

## INVESTIGATION OF THE FLAVONOID CONTENT OF LEAVES, STEMS AND FLOWERS OF EGYPTIAN POLYGONUM SALCIFOLIUM L.

S.I. Balbaa and M.S. Afifi

Pharmacognosy Dept., Faculty of Pharmacy, Mansoura and  
Cairo Universities

### ABSTRACT

The investigation of the flavonoid content of the leaves, stems and flowers of *Polygonum salcifolium* L. (Family : Polygonaceae) was carried out adopting both chromatographic and spectroscopic techniques. This study revealed the presence of kaempferol-7-O-rhamnoglucoside, quercetin-3-O-galactoside (hyperoside), orobol-7-O-glucoside and isorhamnetin-3-O-galactoside in all investigated parts.

### INTRODUCTION

Several authors isolated different flavonoid constituents from the leaves of plants belonging to *Polygonum* species. These are isoquercetin and hyperoside from *P. persicaria* L.<sup>1</sup>; quercetin, avicularin and quercitrin from *P. coriarium* L.<sup>2</sup>; the methanolic extract containing quercetin-3-O-galactoside and hydro-alcoholic extract quercetin-3-arabinogalactoside<sup>3</sup>; quercetin, hyperoside and quercetin-3-O-glucoside from *P. sachalinense* L.<sup>4</sup>; hyperoside, avicularin, quercetin, quercitrin and kaempferol from *P. amphibium* L. and *P. scabium* L. with luteolin-7-O-glucoside in the former and rutin in the latter<sup>5</sup>; quercetin, isorhamnetin, kaempferol and the glucosides of these aglycones from *Polygonum acre* L.<sup>6</sup> and quercetin, avicularin, quercitrin, hyperoside and two unknown quercetin flavonoids from *Polygonum coriarium* L.<sup>7</sup>

From the above, it is clear that nothing was reported throughout the current literature dealing with the flavonoid content of the different organs of Polygonum salcifolium L. So, the authors found it of interest to carry out such study.

## EXPERIMENTAL

### Plant Material :

Wildly-grown plants of Polygonum salcifolium L. were collected at flowering stage from shores of River Nile at mansoura (at period from May to July, 1973). The plant was identified by Prof. Dr. M. Zahran (Prof. of Systematic Botany, Faculty of Science, Mansoura University).

The leaves, stems and flowers were separated, air-dried, powdered, sieved (sieve No. 10) and kept in well-fit glass bottles till use.

### Methods :

#### TLC :

The concentrated methanolic extracts of the defatted powdered leaves, stems and flowers (100 g, each) of Polygonum salcifolium L. were separately subjected to TLC using cellulose plates, alongside with 0.1 % methanolic solutions of authentic flavonoid compounds viz., rutin, hyperoside, narcissin, quercitrin, isorhamnetin and quercetin. Development was carried out by n-Butanol-Acetic acid-Water (4:1:5)<sup>8</sup> (BAW), after development, the chromatoplates were air-dried and examined under U.V. light at wave length of 366 nm before and after spraying with 0.1 M solution of aluminium chloride (alcoholic)<sup>9</sup>.  $R_f$  and fluorescence of each flavonoid compound are recorded in Table 1.

From Table 1, one may conclude that : the leaves, stems and flowers of Polygonum salcifolium L. may contain hyperoside and three unknown flavonoid compounds.

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Spectral analysis of the flavonoid compounds<sup>10</sup> :

In order to identify the unknown flavonoid compounds and to confirm our results, spectral analysis was carried out.

Separation of flavonoid compounds from their methanolic extract, previously prepared, were carried out by preparative TLC using BAW (as solvent system) and cellulose plates. The U.V. spectrum of each compound (0.1 m/10 ml spectroscopic methanol) was carried out. The shifts of the maximum absorption band of each compound after the addition of the following reagents were recorded and listed in Table 11. These reagents are : sodium methoxide (2.5 % in dry spectroscopic methanol), aluminium chloride (5 % in dry spectroscopic methanol), AlCl<sub>3</sub>/HCl (10:1), sodium acetate (saturated methanolic solution) and sodium acetate/boric acid (1:1) (saturated methanolic solutions).

Identification of sugar parts and aglycones of flavonoid compounds :

About 3 ml of the methanolic solution of each flavonoid compound prepared by preparative TLC was separately hydrolysed by refluxing with 3 ml 6 % HCl (aqueous solution) for 45 minutes. The aglycone, in each case, was extracted with ether (3 x 50 ml each). The combined ethereal extracts were washed several times with distilled water (2 x 20 ml) and dehydrated over anhydrous sodium sulphate. The ether was removed by distillation and the aglycone obtained in each case, was dissolved in methanol and subjected to TLC, using the same adsorbent and solvent mentioned before, alongside with authentic quercetin and isorhamnetin. The results obtained are recorded in Table 11.

The  $\lambda$  max of U.V. spectra of methanolic solution of each aglycone was determined. The shifts in absorbance ( $\lambda$  max. in presence of different reagents (previously mentioned) were determined and the results are recorded in Table 11.

The mother liquor of each flavonoid compound after the removal of aglycone was concentrated to syrupy consistency under reduced pressure, dissolved in pyridine and filtered. The pyridine extract was subjected to PC adopting the ascending technique and solvent system, Ethyl acetate-Pyridine-Water (12:5:4)<sup>10</sup> together with authentic sugars viz., D-glucose, D-galactose, D-xylose, L-arabinose, and L-rhamnose. After development, the chromatograms were air-dried and sprayed with aniline phthalate reagent<sup>11</sup> and heated at 105°C for 5 minutes. Results obtained are recorded in Table 111.

## RESULTS AND DISCUSSION

From Table 1, one may conclude that the leaves, stems and flowers of Polygonum salcifolium L., may contain four flavonoid compounds as only four spots were obtained by the use of TLC.

By comparing the results obtained from Tables 11 and 111 and the published data by Mabry et al<sup>10</sup>, the following could be concluded :

- 1- Unknown No. 1, may be kaempferol-7-rhamnoglucoside (m.p. 170°C), as shown from spectral data, chromatographic investigation and its hydrolytic products (aglycone and sugar) :
  - a- It is a flavonol with free 3-OH group (band 1 at 364 nm).
  - b- It contains 4-OH group (a bathochromic shift of band 1 equal to 61 nm in sodium methoxide spectrum without decrease in intensity of the peak).
  - c- It does not contain 0-dihydroxy groups (on change of Al Cl<sub>3</sub> or AlCl<sub>3</sub>/HCl spectrum than that of Al Cl<sub>3</sub> and also of sodium acetate/ boric acid spectrum than that of methanol).
  - d- It contains free OH-group in positions 3 and 5 (an increase in shift of Al Cl<sub>3</sub> spectrum (band 11) than in case of methanol and did not change after addition of HCl).

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- e- It contains substituted 7-OH group (a shoulder in the long side of band 1 in sod. acetate spectrum).
  - f- The sugar part was found to be rhamnose ( $R_f$  0.76) and glucose ( $R_f$  0.45).
  - g- The chromatographic properties of the aglycone ( $R_f$  and the colour of spots, Table 11, in U.V. light) and its m.p. (276-278°C complied with that of kaempferol).
- 2- The unknown No. 11, may be quercetin-3-galactoside (hyperoside) from the chromatographic characters ( $R_f$  in different solvent systems), its m.p. 230-232°C, not depressed when mixed with authentic hyperoside and from spectral data as follows :-
- a- It is a flavonol glycoside (absorption band at 362 nm) with 3-substituted OH group (there is no decrease in the intensity of the peak with sod. methoxide).
  - b- It contains ortho-dihydroxy groups in 3 and 4 positions (there is shifts in both band 1 and 11 of  $AlCl_3$  spectrum than that of the methanol spectrum which is decreased on addition of HCl and also 12 nm bathochromic shift of band 1 in sod. acetate/boric acid spectrum less than that of sodium acetate spectrum).
  - c- It contains free OH in position 5 (an increase in shift of  $AlCl_3$  spectrum than in case of methanol and did not change after addition of HCl).
  - d- It contains free 7-OH group (a 14 nm bathochromic shift of band 11 of sodium acetate spectrum than that of methanolic one).
  - e- The chromatographic characters of the aglycone ( $R_f$  and colour in U.V. light before and after spraying with  $AlCl_3$ , m.p. 310-312°C), comply with those of authentic quercetin.
  - f- The sugar part was found to be galactose ( $R_f$  39) and its osazone melted at 204°C.

- 3- The unknown No. 111, may be orobol-7-glucoside (m.p. 218-220°C), from its chromatographic characters and spectral data as follows:
- a- It is isoflavone (band 11 appears as shoulder of band 1 which appears at 270 nm).
  - b- It contains free 3- and 4-ortho-dihydroxyl groups. This was confirmed from  $AlCl_3$  spectrum, as 4 nm bathochromic shift occurred on addition of HCl and also (3 nm shift) from sodium acetate spectrum on addition of boric acid.
  - c- The free 5-OH group was confirmed by the presence of bathochromic shift (7 nm) on addition of  $AlCl_3$ .
  - d- The presence of 7-substituted OH was confirmed by showing no shift on addition of sodium acetate.
  - e- The sugar part was found to be glucose only ( $R_f$  0.45).
  - f- M.p. of the aglycone was found to be 280-282°C.
- 4- The unknown No. IV was found to be isorhamnetin-3-galactoside (m.p. cannot be determined as no crystalline material could be obtained) from its spectral data as follow :
- a- On the same basis mentioned before, it is a flavonol with substituted OH group at 3 position and free OH group in 4,5 and 7 positions as well as substituted-OH group at 3 position (as no shift in band 1 on addition of HC to  $AlCl_3$  spectrum). In addition, there is a slight shift (17 nm) in methanolic spectrum on addition of sodium methoxide reagent.
  - b- The sugar part was found to be galactose as confirmed by  $R_f$ , in different solvent systems.
  - c- TLC investigation of the aglycone showed that it is similar to authentic isorhamnetin.

N.B. Authentic samples of kaempferol, quercetin, orobol, isorhamnetin and hyperoside (used in this work) were obtained through the courtesy of BDH as well as from Pharmacognosy Dept., Faculty of Pharmacy, Assiut University.

Table 1

Results of TLC investigation of flavonoid content of leaves  
stems and flowers of Egyptian *Polygonum salicifolium* L.

Flavonoid compound No.	R <sub>F</sub> TLC	Fluorescence in U.V. light		<i>Polygonum salicifolium</i> L.		
		before Al Cl <sub>3</sub>	after Al Cl <sub>3</sub>	Leaves	Stems	Flowers
Unknown I	0.35	dark purple	yellow	+	+	+
Hyperoside	0.63	brown	yellow	+	+	+
Unknown II	0.70	"	"	+	+	+
Unknown III	0.80	dark purple	"	+	+	+

Table 2

Results of U.V. spectra of the isolated flavonoid compounds in different reagents

Flavonoid compound / reagent	λ <sub>max</sub> (nm) in					
	Methanol	NaOCH <sub>3</sub>	Al Cl <sub>3</sub>	Al Cl <sub>3</sub> /HCl	NaOAc	NaOAc/H <sub>3</sub> BO <sub>3</sub>
<u>Unknown I</u>						
Glycoside band I	364	340, sh425	343, 430	360, 428	390, 420	364
band II	252, 266	247, 269	266, 298	266, 296	266	258 sh
Aglycone band I	300, 360 sh	403	300, 350sh	300	382	362
band II	260	272	268	272	270	262
<u>Unknown II</u>						
Glycoside band I	362, 410	332, 409	306sh, 335sh 440	364 sh 410	320, 370	380
band II	255, 270sh	270	275	270	272	260
Aglycone band I	370	321	460	354	390	388
band II	255	248	272, 301sh	265	274	261
<u>Unknown III</u>						
Glycoside band I	340 Sh	330	370	375	332	320
band II	260	290	268	274	275	260
Aglycone band I	336	330	365	375	320	290
band II	260	268	270	274	270	266
<u>Unknown IV</u>						
Glycoside band I	358	334	450	400	374	370
band II	270 sh	280	270	268	274, 310	258
Aglycone band I	370	328sh, 435	304sh, 361sh, 432	357, 428	320, 392 sh	306sh, 377 sh
band II	253, 267sh	240sh, 272	264	242sh, 262	260sh, 274	255, 270sh

Table 3

Results of acid hydrolysis of flavonoid compounds

Unknown flavonoid compound (No.)	name	Aglycone R <sub>F</sub> TLC (BAW)	M.P.	Colour in U.V.	Sugars		
					rhamnose (R <sub>F</sub> 0.76)	glucose (R <sub>F</sub> 0.45)	galactose (R <sub>F</sub> 0.39)
I	kaempferol	0.68	276-278°C	Yellow	+	+	-
II	quercetin	0.90	310-312°C	"	-	-	+
III	orobol	0.81	280-282°C	"	-	+	-
IV	isorhamnetin	0.72	---	"	-	-	+

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دراسة المواد الفلافونية لاوراق وسيقان وأزهار  
نبات البوليجونم سالسيفوليم ( لينيه ) الذى ينمو فى مصر

شفيق ابراهيم بلبوع و محمد صفوت عطا عفيفى

قسم العقاقير - كلية الصيدلة - جامعة القاهرة والمنصورة

أسفرت دراسة المواد الفلافونوليه لأوراق ، سيقان وأزهار نبات البوليجونم  
سالسيفوليم ( لينيه ) التابع للعائلة البقضية ( Polygonaceae ) عن وجود  
اربعة مركبات وهى / كامبفيرول - ١٧ منوحلو كوزيد ، كوارستين-٣ - حالكوتوزايد  
( هيبروزايد ) ، اوروبول-٧ - جلو كوزايد والاي زورامنتين-٣ - حالكوتوزايد .

وقد تم التعرف على هذه المركبات بواسطة كروماتوجرافيا الورق والطبقة  
الرفيقة والقياس الضوئى باستعمال الاشعة فوق بنفسجية وكذلك تعيين نقطة الانصهار  
لها كما تم التحليل الحمضى لهذه المركبات وفحص النواتج ( السكاكر والجليكونات ) بنفس  
الطرق السابق ذكرها .