

PHYTOCHEMICAL STUDY OF THE CONSTITUENTS OF THE LEAVES OF *FICUS INFECTORIA* (Roxb.)

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تستعمل نباتات الفيكس في علاج العديد من الأمراض مثل الروماتيزم ، الحمى ، السل الرئوى وقد اكتشف حديثا أن بعض الأنواع لها تأثير على الخلايا السرطانية. لذلك فقد تم في هذا البحث تحضير الخلاصة الكحولية لأوراق هذا النبات تم تجزئتها باستخدام الكلوروفورم وخلات الإيثيل والبيوتانول وبعد ذلك تم فصل وفحص مكونات هذه الخلاصات كل على حدة باستخدام كروماتوجرافيا العمود والطبقة الرقيقة. كما تمت دراسة الصفات الطبيعية والكيميائية للمركبات المفصولة باستخدام الأشعة فوق البنفسجية ودون الحمراء ومطياف الكتلة والرنين النووي المغناطيسى الهيدروجينى والكربونى. وقد أمكن التعرف على المركبات التالية: ألفا-أميرين (1) ، ستيجماستيرول (2) ، برجابتين (3) ، ستيجماستيرول-3-جلوكوزيد (4) ، الكامفيرول-3-جلوكوزيد (5) ، بنزيل-جلوكوزيد (6) ، كوارستين-3-جلوكوزيد (7) ، 6-هيدروكسيكامفيرول-7-جلوكوزيد (8) ، كوارستين-3-جلوكوزيد (9) ، كوراستين-3-روتينوزيد (9).

α-Amyrin (1), stigmasterol (2), bergapten (3), stigmasterol-3-O-β-D-glucoside (4), kaempferol-3-O-β-D-glucoside (5), benzyl-glucoside (6), quercetin-3-O-β-D-glucoside (7), 6-hydroxykaempferol-7-O-β-D-glucoside (8), and quercetin-3-O-β-D-rutinoside (9) were isolated for the first time from the ethanolic extract of the leaves of *Ficus infectoria* (Roxb.) cultivated in Egypt. Identification of these compounds has been established by physical and spectral data (UV, IR, MS, ¹H- and ¹³C-NMR) as well as by comparison with authentic samples.

INTRODUCTION

Ficus infectoria (Roxb.), Family Moraceae is a large spreading deciduous, fast growing tree¹ indigenous to South Africa and cultivated in Egypt for its shade. Leaves and roots of some *Ficus* species are used medicinally for treatment of leprosy, ulcers, chest conditions, to reduce fever, as tonic, anti-rheumatic and for the treatment of tuberculosis.^{2,3} The latex of various species has been used as laxative, emollient, diuretic, anthelmintic and in treatment of warts.² Recent studies revealed the anticancer and anti-infective activity^{4,5} of certain *Ficus* species.

Previous studies of different *Ficus* species revealed the isolation and identification of many constituents of different chemical classes; sterols and triterpenes,⁶⁻⁸ (*α*- and *β*-amyirin, *β*-sitosterol, ursolic acid, stigmasterol, stigmasterol-

glucoside; coumarins^{4,6,7,9,10} (umbelliferone, xanthotoxin, bergapten, psoralen, dihydro-psoralen and marmesin); flavonoids⁹ (chalcones, flavones, apigenin-glucosides, luteolin-glucosides, flavan (4,5,7-trihydroxyflavan-3-ol) and rutin; resveratrol^{5,7} (3,5,4'-trihydroxystilbene), cyanidol,⁵ and taxastane-type triterpenes.¹¹

The diverse chemical constituents of a considerable phytochemical interest of different *Ficus* species as well as the important medicinal uses together with the results obtained from our screening encouraged us to carry out the present study. Reviewing the available literature on *Ficus infectoria* (Roxb.) revealed the lack of information on its chemical constituents. Therefore, it was deemed of interest to carry a pharmacognostical study on this plant. We have previously reported the macro- and micro-

morphological characters of the leaves and stems.¹²

In continuation, we present here the isolation and identification of flavonoids, coumarins, and sterols from the leaves of the title plant.

EXPERIMENTAL

General experimental procedures

- 1- Melting points are uncorrected and were measured by Electrothermal 9100 Digital Melting Point Instrument (England Ltd., England).
- 2- UV spectra were measured in methanol and different ionizing and complexing agents using a Uvidec-320 spectrophotometer with matched 1 cm quartz cells, (Jasco, Tokyo, Japan).
- 3- ¹H- and ¹³C-NMR spectra were run in CD₃OD and d₆-DMSO at 400 MHz by JEOL TNM-LA400, FT NMR system, Japan, using TMS as internal standard.
- 4- EIMS spectra were recorded by JEOL, JMS 600 H, Japan.
- 5- UV-Lamp (254, 366 nm, VL, 6 LC, Marine Lavalee-Codex, France).
- 6- Column chromatography using silica gel (E. Merck, Germany).
- 7- TLC was performed on silica gel G₆₀F₂₅₄ activated layers (E. Merck, Germany).
- 8- Authentic samples were obtained from Department of Pharmacognosy, Faculty of Pharmacy, Assiut University.
- 9- Visualization:
Sulphuric acid 50% was used for visualizing sterols and triterpenes, thymol/sulphuric acid mixture for sugars, and AlCl₃ in 5% methanol, UV lamp for coumarins and flavonoids.
- 10- Solvent systems:
The following solvent systems were used for TLC and PC screening:
 - I- Hexane-ethyl acetate (95:5).
 - II- Chloroform-methanol (95:5).
 - III- Chloroform-methanol (90:10).
 - IV- Chloroform-methanol (80:20).
 - V- Ethyl acetate-methanol (80:20).

VI- n-Butanol-acetone-formic acid-water (60:17:8:15).

VII- Acetic acid-water (15:85).

Plant material

The leaves of *Ficus infectoria* (Roxb.) were collected from the trees cultivated in Experimental station of Faculty of Agriculture, Assiut University in October 1997 (at the flowering time) and identified by Prof. Dr. Ibrahim Hassan, Professor of Floriculture and Horticulture, Faculty of Agriculture, Assiut University.

Extraction and isolation

The air-dried leaves (2 Kg) of *Ficus infectoria* (Roxb.) was extracted with ethanol (70%) at room temperature by maceration (6.0 L). The residue left after evaporation of the solvent (200 g) was diluted with water and successively extracted with chloroform (7x500 ml), ethyl acetate (5x500 ml) and n-butanol (6x500 ml). The chloroform soluble fraction (25 g) was chromatographed over silica gel column (750 g, 7x210 cm) and eluted with hexane, hexane-chloroform and chloroform-methanol gradient. The eluted fractions (100 ml each) were collected, concentrated and screened by TLC using solvent system I, similar fractions were combined. n-Hexane elute afforded compound 1 ($R_f = 0.7$, system I). The fractions eluted with hexane-chloroform (2:8) purified and yielded compound 2 ($R_f = 0.14$, system I). The fractions eluted with chloroform-methanol (9:1) were subjected to PTLC using solvent system II where it yielded compound 3 ($R_f = 0.72$) and the fractions eluted with chloroform-methanol (85:15) gave compound 4 ($R_f = 0.51$, system IV). While the fractions eluted with chloroform-methanol (8:2) were subjected to PPC using solvent system VII to afford compound 5 ($R_f = 0.54$).

The ethyl acetate soluble fraction (15 g) was chromatographed over silica gel column (450 g, 5x180 cm). Elution was started with chloroform followed by chloroform-methanol gradient. The fractions eluted with chloroform-methanol (9:1) were rechromatographed over

silica gel column and the fractions eluted with chloroform-methanol (95:5) yielded compound 6 ($R_f = 0.48$, system II), the fractions eluted with chloroform-methanol (85:15) yielded compound 7 ($R_f = 0.44$, system IV) and the fractions eluted with chloroform-methanol (8:2) gave compound 8 ($R_f = 0.35$, system IV).

The n-butanol soluble fraction (20 g) was chromatographed over silica gel column (600 g, 5x180 cm). Elution was started with ethyl acetate followed by ethyl acetate-methanol gradient. The fractions eluted with ethyl acetate-methanol (80:20) afforded compound 9 ($R_f = 0.63$, system V).

Acid hydrolysis

Five-mg portion of each of the isolated glycosides was dissolved in 5-ml methanol to which 5 ml of 5% sulphuric acid is added. The mixture was refluxed for 3 hours on a boiling water-bath, cooled, the aglycone was extracted with chloroform, purified and subjected to TLC. The produced sugars were identified by TLC using silica gel G and solvent system VI.

Compound 1: White crystalline needles [n-hexane], (150 mg), m.p 185-187°, IR (KBr, ν , cm^{-1}): 3420, 2980, 1650, 1460, 1450, 1380, 1050, 1010, 960 and 805.

Compound 2: White crystalline needles [methanol], (100 mg), m.p 160-162°, IR (KBr, ν , cm^{-1}): 3350, 2940, 1460, 1380 and 1360.

Compound 3: White crystalline needles [chloroform], (50 mg), m.p 191-192°, UV (λ_{max} , nm), MeOH: 270, 294sh, 302, IR (KBr, ν , cm^{-1}): 3100 (C-H stretching), 1720-1700 (α -lactone), 1610, 1970, 1530 (aromatic C-C stretching). $^1\text{H-NMR}$ spectrum (d_6 -DMSO): δ 4.24 (3H, s, OCH_3), 6.28 (1H, d, $J = 9.76$ Hz, H-3), 7.29 (1H, s, H-8), 7.37 (1H, d, $J = 2.2$ Hz, H-3'), 8.01 (1H, d, $J = 2.2$ Hz, H-2'), 8.14 (1H, d, $J = 9.76$ Hz, H-4).

Compound 4: White amorphous powder (200 mg), m.p 230-235°, IR (KBr, ν , cm^{-1}): 3450, 2940, 1370, 1610, 1070 and 1025.

Compound 5: Yellow amorphous powder (20

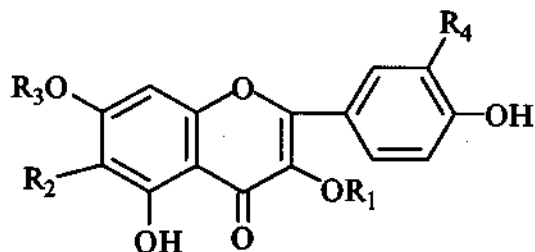
mg), m.p 242-245°, UV (λ_{max} , nm, MeOH): 265, 285sh, 349; NaOMe: 272, 327sh, 401; AlCl_3 : 274, 349sh, 398; AlCl_3/HCl : 274, 349sh, 396; NaOAc: 269, 360; NaOAc/ H_3BO_3 : 265, 300sh, 351. $^1\text{H-NMR}$ spectrum (CD_3OD): δ 3.1-3.61 (sugar protons), 5.12 (1H, d, $J = 7.08$ Hz, H-1''), 6.08 (1H, d, $J = 1.72$ Hz, H-6), 6.27 (1H, d, $J = 1.72$ Hz, H-8), 6.78 (2H, d, $J = 8.80$ Hz, H-3', 5'), 7.94 (2H, d, $J = 8.80$ Hz, H-2', 6').

Compound 6: Colourless needles [methanol-ethyl acetate], (15 mg), m.p 122-123°. $^1\text{H-NMR}$ spectrum (CD_3OD): δ 3.00-4.16 (sugar protons), 4.56 (1H, d, $J = 12.0$ Hz, H- α -benzyl), 4.85 (1H, d, $J = 12.0$ Hz, H- α' -benzyl), 4.87 (1H, d, $J = 7.5$ Hz, H-1'), 7.17 (2H, dd, $J = 7.32, 1.68$ Hz, H-2,6), 7.23 (1H, pseudo t, $J = 7.32$ Hz, H-4), 7.32 (2H, dd, $J = 7.32, 1.68$ Hz, H-3,5). EI^+ -MS: 108 (8.5%) [M] $^+$, other peaks at $m/z = 91$ (100%), 77 (16.6%) and 65 (14.4%).

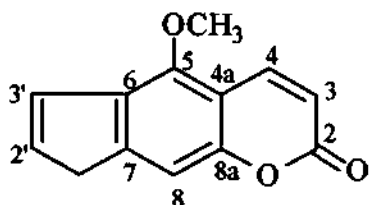
Compound 7: Yellow amorphous powder (25 mg), UV (λ_{max} , nm, MeOH): 257, 356; NaOMe: 268, 328sh, 409; AlCl_3 : 274, 332sh, 430; AlCl_3/HCl : 268, 362sh, 378; NaOAc: 268, 364; NaOAc/ H_3BO_3 : 260, 377. $^1\text{H-NMR}$ spectrum (CD_3OD): δ 3.20-3.63 (sugar protons), 5.16 (1H, d, $J = 7.32$ Hz, H-1''), 6.1 (1H, d, $J = 1.96$ Hz, H-6), 6.29 (1H, d, $J = 1.96$ Hz, H-8), 6.77 (1H, d, $J = 8.56$ Hz, H-5'), 7.49 (1H, dd, $J = 8.56, 2.44$ Hz, H-6'), 7.61 (1H, d, $J = 2.44$ Hz, H-2').

Compound 8: Yellow amorphous powder (150 mg), UV (λ_{max} , nm, MeOH): 265, 289sh, 347; NaOMe: 272, 321sh, 400; AlCl_3 : 273, 348sh, 396; AlCl_3/HCl : 273, 345sh, 395; NaOAc: 265, 289sh, 347; NaOAc/ H_3BO_3 : 265, 289sh, 347. $^1\text{H-NMR}$ spectrum (CD_3OD): δ 3.20-4.00 (sugar protons), 5.23 (1H, d, $J = 7.30$ Hz, H-1''), 6.39 (1H, s, H-8), 6.79 (2H, d, $J = 9.00$ Hz, H-3', 5'), 8.05 (2H, d, $J = 9.00$ Hz, H-2', 6'). EI^+ -MS, m/z 302 (1.0%) [aglycone] $^+$ and other fragments at 284 (1.6%), 167 (57.0%), 149 (100.0%).

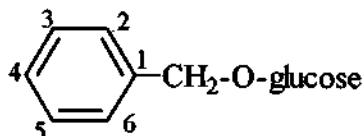
Compound 9: Yellow amorphous powder (15 mg), UV (λ_{max} , nm, MeOH): 258, 353; NaOMe:



	R ₁	R ₂	R ₃	R ₄
5	glucose	H	H	H
7	glucose	H	H	OH
8	H	OH	glucose	H
9	glucose-rhamnose	H	H	OH



Compound 3



Compound 6

265, 403; AlCl₃: 272, 428; AlCl₃/HCl: 268, 358; NaOAc: 265, 363; NaOAc/H₃BO₃: 261, 368. ¹H-NMR spectrum (CD₃OD): δ = 1.02 (3H, d, J = 6.32 Hz, CH₃, rhamnose), 3.25-3.71 (sugar protons), 4.42 (1H, d, J = 1.5 Hz, H-1''), 5.00 (1H, d, J = 7.32 Hz, H-1''), 6.11 (1H, d, J = 2.00 Hz, H-6), 6.30 (1H, d, J = 2.0 Hz, H-8), 6.77 (1H, d, J = 8.32 Hz, H-5'), 7.52 (1H, dd, J = 8.32, 1.96 Hz, H-6'), 7.57 (1H, d, J = 1.96 Hz, H-2').

RESULTS AND DISCUSSION

Compounds 1 and 2 were identified as α-amyrin, and stigmaterol respectively by comparison of their physico-chemical data such as m.p, IR, and co-chromatography with reference sample. Compound 4 was identified as stigmaterol-3-O-glucoside by its physico-chemical data such as m.p, IR, and co-chromatography with reference sample before and after acid hydrolysis. The chromatographic study of sugars in the aqueous fraction revealed

that the sugar appeared as a single spot corresponding to authentic glucose (R_f = 0.43, system VI). The aglycone was identified as stigmaterol.

The ¹H-NMR spectrum of compound 3 showed a pair of one-proton doublet at δ 6.28 and 8.14 (J_{3,4} = 9.76 Hz, H-3 and H-4) characteristic for coumarins with unsubstituted pyrone ring.¹³ In addition, it exhibited two doublets with J value of 2.2 Hz at δ 7.37 and 8.01 characteristic for H-3' and H-2' of the furan ring of furanocoumarins.¹³ It showed also a singlet at δ 4.24 indicative for methoxy function, which was confirmed by the presence of a singlet at 60.16 in ¹³C-NMR, Table 1. The UV bands at 230, 254(sh), 284 (sh) and 324 nm and the IR bands at 1720-1700, 1620, 1570, and 1520 cm⁻¹ supported a coumarin skeleton.¹³ The ¹³C-NMR spectral data of 3 was compared favourably with the reported data for bergapten.¹⁴

The UV spectrum of compound 5 indicated a flavone or C3-OH substituted flavonol

Table 1: ^{13}C -NMR data of compounds isolated from *Ficus infectoria* (Roxb.), (100 MHz, CD_3OD , relative to TMS).

C-Atoms	3*	6	7	8	9
1	----	139.05	----	----	----
2	160.05	129.27	157.00	159.86	158.50
3	112.17	129.20	134.18	136.27	135.62
4	139.36	128.69	177.95	180.27	179.39
5	149.38	129.20	161.72	162.36	162.95
6	112.20	129.27	98.51	130.67	100.00
7	157.72	71.75	164.67	166.88	166.10
8	93.00	----	93.18	95.54	94.90
9	----	----	157.94	159.24	159.35
10	----	----	104.26	106.19	105.61
1'	----	103.27	121.00	123.61	123.12
2'	145.78	75.13	114.55	133.08	116.07
3'	105.61	78.08	144.51	116.88	145.83
4'		71.68	148.35	163.90	149.80
5'		78.01	116.07	116.88	117.71
6'		62.81	121.73	133.08	123.56
1''			102.82	104.92	104.72
2''			74.25	76.53	75.71
3''			76.93	79.65	78.17
4''			69.75	72.16	71.39
5''			76.65	78.83	77.20
6''			61.07	63.43	68.56
1'''					102.40
2'''					72.09
3'''					72.24
4'''					73.93
5'''					69.70
6'''					17.88
4a	105.52				
8a	152.06				
OCH_3	60.16				

* Spectra were measured in d_6 -DMSO.

skeleton. A bathochromic shift (+11 nm) in band I upon the addition of NaOAc indicated a free hydroxyl group at C7. A bathochromic shift (+52 nm) in band I was observed upon the addition of NaOMe indicated a free hydroxyl group at C-4'. Addition of AlCl₃ or AlCl₃/HCl produced a bathochromic shift (+49 nm) in band I indicating the presence of free hydroxyl group at C-5 and absence of ortho-dihydroxy groups at ring B.

No shift was observed upon the addition of NaOAc/H₃BO₃, which confirmed the absence of ortho-dihydroxy groups at ring B. The glycosylation of compound 8 at C-7 was confirmed by the absence of shift upon the addition of NaOAc.

The ¹H-NMR spectra of compounds 5 and 8 showed two doublets at 6.78 (J = 8.8 Hz, H-3', 5'), 7.94 (J = 8.8, H-2', 6') for compound 5 and two doublets at 6.79 (J = 9.0 Hz, H-3', 5'), 8.05 (J = 9.0 Hz, H-2', 6') for compound 8 characteristic for 1,4-disubstituted benzene in kaempferol structure.¹⁵ This was further confirmed with ¹³C-NMR data, Table 1, which were identical with those previously reported.^{16,17} The ¹H-NMR spectrum of compound 5 showed two doublets with meta coupling at 6.08 and 6.27 (J = 1.72 Hz) characteristic for H-6 and H-8.¹⁵ While compound 8 showed only one singlet at 6.39 characteristic for H-8.¹⁵ The absence of H-6 and its substitution with a hydroxyl group was confirmed with the downfield shift of C-6 in ¹³C-NMR (130.67), Table 1, and with [M]⁺ at *m/z* peak at 302 in mass spectrum which was consistent with the formula C₁₅H₁₀O₇.

The glycosidic nature was confirmed by the appearance of anomeric protons, doublets at 5.12 and 5.23 (J = 7.08-7.30) for compounds 5 and 8 respectively.

Acid hydrolysis for each of 5 and 8 gave glucose and kaempferol, which were identified by co-PC and co-TLC with authentic samples.

A study of the effect of ionizing and complexing agents on the UV absorption of compounds 7 and 9 revealed a bathochromic shift (+53, 50 nm) in band I upon the addition of NaOMe indicating the presence of a free hydroxy group at C-4'. Addition of AlCl₃

produced a bathochromic shift (+74, 75 nm) in band I which was decreased to (+22, 5 nm on the addition of AlCl₃/HCl indicating the presence of ortho-dihydroxy groups at ring B. This was also confirmed by a bathochromic shift in band I (+21, 15 nm) upon the addition of NaOAc/H₃BO₃. A free hydroxyl group at C-7 was indicated by the bathochromic shift (+8, 10 nm) in band I upon the addition of NaOAc.

The ¹H-NMR spectra of compounds 7 and 9 showed two doublets at δ 6.1, 6.11 and 6.29, 6.30 with meta coupling assigned to H-6 and H-8 respectively. The spectra showed also three aromatic protons of ABX-type coupling, two doublets at δ 6.77, 6.77 and 7.61, 7.57 which were assigned to H-5' and H-2' respectively, a doublet of doublet at 7.49 and 7.52 was assigned to H-6'. This pattern is typical for quercetin.¹⁵

The glycosylation of compounds 7 and 9 was confirmed from the appearance of one anomeric proton, doublet at 5.16 (J = 7.32 Hz) for 7 characteristic for glucose or two anomeric protons at 4.42, doublet (J = 1.5 Hz) and at 5.0, doublet (J = 7.32 Hz) for 9 characteristic for rhamnose and glucose. The presence of rhamnose was confirmed by the appearance of CH₃ signal at 1.02 (d, J = 6.32 Hz) and at 17.88 in ¹³C-NMR, Table 1. The ¹³C-NMR shifts of C-6'' and 1'''' appeared at 68.56 and 102.40 respectively, Table 1, suggesting that the interglycosidic linkage was (1→6). ¹H- and ¹³C-NMR data are in accordance with the reported values.^{16,17}

Acid hydrolysis of each of compounds 7 and 9 gave glucose and quercetin for compound 7, glucose, rhamnose and quercetin for compound 9, which were identified by co-PC and co-TLC with authentic samples.

From the above data, it could be concluded that compounds 7 and 9 are quercetin-3-O-glucoside and quercetin-3-O-rutinoside respectively.

The ¹H-NMR spectrum of compound 6 exhibited AB-type signals due to benzylic methylene at 4.56 (1H, J = 12 Hz) and 4.85 (1H, d, J = 12 Hz, (confirmed by a signal at 71.75 in ¹³C-NMR), a doublet signal due to an anomeric proton at 4.87 in addition to three

signals integrating for five protons for benzene ring (7.17-7.33). The benzyl structure was further confirmed from the mass spectrum by the appearance of mass fragment at 91 (base peak) for the most stable tropylium cation. It also showed a molecular ion peak $[M]^+$ at m/z 108 consistent with the formula C_7H_8O (benzyl alcohol). Other peaks appeared at 91, 77 and 65. These data led us to conclude the structure of this compound to be benzyl-glucoside.

It is noteworthy that, benzyl glucoside was previously isolated from other representatives.^{18,19}

In conclusion, quercetin glycosides, α -amyrin, stigmasterol, its 3-glucoside, and bergapten were previously reported from other *Ficus* species. On the other hand, kaempferol, its 6-hydroxy derivative and their glucosides, as well as benzyl-glucoside have not been previously reported in any *Ficus* species. Furthermore, all the isolated compounds were reported here to be isolated for the first time from *Ficus infectoria* (Roxb.).

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