrant flowers and for perfume-making. The last 2 species grow **widely** in Gebel Elba and surrounding mountains and are very rare (5).

In previous communications (7-10) on Jasminum plants growing in Egypt (J. mesnyi, Hance; J. sambac, Ait. and J. azoricum, L.), the following compounds were isolated and identified: flavonoids (quercetrin, rutin, quercetin-3-dirhamnoglucoside, kaempferol-3-rhamnoside and kaempferol-3-rhamnoglucoside), iridoids(Jasminin,sambacin and azoricin), triterpenoids (α-and β-amyrins),β-sitosterol, ceryl alcohol, fatty acids, mannitol and sucrose. The aim of the present investigation is to study the distribution of iridoids and flavonoids qualitatively and quantitatively as well as mannitol in the different organs of the cultivated Jasmins in Egypt to throw light on the chemotaxonomy of this genus.

RESULTS AND DISCUSSION

Chromatographic investigation of the methanolic extracts of the different organs of 5 Jasminum plants revealed the presence of at least 6 iridoidal spots {pinkish-violet to pinkish-brown with vanillin/H_2SO_4 reagent (1) and blue colour with Trim Hills reagent (12)}(Table 1) and 8 flavonoidal spots { brownish-yellow to yellow or yellowish-green with ammonia vapour in UV and orange to red with Shinoda's test (13)} (Table 2). Iridoids, Jasminin and azoricin as well as flavonol glycosides rutin and quercetrin were detected in all samples under investigation. Kaempferol-3-O-rhamnoside and kaempferol-3-O-rhamnoglucoside were restricted mainly in the flowers and leaves of J.azoricum, L. , while traces

of kaempferol-3-O-rhamnoside had been detected in the leaves of J. mesnyi, Hance (Table 2). The trisaccharide flavonol quercetin-3-O-dirhamnoglucoside was found mainly in the leaves of the 2 varieties of J.sambac Ait., while J. azoricum L. lack its presence.

Chromatospectrophotometric methods were carried out for quantitative estimation of total iridoids(calculated as Jasminin, g% w/w), total flavonoids(calculated as rutin) as well as for rutin and quercetrin in the different organs of plants under investigation (Table 3). The highest percentage of total iridoids was found in the flowers (3.70) of J.mesnyi,Hance followed by the flowers(3.10) of J.azoricum, L. . and the flowers (2.25) of J.officinale Var. grandiflorum, Bailey. Both the leaves and stems of J. mesnyi Hance contain a significant percentage of total iridoids (2.15 and 2.05, respectively). Concerning flavonoids, the highest percentage of total flavonoids was found in the flowers (2.40) and leaves (2.08) of J.mesnyi, Hance followed by the leaves (1.39) of J.officinale Var.grandiflorum, Bailey.

Rutin is the major flavonol glycoside present in Jasminum spp.(constitutes about 51-83% from the total flavonoids present) followed by quercetrin(21-30% from the total flavonoids). In other words rutin and quercetrin together consitute about 65-96% from the total flavonoids present.

The flowers of J.mesnyi Hance are yellow in colour, while those of the other spp. are white. The yellow colour may be attributed to the presence of a significantly high percentage of flavonoids in the flowers (2.40%).

The hexahydric alcohol mannitol was proved to be present in large amounts in all Jasminum plants under investigation

(Table 3). Its percentage (g% w/w) in the leaves ranges from 2.00-4.74, while in the stems from 0.87-2.43. The leaves of J.azoricum, L. , J.sambac, Ait. C.V. Single flowers and J. sambac, Ait. C.V. double flowers contain the highest percentage of mannitol (4.74, 4.56 and 4.18 respectively)

In conclusion, all Jasminum plants under investigation contain both iridoids and flavonol glycosides. They are free from flavonoid aglycones. Jasminin, azoricin , rutin, quercetrin and mannitol are present in significant amounts and can be considered as finger prints for all of them.

EXPERIMENTAL

Plant material:

Samples of flowers, leaves and stems of 5 Jasmins were collected from the growing plants at the Experimental Farm of Floriculture, Faculty of Agriculture , Assiut University, Assiut, Egypt. The samples were collected at the flowering stage of J.mesnyi, H.; J.azoricum, L. ; J. officinale Var. grandiflorum, Bailey; J. sambac, Ait. C.V. single flower and J. sambac, Ait. C.V. double flower during April and May 1982. The plants were identified by Dr. N.E. El-Keltawi, Associate Prof. of Floriculture, Hort. Dept., Fac. of Agric., Assiut Univ., Assiut, Egypt.

Solvent System:

System 1, $CHCl_3$-CH_3OH (82:18) and system 2, $CHCl_3$-CH_3OH (90:10) were used for TLC (silica gel G, E. Merck). System 3, n-butanol-acetic acid-water (4:1:2) and system 4, 15%aqueous acetic acid were used for PC (Whatman 3 MM).

Qualitative Study:

Ten grams of the air-dried powder of each of flowers, leaves and stems of the Jasminum plants under investigation were separately extracted with methanol. The extracts

were separately concentrated and chromatographed alongside with authentic reference iridoids (systems,1and 2) and reference flavonoids (systems 3 and 4). Results are shown in Tables 1 and 2.

Quantitative Study:

A) Estimation of total iridoids calculated as Jasminin:

Sixty grams of each of the air-dried powdered flowers, stems and leaves of each plant (P) were separately extracted with methanol in soxhlet apparatus. Each methanolic extract was concentrated and separately chromatographed on alumina column in order to remove flavonoids and polysaccharides(150 g alumina, E. Merck, 150 x 2 cm column). The column was washed with 2 liters chloroform (to remove lipids), then eluted with 3 liters of chloroform-methanol (1:1) at a slow rate (15 drops per minute). The chloroform-methanol eluate (containing iridoids) was concentrated to 25 ml (V). One ml (V_1) of the adjusted concentrated eluate was preparatively chromatographed on TLC (silica gel G,E. Merck, 20 x 20 cm) in system 1. Zones containing iridoids were scraped, eluted with methanol and the eluate was concentrated to 50 ml (W). The absorbance of the eluate was determined at λ 238 nm and the corresponding concentration was calculated (C)(mg%) from the standard curve of Jasminin. Percentage (g% w/w) of total iridoids (X) was deduced from the equation (14):

$$X = \frac{W.\ C.\ V.\ 100}{P.\ V_1 \cdot R.\ 1000}$$

R=percentage of recovery of Jasminin from TLC which was found to be 90.7%

B) Estimation of total flavonoids calculated as rutin (14):

Twenty grams of each of the air-dried powdered flowers, stems & leaves of each plant (P) were separately and successively extracted with pet.ether (b.r. 40-60°), chloroform & methanol in soxhlet apparatus. Each methanolic extract was concentrated to 25 ml (V); 0.1 ml of the extract (V_1)was chromatographed by PC (3mm) using system 3. Flavonoidal spots were cut,

eluted with methanol and the eluate was concentrated to 25 ml (w). The absorbance of the eluate was determined at λ359 nm and the corresponding concentration was calculated (C)(mg%) from the standard curve of rutin. Percentage (g% w/w) of total flavonoids (X) was deduced from the equation (14): $X = \frac{W.\ C.\ V.100}{P.V_1.R.1000}$ where

R= percentage of recovery of rutin from PC which was found to be 80.0%

C) Estimation of rutin and quercetrin (14):

The same procedure for total flavonoids was followed. Elution was done only for the spots corresponding to rutin and quercetrin (spots with R_f 0.50 and 0.65,respectively). The absorbance of the eluate was determined at λ 359 nm for rutin and 350 nm for quercetrin.

R for quercetrin was found to be 82.5%

D) Estimation of mannitol (15):

Fourty grams of each organ under investigation (air-dried and powdered) were successively extracted with pet.ether, chloroform and methanol in soxhlet apparatus. The methanolic extracts were concentrated to 100 ml and left in refrigerator overnight. The ppt. (mannitol) was filtered, washed several times with cold methanol, dried in hot oven at 40° for 2 hours and weighed.

The percentages (g% w/w) of total iridoids, total flavonoids, rutin, quercetrin and mannitol in the different organs of plants under investigation are illustrated in Table 3. The procedure for each assay was done in triplicate.

Statistical analysis

Data were statistically analysed according to Snedecor and Cochran(16) and the least significant difference at 5% probability (L. S. D. at 5%) were calculated (Table 3).

Table 1: Qualitative analyses of iridoids in the methanolic extracts of Jasmins

Species	Organ	hR_f* System 1: 88	83	69	60	54	28
		System 2: 82	80	61	55	37	20
Jasminum mesnyi, Hance	F	±	+	+	±	+	±
	L	±	+	+	+	+	+
	S	-	+	+	-	+	+
J.azoricum, L.	F	±	±	+	-	+	-
	L	±	+	+	-	+	+
	S	-	+	+	-	+	-
J.officinale Var.grandiflorum, Bailey	F	±	±	+	-	+	-
	L	-	+	+	-	+	±
	S	-	+	±	-	+	-
J.sambac,Ait.C.V. single flower	F	±	+	+	-	+	-
	L	±	+	+	±	+	+
	S	-	+	-	-	±	-
J.sambac,Ait.C.V. double flower	F	±	+	+	±	+	+
	L	±	+	+	±	+	+
	S	-	+	-	-	+	-

Legend:

F, flower; L, leaves; S, stems

-, absent; ±, traces; +, present.

*, TLC; silica gel G; systems 1, $CHCl_3$-CH_3OH (82:18 v/v), system 2, $CHCl_3$-CH_3OH (90:10 v/v); spots gave from pinkish-violet to pinkish-brown with vanillin H_2SO_4 reagent and blue colour with Trim Hill reagent

Identification: (corresponding Reference)	hR_f: System 1	System 2	
	88	82	unknown
	83	80	azoricum
	69	61	unknown
	60	55	unknown
	54	37	Jasminin
	28	20	sambacin

eluted with methanol and the eluate was concentrated to 25 ml (w). The absorbance of the eluate was determined at λ359 nm and the corresponding concentration was calculated (C)(mg%) from the standard curve of rutin. Percentage (g% w/w) of total flavonoids (X) was deduced from the equation (14): $X = \frac{W.\ C.\ V.100}{P.V_1.R.1000}$ where

R= percentage of recovery of rutin from PC which was found to be 80.0%

C) Estimation of rutin and quercetrin (14):

The same procedure for total flavonoids was followed. Elution was done only for the spots corresponding to rutin and quercetrin (spots with R_f 0.50 and 0.65,respectively). The absorbance of the eluate was determined at λ 359 nm for rutin and 350 nm for quercetrin.

R for quercetrin was found to be 82.5%

D) Estimation of mannitol (15):

Fourty grams of each organ under investigation (air-dried and powdered) were successively extracted with pet.ether, chloroform and methanol in soxhlet apparatus. The methanolic extracts were concentrated to 100 ml and left in refrigerator overnight. The ppt. (mannitol) was filtered, washed several times with cold methanol, dried in hot oven at 40° for 2 hours and weighed.

The percentages (g% w/w) of total iridoids, total flavonoids, rutin, quercetrin and mannitol in the different organs of plants under investigation are illustrated in Table 3. The procedure for each assay was done in triplicate.

Statistical analysis

Data were statistically analysed according to Snedecor and Cochran(16) and the least significant difference at 5% probability (L. S. D. at 5%) were calculated (Table 3).

Table 2: Qualitative analyses of flavonoids present in the methanolic extract of Jasmins

Species	Organ	hR_f*							
		System 3	79	65	60	54	50	44	40	33
		System 4	50	56	58	60	63	67	78	83
Jasminum mesnyi, Hance	F		−	+	−	−	+	+	−	±
	L		±	+	−	−	+	−	±	−
	S		−	±	−	−	+	±	−	−
J. azoricum, L.	F		+	+	+	−	+	−	−	−
	L		+	+	+	−	+	−	−	+
	S		−	±	−	−	+	−	−	−
J. officinale Var. grandiflorum, Bailey	F		−	+	−	−	+	±	−	±
	L		−	+	−	−	+	−	±	+
	S		−	+	−	−	+	±	−	−
J. sambac, Ait. C.V. single flower	F		−	+	−	±	+	−	−	−
	L		−	+	−	±	+	−	+	+
	S		−	±	−	±	+	−	−	−
J. sambac, Ait. C.V. double flower	F		−	+	−	+	+	−	−	−
	L		−	+	−	+	+	−	+	+
	S		−	±	−	+	+	−	−	−

Legend: F, flowers; L, leaves; S, stems.
−, absent; ±, traces; +, present.
*: PC; system 3, n-butanol-acetic acid-water (4:1:2 v/v); system 4, 15% aqueous acetic-acid; spots gave from brownish-yellow to yellow or yellowish-green with ammonia vapour in UV.

Identification: (corresponding reference)	hR_f* System 3	hR_f* System 4	
	79	50	Kaempferol-3-O-rhamnoside
	65	56	Quercetin-3-O-rhamnoside
	60	58	Kaempferol-3-O-rhamnoglucoside
	54	60	unknown
	50	63	Rutin
	44	67	Unknown
	40	78	Quercetin-3-O-dirhamnoglucoside
	33	83	Unknown

Table 3: Percentages of total iridoids, total flavonoids, rutin, quercetrin and mannitol in different organs of Jasmins (g% w/w)

Species	Organ*	Percentages				
		Total iridoids	total flavonoids	Rutin	Quercetrin	Mannitol
J. mesnyi, Hance	F	3.70	2.40	2.00	0.30	
	L	2.15	2.08	1.38	0.50	2.67
	S	2.05	0.26	0.20	0.05	2.43
J. azoricum, L.	F	3.10	1.06	0.55	0.30	
	L	1.02	0.72	0.37	0.10	4.74
	S	0.85	0.07	0.05	0.02	1.12
J. officinale Var. grandiflorum, Bailey	F	2.25	0.70	0.38	0.15	
	L	1.66	1.39	0.86	0.39	2.00
	S	1.28	0.29	0.23	0.05	1.89
J. sambac, Ait. C.V. single flowers	F	1.17	0.43	0.31	0.06	
	L	0.99	1.06	0.68	0.14	4.56
	S	0.81	0.06	0.04	0.02	2.43
J. sambac, Ait. C.V. double flowers	F	0.40	0.27	0.18	0.08	
	L	1.54	0.98	0.50	0.20	4.18
	S	0.35	0.10	0.06	0.03	0.87
L.S.D. 5%	F	0.17	0.15	0.24	0.07	
	L	0.15	0.14	0.17	0.07	0.58
	S	0.10	0.08	0.02	0.03	0.47

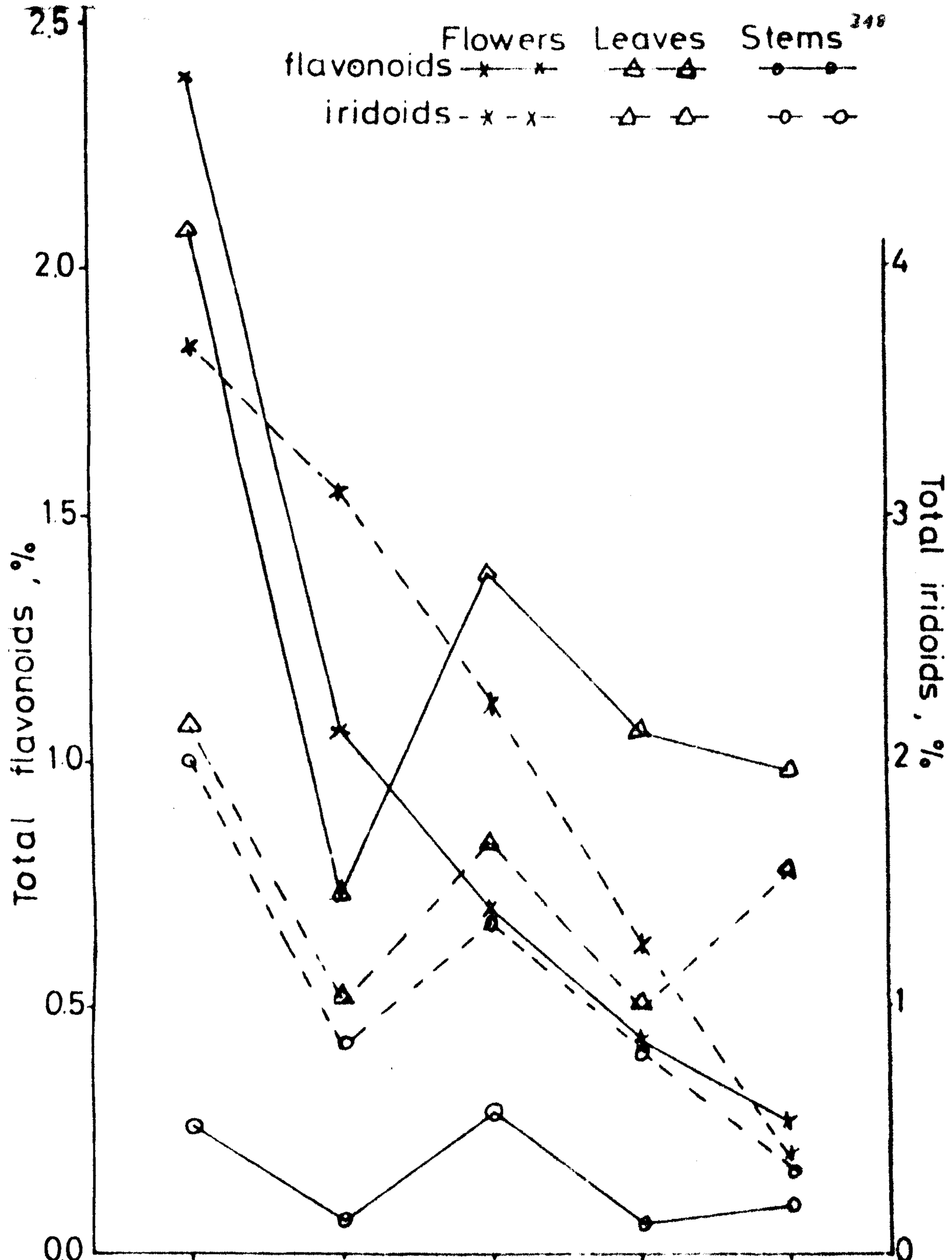
* : F, flowers ; L, leaves; S, stems

Table 4 : Distribution of iridoids and flavonoids in Jasmins flowers

Botanical taxonomy of Genus: Jasminum {according to Bailey 1963}	Total * iridoids	Total * flavonoids	* Rutin	* Quercetrin
A. Colour of flowers yellow:				
1. J. mesnyi	+++++++	++++++	++++++	++
AA. Colour of flowers white or pink				
B. Leaves 3-7 leaflets				
C. Calyx-teeth very short				
2. J. azoricum	+++++++	++++	+++	++
CC. Calyx-teeth linear				
3. J. officinale Var. grandiflorum	++++++	+++	++	+
BB. Leaves apparently simple				
C. Flowers white				
D. Calyx and branchlets pubescent .				
E. Teeth of calyx $\frac{1}{4}$ - $\frac{1}{2}$ inch long .				
F. Corolla double, lobes short & obtuse .				
G. Corolla not more than 2 circles				
4. J. sambac C.V. single flower	++++	++	++	±
GG. Corolla more than 2 circles				
5. J. sambac C.V. double flower	++	++	+	±

Designations :

±: Less than 0.1%
+: From 0.10-0.19%
++: From 0.20-0.49%
+++: From 0.50-0.99%
++++: From 1.00-1.49%
+++++: From 1.50-1.99%
++++++: From 2.00-2.49%
+++++++: From 2.50-3.99%



Fig(1): The relations between percentages of total flavonoids and iridoids in the organs of Jasmins and their botanical taxonomy of Bailey(1963).

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التقدير الكمى والكيفى للايرويدويدات والفلافونويدات والمانيثـــــــــول الموجوده فى نبـاتـات الـيـاسمين الـمنزرعه فى مصر

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أسفرت الدراسات الكيفيه لنبـاتـات الـيـاسمين الـمنزرعه فى مصر عما يـأتى:

١ - جميع نبـاتـات الـيـاسمين الـمنزرعه فى مصر تحتوى على ايرودي ـــــدات الجاسمينين والأزورسين وكذلك على جلوكوزيدات الروتين والكوارسيتريـــن بكميـات كبيـــــــره .

٢ - نبـاتـات الـيـاسمين الـمنزرعه فى مصر لاتحتوى على الفلافونيدات الحـــــره ولكنها تحتوى على جلوكوزيدات فلافونوليـــه .

٣ - نبـات الجاسمينيم ازوريكم لينيه هو النوع الوحيد من بين نبـاتـــــات الـيـاسمين الذى يحتوى على جلوكوزيدات الكامفيرول .

٤ - نبـات الفل بنوعيه المفرد والمجوز (جاسمنيم سامبك آيت مفرد الأزهـــار جاسمينيم سامبك آيت مجوز الأزهـــار) يحتوى على كوارسيتين -٣ أ - داى رامنوجلوكوزيـــــد .

وقـــد تم أيضـــا تعيين نسب المواد الآتيه كميـــا فى الأجـــــزاء المختلفه (الأزهـــار - الأوراق - الســـيقان) لنبـاتـات الـيـاسمين تحـــــت البحـــــث :

أ - النســبه المئويه الكلية للايروديدات محســوبه على أنها جاسمينيـــــن

ب - النسبه المئويه الكلية للمواد الفلافونوليـــه على هيئـة ســـــكاكر محســـوبه على انهاروتيـــــن .

ج - النسبه المئويه لكل من الروتين والكوارسيتريـــــن .

د - النســـــبه المئويـــه للمانيتـــــــول .

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