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The flavonoids of *Balanites aegyptiaca* (*Balanitaceae*) from Egypt

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Abstract: Six flavonoid glycosides: quercetin 3-glucoside, quercetin-3-rutinoside; 3-glucoside, 3-rutinoside, 3-7-diglucoside and 3-rhamnogalactoside of isorhamnetin were extracted and identified from the leaves and branches of Egyptian material of *Balanites aegyptiaca.* Only isorhamnetin: 3-rutinoside and 3-rhamnogalactoside were recorded from the fruits of the same plant. – Phytochemical aspects of *Balanites aegyptiaca* and some genera of *Zygophylaceae* s. l. viz. *Nitraria, Fagonia, Zygophyllum, Seetzenia* and *Tribulus* support its affinities with that family.

DELILE (1813) founded the genus *Balanites* to include *B. aegyptiaca* (L.) DEL. which is a thorny shrub or tree with alternate compound (rarely simple) coriaceous leaves, each composed of 2 leaflets. The flowers, disposed in axillary corymbs, are small, green and pentamerous. The fruit is an edible drupe of about plum-size.

The genus was variously treated, first as a member of *Simaroubaceae*, then by ENGLER (1931) as the monogeneric taxon of his *Balanitoideae*, one of the 7 subfamilies of *Zygophyllaceae* s. l., and currently as a monogeneric family *Balanitaceae* ENDL.

According to SANDS (1983), a study of the genus is currently being completed and a full revision of the genus will be published shortly. Nine species are known at present of which seven occur in Africa. SANDS (pers. comm.) recognizes 5 varieties of *B. aegyptiaca* which vary in respect to the colour of the leaflets, pubescence, length of the spines, the number of flowers per corymb, as well as the size and shape of the drupe.

Most of the varieties have a limited geographical range. The typical variety grows in the southern deserts of Egypt and is perhaps the most wide-spread taxon, ranging from the Sudan Republic, Ethiopia, and Egypt through the Arabian Peninsula to the Jordan valley.

This account deals with the flavonoid chemistry of the Egyptian variety of *Balanites aegyptiaca.* It is hoped to continue this study in the future to include the other varieties of this species. The chemistry of our taxon will be compared and briefly discussed with other investigated taxa of the *Zygophyllaceae* s. l.

Table 1. Distribution of flavonoid glycosides in *Balanites aegyptiaca*. + + + major, + + strong, + present

Compound	Branches and leaves	Fruit
Quercetin-3-glucoside	+	−
Quercetin-3-rutinoside	+	−
Isorhamnetin-3-glucoside	+ +	−
Isorhamnetin-3-rutinoside	+ + +	+ +
Isorhamnetin-3,7-diglucoside	+	−
Isorhamnetin-3-rhamnogalactoside	+ +	+

Table 2. Distribution of flavonoid glycosides in investigated

	Quercetin							
	3-galactoside	3-rhamnogalactoside	3-rutinoside	3-dirhamnoglucoside	3-gentiobioside	3,7-diglucoside	3-glucoside	3-gentiobioside-7-glucoside
Balanites aegyptiaca (L.) DEL.			+				+	
Nitraria retusa (FORSSK.) ASCH.								
Fagonia bruguieri DC.	+	+	+					
F. arabica L.								
F. mollis DEL.								
Zygophyllum simplex L.			+			+	+	
Z. decumbens DEL.			+					
Z. coccineum L.			+					
Z. album L. fil.			+					
Z. aegyptium A. HOSNY			+					
Seetzenia lanata (WILLD.) BULLOCK			+	+				
Tribulus pentandrus FORSSK.			+		(+)		+	+
T. terrestris L.			+		(+)			+

Materials and methods

Leaves, branches and fruits of *Balanites aegyptiaca* were collected from the Arabian desert: Idfu-Mersa Alam road, 44 km from Mersa Alam; January 1984. Voucher specimens are deposited in the Herbarium, Faculty of Science, Cairo University (CAI).

Standard procedures for the separation and identification of flavonoids were followed (HARBORNE 1967 and MABRY & al. 1970). The extract was subjected to column chromatography on polyamide (MN polyamide SC 6 Macherey Nagel). Acid hydrolysis was done with 2 N HCl, while mild acid hydrolysis was carried out with 0.1 N HCl. Enzymatic hydrolysis was done at pH 5 (acetate buffer) at 37 °C (HARBORNE 1965). For H_2O_2 oxidation, the method described by CHANDLER & HARPER (1961) was applied. Chromatography was carried out on Whatman No. 1 paper using the following solvent systems BAW (n BuOH – HOAc – H_2O, 4 : 1 : 5), H_2O, 15% HOAc, 80% PhOH. Sugars were identified

taxa of *Zygophyllaceae* s.l. (+)major component

Isorhamnetin							
3-galactoside	3-glucoside	3-rutinoside	3-rhamnogalactoside	3-galactorhamnogalactoside	3-xylorhamnogalactoside	3,7-diglucoside	7-glucoside
	+	(+)	+			+	
+	+	+	(+)	+	+	+	
		+					
	+	+					
		(+)					
	+	(+)				+	+
	+	(+)					
		(+)					
		(+)					
		(+)					

by cellulose TLC against standard markers in pyridine EtOAc : HOAc : H_2O, 36 : 36 : 7 : 21) and detected by spraying with aniline phthalate. All fractions were purified by standard procedures (MABRY & al. 1970 and MARKHAM & MABRY 1975) over Sephadex L-H-20 (Pharmacia) using MeOH prior to spectral analysis. Chemical structures of compounds were elucidated using UV, 'H NMR and chromatographic techniques in conjunction with comparison of appropriate reference compound (when available, using the standard procedure described by MABRY & al. 1970, MARKHAM & MABRY 1975). UV spectra were recorded in Beckman 34 ultraviolet spectrophotometer, 'H NMR spectra of trimethylsilyl ether were recorded on Varian EM 390,90 MHz spectrometer in CCl_4 with TMS as internal standard.

Results and discussion

Table 1 gives the distribution of the flavonoid glycosides in *Balanites aegyptiaca*. Six flavonoid glycosides are identified among which isorhamnetin 3,7-diglucoside and isorhamnetin 3-rhamnogalactoside are new records (cf. SALEH & EL HADIDI 1977). The identity of the isolated flavonoid glycosides were determined by UV, 'H NMR, acid hydrolysis, H_2O_2 oxidation, enzymatic hydrolysis and finally by comparison of its data with authentic samples and also with those reported in the literature (MABRY & al. 1970, SALEH & EL HADIDI 1977, RÖSLER & al. 1966).

Isorhamnetin 3-rhamnogalactoside gave isorhamnetin, galactose and rhamnose on acid hydrolysis, while mild acid hydrolysis gave rise to isorhamnetin 3-galactoside and 3-rhamnogalactoside. The aglycone was identical as previously reported (MABRY & al. 1970). R_f values: BAW = 47, H_2O = 33, HOAc = 52 and PhOH = 90 · UV λ_{max}, nm MeOH: 350, 265 (sh), 255; NaOMe: 405, 325, 267; $AlCl_3$: 400, 367, 300, 265; $AlCl_3$ – HCl: 400, 357, 300, 265; NaOAc: 375, 315, 272 and NaOAc – H_3BO_3: 350, 310 (sh) 265 (sh), 255. NMR of trimethylsilyl ether of isorhamnetin 3-rhamnogalactoside gave a methoxyl signal at 3.9 ppm, flavonoid proton signals at 7.8, 7.42, 6.9, 6.45, and 6.15 ppm for H-2', H-6', H-5', H-8, and H-6, respectively. Signals for anomeric protons of the sugars were observed at 5.7 and 4.35 for H_I'' and H_{II}'' (C-1 rhamnosyl) respectively and for non anomeric at 3.3 – 3.65 ppm.

Isorhamnetin 3,7 diglucoside gave isorhamnetin and glucose on acid hydrolysis, mild acid hydrolysis gave rise to isorhamnetin, and isorhamnetin 7-glucoside, while H_2O_2 oxidation gave glucose and enzymic hydrolysis (B-glucosidase) gave isorhamnetin 3-glucoside. The aglycone was identical as previously reported (MABRY & al. 1970) R_f values: BAW = 25, H_2O = 34 HOAc = 53 and PhOH = 75 · UV λ_{max}, nm MeOH: 350, 270, 254; NaOMe: 420, 277; $AlCl_3$: 400, 350, 300 (sh), 268; $AlCl_3$-HCl: 400, 350, 300 (sh) 268; NaOAc: 350, 267, 254 and NaOAc – H_3BO_3: 350, 267 (sh), 254. NMR of trimethylsilyl ether of isorhamnetin 3,7 diglucoside gave a methoxyl signal at 3.92 ppm; flavonoid proton signals at 7.8, 7.41, 6.85, 6.45, and 6.14 ppm for H-2', H-6', H-5', H-8, and H-6, respectively. Signals for the anomeric protons of the two glucosyls were observed at 5.85 and 4.9 ppm. These signals are in agreement with sugars attached at C-3 and C-7 (MABRY & al. 1970).

All of the six flavonoid glycosides were identified from extracts of the leaves and branches, while isorhamnetin 3-rutinoside and isorhamnetin 3-rhamnogalactoside are recorded from the fruit extract. The accumulation of these flavonoid glycosides in the fruits may be related to the process of fruit development.

In addition, considerable amounts of free quercetin were identified in the extracts of the vegetative parts of the plant.

The chemotaxonomy of *Balanites aegyptiaca* may be discussed in relation to the other investigated groups of *Zygophyllaceae* s. l. Five aglycone groups are already identified from the taxa investigated. Of these quercetin and isorhamnetin are of major importance while kaempferol, herbacetin and tricin are of minor importance and are restricted to certain taxa (SALAH & EL HADIDI 1977, SALEH & al. 1982, EL NEGOUMY & al. 1986, AL WAKEEL & al., 1987, MAKSOUD & EL HADIDI 1987.

In this account, the distribution of quercetin and isorhamnetin glycosides will be discussed. Both groups are identified from the extracts of *Balanites aegyptiaca.*

Table 2 summarizes the available information about the distribution of quercetin and isorhamnetin glycosides among the investigated taxa of *Zygophyllaceae* s. l. It may be noticed that the 3-rutinoside of both quercetin and isorhamnetin are the major flavonoid glycosides among the investigated taxa. Quercetin 3-rutinoside was not detected in *Nitraria* and some species of *Fagonia* and *Tribulus.* On the other hand, isorhamnetin 3-rutinoside was not detected in *Seetzenia* and *Tribulus.* In addition, quercetin 3-glucoside is identified in small amounts in *Balanites, Zygophyllum simplex* and *Tribulus pentandrus.* Also isorhamnetin 3,7 diglucoside is recorded in small amounts in *Balanites, Nitraria* and *Z. simplex.*

This flavonoid pattern shows affinities in the chemistry between *Balanites* and certain groups of *Zygophyllaceae* s. l., viz. *Nitraria, Zygophyllum* and *Tribulus.* Such affinities are also supported by the restricted occurrence of steroidal sapogenins in *Balanites* and members of the *Zygophyllaceae* s. l., viz. *Kallstroemia* and *Tribulus* (HEGNAUER 1983). Still, forthcoming evidence on leaf vascularization, floral morphology and pollen structure favours a very isolated positionof *Balanites.*

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