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ANTHRAQUINONES AND FLAVONOIDS FROM *RUMEX TINGITANUS* GROWING IN LIBYA

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ABSTRACT

A phytochemical invesitgation of the ether soluble portion of alcoholic extract of *Rumex tingiranus* L. has resulted in the isolation of five anthraquinone pigments; chrysophanol, physcion, emodin , aloe - emodin and chrysophanein. In addition, three flavonoids namely , apigenin, luteolin and catechin were also isolated from the extract. Identification of these compounds were performed through their physical and spectral data as well as comparison with reference samples . This is the first report for the isolation of all these compounds from this plant . The anti-bacterial activity of the compounds isolated in sufficient amounts was carried out.

INTRODUCTION

Rumex tingitanus L. (perennial herb) is among the species of genus *Rumex* which belongs to the family Polygonaceae (1-3). Genus *Rumex* is represented in Libya by ten species (1) . This family is characterized by its anthraquinones contents which are also considered as a chemotaxonomic marker of this genus (4-7) . Flavonoids also , have been detected in many *Rumex* species (8-11). Some species of *Rumex* are used as laxatives and purgative for their anthraquinones contents (12-14) while other species were reported to have antiumor activities (15,16).

On reviewing the appropriate literature, it was apparent that there are no previous scientific reports of *R. tingitanus* L. growing in Libya , therefore it was considered to be of interest to carry out the present study on this plant.

EXPERIMENTAL

Plant material :

The aerial parts of *R. tingitanus* L. were collected at the flowering stage from the wild plants growing at the Mediterranean Costal strip near Benghazi, Libya during the period from January to February , 1997 . The plant material was identified by **Prof. Dr. A. El-Gadi,** Department of Botany , Faculty of Science, Al-Faateh University, Tripoli, Libya.

General experimental procedures :

CC silica gel Merck, 70-230 mesh TLC silica gel 60 F 254 precoated plates (E. Merk, Germany) . Agar (Mikrobiologic , nutrient agar . 20 g/L, pH = 7.0 + 0.2 . Germany). UV spectra : UV -Visible spectrophotometer (UV- 1601 PC. Schimadzu , Japan) . IR spectra: Nicolet Mx-1 FT-IR Spectrophotometer. USA. Authentic samples from previously isolated and identified compounds in Department of Pharmacognosy, College of Pharmacy, Mansoura University. ^{1}H-NMR spectra (Varian VXR 300 Spectrometer, 300 MHz) and JNM -LA Series, FT-NMR 400 MHz, JEOL, CO, Japan.

Extraction and isolation :

The aerial parts of *R. tingitanus* L. (0.5 kg) were extracted with cold methanol in a syphone and the extract (6L) was evaporated to dryness in a rotary evaporator at 40°C . The residue (25 gm) was suspended in distilled H_2O . Successive extraction was done with petroleum ether , ether and ethyl acetate . The ether extract was evaporated to dryness (5g) and applied as a band to the top of a silica gel column (2 x 75 cm, 80 gm) and gradient elution was performed using pet. ether- EtOAc (9.5 : 0.5), (9:1) , (8.5 : 1.5) , (8 : 2) , (7.5 : 2.5) and (7:3) . Fractions, 50 ml each, were collected. Each fraction was examined by TLC , similar fractions were pooled together and concentrated to give fractions from A to H . Fraction A contained pure compound 1 (7 mg). Fraction B contained pure compound 2 (5 mg). Fractions C and D were re-chromatographed by another silica gel G columns (1 x 50 cm, 30 gm) using the same solvent system to obtain pure compound 3 (8 mg) and compound 4 (6 mg) respectively . Fraction E was concentrated and applied to the top of a small silica gel column and elution was performed using $CHCl_3$-MeOH gradient elution 1%, 2%, 3% to obtain pure compound 5 (4mg) . Fraction F was purified on silica gel column using gradient elutions of $CHCl_3$ - MeOH to obtain pure compounds 6 (12 mg) and 7 (6 mg) . Fraction G was purified as fraction F to obtaine pure compound 8 (2 mg).

Characterization of the isolated compounds:

Compound **1** (chrysophanol, 0.0014% w/w) occurred as yellow plates (chloroform), m.p. 195-197°C R_f 0.91 [pet. ether - EtOAc (8 : 2)] . IR ν max (KBr) 3500 (OH), 1682 (CO), 1630 (hydrogen bonded CO), 1610 and 1570 (aromatic system), 1480, 1460, 1380, 1210, 1090 and 900 cm^{-1} . UV λ max (MeOH) : 228, 245, 273 and 434 nm. The ^{1}H-NMR ($CDCl_3$, 400 MHz) spectral data (Table 1).

Compound **2** (physcion, 0.001% w/w) occurred as orange plates (methanol) m.p. 204-206°C . R_f 0.84 [pet. ether - EtOAc (8 : 2)] IR ν max (KBr): 3500 (OH), 1680 (CO), 1630 (hydrogen-bonded CO), 1615 and 1570 (aromatic system), 1480,1325, 1230, 1160 and 895

cm^{-1}; UV λ_{max} (MeOH) : 224 , 255 , 290 and 430 nm. The 1H-NMR ($CDCl_3$, 400 MHz) spectral data (Table 1).

Compound **3** (emodin, 0.0016% w/w) occurred as reddish-brown prisms (methanol) , m.p. 251- 253°C . R_f 0.46 [pet. ether- EtOAc) (7.3)] . IR ν_{max} (KBr): 3490 (OH), 2940 (CH), 1680 (CO), 1635 (hydrogen bonded CO), 1605 and 1570 (aromatic system), 1480, 1340, 1300, 1230, 1170 and 910 cm^{-1}. UV λ_{max} (MeOH) : 224, 292 and 442 nm. The ^{1}H-NMR ($CDCl_3$, 400 MHz) spectral data (Table 1).

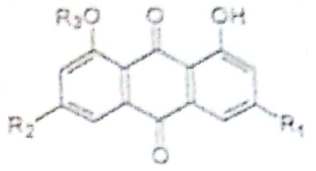
Compound **4** (aloe - emodin, 0.0016% w/w) occured as brown crystals (methanol), m.p. 218-220°C; R_f 0.30 [pet. ether-EtOAc (7: 3)]. IR ν_{max} (KBr): 3400 (OH), 1680 (CO), 1630 (hydrogen bonded CO), 1575, 1460, 1395, 1280, 1090, 1060, 1040 and 870 cm^{-1}. UV λ_{max} (MeOH) : 214, 226, 256, 276 and 432 nm. The ^{1}H-NMR ($CDCl_2$, 400 MHz) spectral data (Table 1).

Compound **5** (apigenin, 0.0008% w/w) occurred as a yellow powder (methanol). It gave yellow colour after spraying with NaOH and vanilin - H_2SO_4 spray reagents R_f 0.34 [CH_2Cl_2-MeOH (9.5:0.5)]. UV λ_{max} (MeOH): 336 , 298 and 268; + $NaOCH_3$: 392, 322 and 276; + $AlCl_3$: 380 , 342 , 301 and 276; + $AlCl_3$ /HCl : 382, 342, 298 and 276;+ NaOAc : 375, 302, 275 and+ NaOAc / H_3BO_3 : 334, 304 and 269 nm ^{1}H-NMR (DMSO, 300 MHz) spectral data (Table 2).

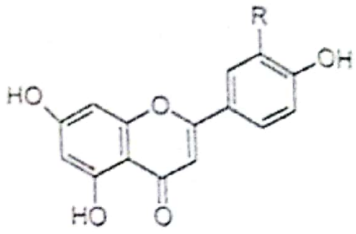
Compound **6** (luteolin 0.0024% w/w) occurred as a yellow powder (methanol), R_f 0.37 [CH_2Cl_2-MeOH-H_2O (80 : 20: 2)]. UV λ_{max} (MeOH) : 349 , 293 and 268; + $NaOCH_3$:402 and 268 : + $AlCl_3$ 415 , 332 and 273; $AlCl_3$/Hcl: 383, 356, 295 and 275 ;+ NaOAc: 368, 300 and 270 and +NaOAc/H_3BO_3:370 and 269 nm. ^{1}H-NMR (DMSO, 400 MHz) spectral data (Table 2).

Compound **7** (catechin, 0.0012% w/w) occurred as yellowish - brown crystals (methanol), m.p. 148 -150°C. It gives orange colour with KOH and vanillin -H_2SO_4 spray reagents. R_f 0.49 [CH_2Cl_2- MeOH (8.5: 1.5)] . IR ν_{max} (KBr) : 3405, 2962, 1636, 1534, 1476, 1295, 1154, 1085, 988 and 825 cm^{-1}. UV λ_{max} (MeOH) : 277 and 227 nm. ^{1}H-NMR (DMSO, 400 MHz) spectral data (Table 2).

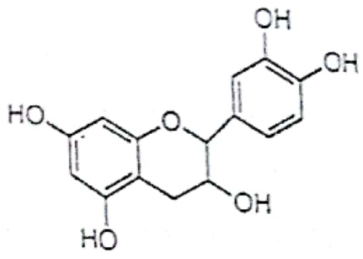
Compound **8** (chrysophanein, 0.0004% w/w) occurred as yellow needles (methanol), m.p. 240-242°C. R_f 0.35 [CH_2Cl_2-MeOH(8.5 :1.5)]. IR ν_{max} (KBr): 3450 (OH), 2960 (CH), 1670 (CO), 1620 (hydrogen bonded CO), 1454 , 1270 cm^{-1}. UV λ_{max} (MeOH) 228, 278 , 280 , 408 and 430 nm.



Compound	R1	R2	R3
1 (chrysophanol)	CH_3	H	H
2 (physcion)	CH_3	OCH_3	H
3 (emodin)	CH_3	OH	H
4 (aloe-emodin)	CH_2OH	H	H
8 (chrysophanein)	CH_3	H	β-D-glucose



Compound	R
5 (apigenin)	H
6 (luteolin)	OH



Compound 7 (catechin)

Acid hydrolysis :

About 1 mg of compound 8 was refluxed with 2ml of a mixture of 6% hydrochloric acid and methanol (1: 1) for two hours on a water path. The mixture was cooled and neutralized with silver oxide and centrifuged. The supernatant was evaporated to dryness and the residue was dissolved in pyridine and examined for the sugar moiety by TLC alongside authentic sugars using cellulose plates (Merck) and ethyl acetate-pyridine - water - n-butanol - acetic acid (25: 20: 20 : 50 : 10) as solvent system and aniline hydrogen phthalate as spraying reagent and heated at 110 °C for 5 minutes.

The anti-bacterial activity :

The agar diffusion method was used . Bacterial strains used (Table 3) were *Staphylococcus aureus*

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Table (1): ^{1}H-NMR spectral data of compounds 1, 2, 3 and 4.

Proton at C-atom	δ (ppm)			
	Compound 1	Compound 2	Compound 3	Compound 4
2	7.22, d (2)	7.06, d (2.5)	7.07, d (2.5)	7.27, d (2)
4	7.58, d (2)	7.34, d (2.5)	7.23, d (2.5)	7.62, d (2)
5	7.68, dd (8, 2)	7.60, d (2.5)	7.59, d (2.5)	7.69, dd (8, 2)
6	7.78, t (8)	---	---	7.79, t (8)
7	7.27, dd (8, 2)	6.66, d (2.5)	6.69, d (2.5)	7.25, dd (8, 2)
1-OH	11.98, s	12.08, s	12.19, s	12.18, s
8-OH	12.05, s	12.28, s	12.31, s	12.0, s
3-CH_3	2.42, s	2.44, s	2.44, s	---
3-CH_2OH	---	---	---	4.48, m
O-CH_3	---	3.93	---	---

Table (2): ^{1}H-NMR spectral data of compounds 5, 6 and 7.

Proton at C-atom	δ (ppm)		
	Compound 5	Compound 6	Compound 7
2	---	---	4.56, d (7)
3	6.59, s	6.66, s	3.95, m
4	---	---	2.82, dd (16, 5)
			2.48, dd (16, 8)
6	6.20, d (1.8)	6.17, s	5.86, d (2)
8	6.45, d (1.8)	6.43, s	5.92, d (2)
2'	7.85, d (9.0)	7.39, m	6.84, d (2)
3'	6.92, d (9.0)	---	---
5'	6.92, d (9.0)	6.87, d (8.0)	6.74, d (8)
6'	7.85, d (9.0)	7.39, m	6.70, dd (2, 8)

Table 3. Anti-microbial activity of compounds isolated in sufficient amount.

Tested compounds	Inhibition zone in mm.	
	Staph. aureus	*Escherichia coli*
1 (chrysophanol)	+	++
2 (physcion)	+	---
3 (emodin)	+	++
4 (aloe-emodin)	---	++
6 (luteolin)	+	+++
7 (catechin)	+	++

Control (DMF) = 10 mm, + 12-15 mm, ++ 16-25 mm, +++ 26-35 mm.

NTCC 6538 (Gram positive) and *Escherichia coli* NTCC 10536 (Gram negative). Nutrient agar plates were seeded using 0.1 ml of diluted organisms (a plate for each bacterial strain). Cylindrical plugs were removed from agar plates using a sterile cork borer. From each tested compound, 50 µl of each of the tested compounds (1 mg/ml dimethyl formamide) and blank solvent were added to each well in the plates which were kept in the incubator at 37°C for 24 hours, and the sizes of the inhibition zones were measured in mm (Table 3).

RESULTS AND DISCUSSION

The ether - soluble fraction of the alcoholic extract of the aerial parts of *Rumex tingitanus* was chromatographed to afford compounds 1-8. Compound 1, 2, 3, 4, and 8 gave red colour with NaOH spray reagent indicating their anthraquinonoid nature. The UV spectra revealed the presence of absorption bands characteristic of α- hydroxyanthraquinone [17] which was confirmed by the presence of two downfield singlets in the ^{1}H-NMR around δ 12 ppm assigned to OH - groups at C-1 and C-8. Substitution in ring C is identical in all compounds with C-3 methyl group (compounds 1-3) or hydroxymethyl group (compound 4) and two *meta* -coupled aromatic protons at C-2 and C-4 (Table 1). Ring A showed significant differnce where compounds 1 and 4 showed one ABC system for H-5, H-6 and H-7 (Table 1) which confirm the identity with chrysophanol and aloe - emodin respectively. On the other hand, compounds 2 and 3 were closely related to each other (compound 3 showed additional signal at δ 3.93 ppm assigned for OCH_3 group) and showed another pair of *meta* -coupling for H-5 and H-7 which confirm the identity with physcion and emodin, respectively. The interpretation of data (UV, IR, m.p. ^{1}H-NMR spectra and co-chromatography with authentic samples) and comparing it with the published ones confirmed the identification of compounds 1, 2, 3, and 4 as chrysophanol, physcion, emodin and aloe-emodin respectively [7,11, 17, 18]. Molish's test for compound 8 indicated its glycosidic nature. Acid hydrolysis and TLC alongside authentic sugars indicated that the sugar moiety is D-glucose. From UV, IR, m.p. and co-chromatography with authentic sample, compound 8 was identified as chrysophanein.

Compounds 5 and 6 gave yellow colour and compound 7 gave orange colour with NaOH and vanillin - H_2SO_4 spray reagents indicating their flavonoidal nature. The UV spectra of compounds 5 and 6 in MeOH indicating a flavone nucleus. A bathochromic shift after the addition of $NaOCH_3$ indicated the presence of a free OH group at C-4` which was confirmed by the bathochromic shift in band I after the addition of NaOAc where the bathochromic shift in band II and after the addtion of $NaOAc$ indicated the presence of a free OH groiup at C-7 for both compounds. The bathochromic shift in band I after addition of $AlCl_3$ indicated the presence of OH group at C-5 for both compounds. The absence of a clear shift in band I with $AlCl_3$/HCl relative to $AlCl_3$ which was confirmed by the absence of a clear shift in band I with $NaOAc/H_3BO_3$ indicated the absence of *ortho*-dihydroxy group in ring B for compound 5 (c.f. compound 6 which exhibited a clear shift indicating the presence of *ortho* - dihydroxy group in ring B) the ^{1}H-NMR spectra of both compounds showed a *meta*-coupling between H-6 and H-8 (Table 2). For compound 5 there was two coupled doublets each intergrated for two protons assigned for H-2` / H-6` and H-3` / H-5` but for compound 6 there was a *meta*- coupling between H-2` and H-6` and another ortho-coupling between H-5` and H-5`. From the anlaysis of these data as well as by comparing it with the published literature, compound 5 and 6 were confirmed as apigenin and luteolin respectively [19, 20]. The UV λ max (MeOH) at 277 and 227 nm of compound 7 indicating the presence of two isolated benzenoid chromophores. The ^{1}H-NMR spectrum revealed the presence of five aromatic protones assigned to positions 6, 8, 2`, 5` and 6` of a flavonoid skeleton. The doublet at δ 5.92 ( 1H, $J$ = 2 Hz) assigned to H-8 showed a *meta* - coupling with the doublet at δ :5.86 (1H, $J$ = 2Hz) assigned to H-6. On the other hand the doublet at δ 6.84 ( 1H , $J$ = 2 Hz) assigned to H-2` showed a meta - coupling with the doublet of doublet at δ 6.70 (1H J = 2 and 8= Hz) assigned to H-6` and the later exhibited an ortho-coupling with the doublet at δ 6.74 (1H , $J$ = 8 Hz) assigned to H-5`. The ^{1}H-NMR spectrum (Table 2) indicated that it is a catechin type flavanol with a trans-configuration at C-2 and C-3 (the coupling constant between H-2 and H-3 was 7Hz). From the interpretation of these data and by comparing it with the published data [21-23], compound 7 was identified as catechin.

In this study, the preliminary anti-bacterial screening (Table 3) of the compounds isolated in sufficient amount using agar diffusion method showed that compounds 1 (chrysophanol), 2 (physcion), 3 (emodin), 6 (luteolin) and 7 (catechin) exhibited a weak activity against Gram positive (*Staphylococcus aureus*) bacteria (inhibition zone = 12 to 15 mm). On the other hand compounds 1 (chrysophanol), 3 (emodin), 4 (aloe-emodin) and 7 (catechin) exhibited a moderate activity against Gram negative (*Escherichia coli*) bacteria (inhibition zone = 16-25 mm) while compound 6 (luteolin) exhibited a high activity against Gram negative (*Escherichia coli*) bacteria (inhibition zone = 28 mm).

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REFERENCES

1-Siddiqi, M. A. and El-Taife, A. ; "Flora of Libya" Jafri. S. M. H. and El-Gadi, A. (eds). Al Faateh University , 106 , 21 (1983).

2-Bailey, L. H.; " Manual of cultivated plants". 15th ed. The MacMillan Publishing Co., New York, p. 347 (1975).

3-Pandey, B. P. ; "Taxonomy of Angiosperms" . S. Chand and Company LTD , Ram Nagar, New Delhi - 110055 , p. 340 (1982) .

4-Fairbairn, J. W. and El-Muhtadi, F. J.; **Phytochemistry** , 11 , 263 (1972) .

5-Mohamed, K. F.; M. Sc. Pharm. Science Thesis. Mansoura Univ. (1989).

6-Abd El-Fattah, H.; **Mans. J. of Pharm. Sci.** , 6, 141 (1989).

7-Abd El-Fattah, H. , El-Dahmy, G. S. and Hubaishi, A.; **Acta Pharm. Hungarica,** 64 , 83 (1994).

8-El-Keiey , M. A. ; Sayed, M. D. and Moustafa , M. A.; **Egypt. J. Pharm. Sci.,** 5, 197 (1964) .

9-Afif, M. S.; Ph. D. Pharm. Sci. Thesis. Cairo Univ. (1972).

10-Rizk , A. M.; The Phytochemistry of the Flora of Qatar . The Scientific ANF Applied. Research Center. University of Qatar, 321 (1986).

11-El-Gamal, A. A. H. ; M. Sc. Pharm. Sci. Thesis Mansoura Univ. (1991).

12-Watt J. M. and Breyer - Brandwijk , M. G.; "The Medicinal and Poisonous Plants of Southern and Eastern Africa" 2nd ed. E & S Levingstone Ltd. Edinburgh , London (1962) .

13-Wallis, T. E. ; "Texbook of Pharmacognosy" . Churchill LTD , London p. 365 (1967).

14-Balbaa, S. I. , Hilal , S. H. and Zaki, A. Y. ; "Medicinal Plants Constituents" . General Agency for University and School Books , Cario, p. 275 (1976).

15-Belkin, M. , Gerald, D. B. and Cogan, G. W.; **J. Nat. Cancer,** 13 , 139 (1952) .

16-Kabiev, O. K. and Vermenichev, S. M.; **Vopr, Onkol.,** 12 , 61 (1966) (**Through C. A.** 65 , 2875 e, 1966).

17-Thomson, R. H. ; "Naturally Occuring Qninones". 2nd ed. Academic Press, London and New York (1971) .

18-Abd El-Fattah, H., Zaghloul , A. M., Mansour, E. S. , Halim, A. F. and Waight, E. S. ; **Egypt J. Pharm. Sci.** ,31 , 93 (1990) .

19-Mabry, J. J. Markham, K. R. and Thomas, M. B.; "The Systematic Identification of Flavonoids" . Springer - Verlag . New York (1970) .

20-Harborne, J. B. and Mabry, T. J.; "The Flavonoid Advances in Research" . 1st ed., Chapman and Hall LTD , London, New York (1982).

21-Nonaka, G. , Ezaki, E. , Hayashi, K. and Nishioka, J. ;. **Phytochemistry** 22 , 1659 (1983).

22-Ikuta , J. Hano, Y. and Namura T. ; **Heterocycles** , 23 , 2835 (1985).

23-El-Gamal, A. A. H. ; Ph. D. Pharm. Sci. Thesis Mansoura Univ. (1996).

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فصل أنثر اكينونات وفلافونيدات من نبات رومكس تنجيتانس والذى ينمو فى ليبيا

منى جودة زغلول ، حسنى عبدالفتاح

قسم العقاقير - كلية الصيدلة - جامعة المنصورة - المنصورة - مصر

أدت الدراسة الكيميائية لخلاصة الأثير لنبات رومكس تنجيتانس إلى فصل خمسة أنثر اكينونات وهى كريزوفانول، فيسيكون ، ايمودين ، ألوايمودين ، كريزوفانين كما تم فصل ثلاثة فلافونيدات وهى أبيجينين ، ليتيولين ، كاتيشين من نفس الخلاصة وقد تم التعرف على هذه المركبات بدراسة خواصها الطبيعية والطيفية وهذه هى المرة الأولى التى يتم فيها فصل هذه المركبات من هذا النبات .

وقد تم فى هذا البحث أيضا دراسة قدرة المركبات التى فصلت بكميات كافية على مقاومة نشاط بعض البكتيريا موجبة الجرام وسالبة الجرام المسببة للأمراض، حيث أظهر كل من كريزوفانول ، فيسيكون ، ايمودين ، ليتيولين ، كاتيشين قدرة ضعيفة على مقاومة نشاط البكتيريا موجبة الجرام ، ومن ناحية أخرى أظهر كل من كريزوفانول ، ايمودين ، الوايمودين، كاتيشين قدرة متوسطة على مقاومة نشاط البكتيريا سالبة الجرام ، بينما أظهر ليتيولين قدرة عالية على مقاومة نشاط البكتيريا سالبة الجرام.