ejpmr, 2015,2(4), 618-634.

SJIF Impact Factor 2.026

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 3294-3211

EJPMR

A NEW FLAVONE GLYCOSIDE AND THE STUDY OF ANALGESIC AND ANTI-INFLAMMATORY ACTIVITIES OF *FICUS LAURIFOLIA*

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Article Received on 18/05/2015 **Article Revised on 10/06/2015** **Article Accepted on 01/07/2015**

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ABSTRACT

A new compound; chrysoeriol 7-*O*-(6``-methylglucuronoide) (**1**) and seven known compounds; chrysoeriol 7-*O*-glucuronoide (**2**), luteolin 7-*O*-*β*-D-glucopyranoside (cinaroside) (**3**), salicyl alcohol glucoside (salicin) (**4**), 2`-acetylsalicortin (**5**), 2-(*β*-D-glucopyranosyloxy)-benzyl (3,4-dihydroxy) cinnamoate (populoside) (**6**), 2-(*β*-D-glucopyranosyloxy)-benzyl (4-hydroxy) cinnamoate (populoside B) (**7**) and β-sitosterol (**8**) were isolated for the first time from *Ficus laurifolia*. The identification of these compounds was based on their NMR, UV, IR and ESIMS analyses. The alcoholic extract, ethyl acetate and *n*-butanol fractions showed good analgesic and anti-inflammatory activities. The *n*-butanol fraction appeared to be the most active one.

KEYWORDS: *Ficus laurifolia*, phytoconstituents, analgesic and anti-inflammatory activities.

INTRODUCTION

Ficus constitutes one of the largest genera of the family Moraceae with about 1,000 species occurring in tropical and subtropical regions.[1] The therapeutic utilities of this genus have been indicated in traditional medicine. It has been used to cure the disorders of the central nervous system (migraine, etc.), endocrine system (diabetes, etc.), gastrointestinal tract (ulcers, stomatitis, liver diseases, etc.), respiratory system (asthma, cough, etc.), infectious diseases (chickenpox, gonorrhea, scabies, etc.) and several inflammatory processes.[2-4] Previously, flavonoids,[5] phenolics,[6,7] sterols and triterpenoids,[8,9] and alkaloids,[10] have been isolated and identified from different species of Ficus. *Ficus laurifolia* Hort. Ex Lam. is a popular ornamental plant in the Orient. Surprisingly no intensive research work has been

reported on this species. In this paper, we report the isolation and structure elucidation of a new flavone glycoside, chrysoeriol-7-*O*-(6``-methylglucuronoide) (**1**), along with seven known compounds (**2**-**8**) from the leaves of *Ficus laurifolia*. In addition to evaluation of analgesic and anti-inflammatory activities of the titled plant.

MATERIAL AND METHODS

General experimental procedures. UV spectra were determined with Pye Unicam spp. 1750 spectrophotometer. ESIMS was carried out on a XEVO TQD triple quadruple instrument (Waters Corporation, Milford, MA 01757, USA) mass spectrometer. The ^{1}H- and ^{13}C NMR measurements were obtained with a Bruker Avance III (400) NMR spectrometer operating at 400 MHz (for ^{1}H) and 100 MHz (for ^{13}C) in $CDCl_3$ or DMSO-d_6 solution, and chemical shifts were expressed in δ (ppm) with reference to TMS, and coupling constant (*J*) in Hertz. ^{13}C multiplicities were determined by the DEPT pulse sequence (135^{o}). HMBC and HSQC NMR experiments were carried out using a Bruker Avance III (400) high field spectrometer. Si gel (Si gel 60, Merck) and Sephadex LH-20 (Pharmacia) were used for open column chromatography. TLC was carried out on precoated silica gel 60 F_{254} (Merck) plates. Developed chromatograms were visualized by spraying with 1% vanillin-H_2SO_4, followed by heating at 100 ^{0}C for 5 min, or spraying with ammonia or aluminum chloride solutions.

Plant material

Ficus laurifolia Hort. Ex Lam. leaves were collected from Giza zoo, Giza, Cairo, Egypt during March 2012. The plant was kindly identified by Dr. Mohamed El-Gebaly, National Research Institute, Dokki, Giza, Egypt.

Extraction and isolation

Air dried powder of *Ficus laurifolia* Hort. Ex Lam. leaves (3kg) were subjected to exhaustive extraction with 70% MeOH (3 x 9L). The combined methanolic extracts were concentrated under vacuum at 40^{o}C to dryness. The concentrated methanolic extract was suspended in distilled water (500 ml) and was fractionated with petroleum ether to yield 90g. The defatted crude extract (400g) was fractionated with ethyl acetate and *n*-butanol to give 110 and 130g, respectively. The petroleum ether fraction (90g) was applied to Si gel column and eluted with *n*-hexan-ethyl acetate (100:0 → 50:50) to give four fractions of A (900mg), B (610mg) C (700mg) and D (1.37g). Fraction D (1.37g) was rechromatographed over Si gel column eluted with *n*-hexan-ethyl acetate (97 : 3) to give compound (**8**) (70mg). The ethyl acetate fraction (110g) was applied to Si gel column and eluted with chloroform-methanol (100:0 → 80:20)

to give four fractions of A (1.6g), B (1.3g) C (1.75g) and D (1.83g). Fraction B (1.3g) was rechromatographed over Si gel column eluted with chloroform-methanol (97:3) to give compound (**5**) (18mg). Fraction C (1.75g) was rechromatographed over Si gel column eluted with chloroform-methanol (95:5) to give compound (**7**) (67mg). Fraction D (1.83g) was rechromatographed over Si gel column eluted with chloroform-methanol (92:8) to give compound (**1**) (58mg) and compound (**6**) (30mg), respectively. The *n*-butanol fraction (130g) was chromatographed over Si gel column chromatography eluted with chloroform-methanol (100:0 → 50:50) to give four fractions of A (2.3g), B (3.57g), C (4.5g) and D (7.0g). Fraction A (2.3g) was rechromatographed over Si gel column eluted with chloroform-methanol (90:10) to give compound (**2**) (50mg). Fraction B (3.57g) was rechromatographed over Si gel column eluted with chloroform-methanol (90:10) to give compound (**3**) (85mg) and compound (**4**) (130mg), respectively. All isolated compounds (**1-8**) were further purified by gel filtration using Sephadex LH-20, eluted with methanol.

Compound (1): Yellow crystals from MeOH; UV λ_{max} (MeOH) nm: 266, 346, 354; λ_{max} (MeONa) nm: 270, 399, λ_{max} ($AlCl_3$) nm: 273, 387, λ_{max} ($AlCl_3$/HCl) nm: 275, 357, 387, λ_{max} (AcONa) nm: 270, 357, 407; λ_{max} (AcONa/Boric acid) nm: 270, 349; IR υ_{max} (KBr) cm^{-1}: 3360 (OH), 1627 (CO, ester), 1655 (CO, γ-pyrone) and 1610, 1578, 1497 (aromatic); ^{1}H NMR (DMSO-d_6, 400 MHz) aglycon δ 13.02 (1H, brs, OH-5), 7.60 (1H, d, *J* = 7.8 Hz, H-6`), 7.58 (1H, brs, H-2`), 6.98 (2H, brs, H-3, H-5`), 6.89 (1H, brs, H-8), 6.48 (1H, brs, H-6), 3.90 (3H, s, 3`-OMe), 3.67 (3H, s, 6``-$OCH_3$), sugar δ 5.33 (1H, d, *J* = 6.9 Hz, H-1``), 4.21 (1H, d, *J* = 9.0 Hz, H-5``), 3.40 (1H, t, *J* = 9.2 Hz, H-4``), 3.37 (1H, d, *J* = 8.6 Hz, H-3``), 3.33 (1H, d, *J* = 7.2 Hz, H-2``); ^{13}C NMR (DMSO-d_6, 100 MHz) aglycon δ 182.49 (C, C-4), 164.37 (C, C-2), 162.88 (C, C-7), 161.66 (C, C-5), 157.42 (C, C-9), 151.50 (C, C-4`), 148.57 (C, C-3`), 121.56 (C, C-1`), 121.01 (CH, C-6`), 116.29 (CH, C-5`), 110.80 (CH, C-2`), 105.96 (C, C-10), 103.87 (CH, C-3), 99.73 (CH, C-6), 95.0 (CH, C-8), sugar δ 169.70 (C, C-6``), 99.54 (CH, C-1``), 75.91 (CH, C-3``), 75.58 (CH, C-5``), 73.19 (CH, C-2``), 71.81 (CH, C-4``), 56.43 (CH_3, 3`-OMe), 52.46 (CH_3, 6``-OMe); ESIMS *m\z* 491 $[M+H]^+$.

Compound (2): Pale yellow amorphous powder; UV λ_{max} (MeOH) nm: 270, 345, 354; λ_{max} (MeONa) nm: 267, 335, 402, λ_{max} ($AlCl_3$) nm: 272, 305, 372, 391, λ_{max} ($AlCl_3$/HCl) nm: 272, 305, 357, 391, λ_{max} (AcONa) nm: 278, 357, 405, λ_{max} (AcONa/Boric acid) nm: 270, 355; IR υ_{max} (KBr) cm^{-1}: 3330 (OH), 1715 (acid CO), 1652 (CO, γ-pyrone) and 1602, 1498 (aromatic); ^{1}H NMR (DMSO-d_6, 400 MHz) aglycon δ 13.02 (1H, brs, OH-5), 7.59 (1H, m,

H-6`), 7.55 (1H, brs, H-2`), 6.98 (1H, d, *J* = 6.9 Hz, H-5`), 6.95 (1H, brs, H-3), 6.86 (1H, brs, H-8), 6.43 (1H, brs, H-6), 3.87 (3H, s, 3`-OMe), sugar δ 5.09 (1H, d, *J* = 7.2 Hz, H-1``), 3.16-3.75 (4H, m, H-2``-5``); $^{13}$C-DEPT NMR (DMSO-$d_6$, 100 MHz) aglycon δ 121.04 (CH, C-6`), 116.27 (CH, C-5`), 110.62 (CH, C-2`), 103.90 (CH, C-3), 100.16 (CH, C-6), 95.10 (CH, C-8), 56.43 ($CH_3$, 3`-OMe), sugar δ 99.96 (CH, C-1``), 76.69 (CH, C-3``), 73.90 (CH, C-5``), 73.31 (CH, C-2``), 72.25 (CH, C-4``); ESIMS *m\z* 477 $[M+H]^+$, 499 $[M+Na]^+$.

Compound (3): Yellow amorphous powder; UV λ_{max} (MeOH) nm: 254, 266sh, 348, (NaOMe) nm: 264, 300sh, 395, ($AlCl_3$) nm: 275, 299sh, 329, 430, ($AlCl_3$/HCl) nm: 274, 294sh, 358, 388, (NaOAc) nm: 259, 266sh, 364, 405, (NaOAc/boric acid) nm: 259, 374; IR υ_{max} (KBr) cm^{-1}: 3460 (OH), 1665 (CO), 1560, 1515 (C=C aromatic); ^{1}H NMR (DMSO-d_6, 400 MHz) aglycon δ 13.01 (1H, brs, OH-5), 7.45 (1H, dd, *J*=8.3, 1.9 Hz, H-6`), 7.43 (1H, d, *J*=1.9 Hz, H-2`), 6.90 (1H, d, *J*=8.3 Hz, H-5`), 6.79 (1H, d, *J*=1.9 Hz, H-8), 6.45 (1H, d, *J*=2.0 Hz, H-6), sugar δ 5.08 (1H, d, *J*=7.2 Hz, H-1``), 3.72 (1H, d, *J*=10.4 Hz, H-6``a), 3.48 (1H, d, *J*=10.5 Hz, H-6``b), 3.44 (1H, m, H-5``), 3.30 (1H, d, *t*=8.8 Hz, H-3``), 3.27 (1H, dd, *J*=8.8, 7.4 Hz, H-2``), 3.18 (1H, d, *J*=8.8 Hz, H-4``); ^{13}C NMR (DMSO-d_6, 100 MHz) aglycon δ 182.35 (C, C-4), 164.95 (C, C-2), 163.40 (C, C-7), 161.59 (C, C-5), 157.40 (C, C-9), 150.52 (C, C-4`), 146.28 (C, C-3`), 121.74 (C, C-1`), 119.64 (CH, C-6`), 116.44 (CH, C-5`), 113.98 (CH, C-2`), 105.79 (C, C-10), 103.59 (CH, C-3), 99.99 (CH, C-6), 95.18 (CH, C-8), sugar δ 100.34 (CH, C-1``), 77.62 (CH, C-5``), 76.85 (CH, C-3``), 73.58 (CH, C-2``), 70.00 (CH, C-4``), 61.07 ($CH_2$, C-6``); ESIMS *m\z* 471 $[M+Na]^+$.

Compound (4): Colorless crystals; UV λ_{max} (MeOH) nm: 212, 267 ; IR υ_{max} (KBr) cm^{-1}: 3415 (OH), 2920 (CH), 1610, 1596 (aromatic); ^{1}H NMR (DMSO-d_6, 400 MHz) aglycon δ 7.37 (1H, brd, *J* = 7.4 Hz, H-3), 7.20 (1H, td, *J* = 8.1, 1.4 Hz, H-5), 7.10 (1H, brd, *J* = 7.9 Hz, H-6), 7.01 (1H, brt, *J* = 7.3 Hz, H-4), 5.11, (1H, brs, OH), 5.05 (1H, brs, OH), 4.65 (1H, d, *J* = 14.3 Hz, H-7a), 4.46 (1H, d, *J* = 14.2 Hz, H-7b), sugar δ 5.37 (1H, brs, 4`-OH), 4.77 (1H, d, *J* = 7.4 Hz, H-1`), 3.71 (1H, d, *J* = 11.5 Hz, H-6`a), 3.47 (1H, dd, *J* = 12.00, 5.8 Hz, H-6`b), 3.36 (1H, m, H-3`), 3.33 (1H, m, H-4`), 3.29 (1H, m, H-5`), 3.16 (1H, dd, *J* = 9.0, 7.8 Hz, H-2`); ^{13}C NMR (DMSO-d_6, 100 MHz) aglycon δ 155.13 (C, C-1), 131.94 (C, C-2), 128.17 (CH, C-3), 127.64 (CH, C-5), 122.21 (CH, C-4), 115.23 (CH, C-6), 58.71 (CH_2, C-7), sugar δ 101.86 (CH, C-1`), 77.53 (CH, C-5`), 76.95 (CH, C-3`), 73.86 (CH, C-2`), 70.20 (CH, C-4`), 61.22 ($CH_2$, C-6`); ESIMS *m\z* 287 $[M+H]^+$, 309 $[M+Na]^+$.

Compound (5): Colorless crystals; UV λ_{max} (MeOH) nm: 213, 269; IR υ_{max} (KBr) cm^{-1}: 3420 (OH), 2920 (CH), 1745, 1723 (CO), 1610, 1590 (aromatic); ^{1}H NMR (DMSO-d_6, 400 MHz) aglycon δ 7.31 (1H, td, *J* = 8.2, 1.2 Hz, H-5), 7.24 (1H, dd, *J* = 7.5, 1.2 Hz, H-3), 7.17 (1H, brd, *J* = 8.2 Hz, H-6), 7.05 (1H, brt, *J* = 7.5 Hz, H-4), 6.50 (1H, brs, 9-OH), 6.12 (1H, dt, *J* = 9.7, 3.8 Hz, H-11), 5.75 (1H, d, *J* = 9.8 Hz, H-10), 5.08 (1H, d, *J* = 13.4 Hz, H-7a), 5.03 (1H, d, *J* = 13.2 Hz, H-7b), 2.72 (2H, t, *J* = 7.1 Hz, H-13), 2.50-2.57 (2H, m, H-12), sugar δ 5.45 (1H, brs, 3`-OH), 5.34 (1H, brs, 4`-OH), 5.12 (1H, d, *J* = 8.0 Hz, H-1`), 4.82 (1H, dd, *J* = 9.4, 8.2 Hz, H-2`), 3.76 (1H, d, *J* = 10.6 Hz, H-6`a), 3.47 (1H, m, H-6`b), 3.31 (1H, m, H-3`), 3.29 (1H, m, H-5`), 3.18 (1H, m, H-4`), 2.05 (3H, s, CH_3, acetyl); ^{13}C NMR (DMSO-d_6, 100 MHz) aglycon δ 206.47 (C, C-14), 170.51 (C, CO, acetyl), 170.01 (C, C-8), 154.68 (C, C-1), 132.12 (CH, C-10), 129.88 (CH, C-3), 129.11 (CH, C-11),128.53 (CH, C-5), 124.86 (C, C-2), 122.79 (CH, C-4), 115.38 (CH, C-6), 77.77 (C, C-9), 61.88 (CH_2, C-7), 36.03 (CH_2, C-13), 26.34 (CH_2, C-12), sugar δ 98.80 (CH, C-1`), 77.63 (CH, C-5`), 74.17 (CH, C-2`), 73.92 (CH, C-3`), 70.37 (CH, C-4`), 60.99 ($CH_2$, C-6`), 21.26 (CH_3, CH_3, acetyl); ESIMS *m\z* 489 $[M+Na]^+$.

Compound (6): White crystal needles; UV λ_{max} (MeOH) nm: 215, 325; IR υ_{max} (KBr) cm^{-1}: 3450 (OH), 2925 (CH), 1690 (CO), 1600, 1585 (aromatic); ^{1}H NMR (DMSO-d_6, 400 MHz) aglycon δ 7.33 (1H, d, *J* = 7.2 Hz, H-3), 7.29 (1H, dd, *J* = 7.6, 1.4 Hz, H-5), 7.16 (1H, d, *J* = 8.1 Hz, H-6), 7.03 (1H, t, *J* = 7.1 Hz, H-4), 5.30 (1H, d, *J* = 13.0 Hz, H-7a), 5.25 (1H, d, *J* = 13.1 Hz, H-7b), sugar δ 4.85 (1H, d, *J* = 7.4 Hz, H-1`), 3.71 (1H, dd, *J* = 12.0, 6.2 Hz, H-6`a), 3.48 (1H, dd, *J* = 11.6, 5.5 Hz, H-6`b), 3.36 (1H, t, *J* = 9.5 Hz, H-3`), 3.33 (1H, dd, *J* = 9.7, 7.4 Hz, H-2`), 3.28 (1H, m, H-5`), 3.20 (1H, t, *J* = 8.9 Hz, H-4`), caffoyl δ 7.53 (1H, d, *J* = 15.8 Hz, H-*β*), 7.16 (1H, d, *J* = 8.1 Hz, H-6``), 7.08 (1H, d, *J* = 1.8 Hz, H-2``), 6.77 (1H, d, *J* = 8.2 Hz, H-5``), 6.34 (1H, d, *J* = 15.9 Hz, H-*α*); ^{13}C NMR (DMSO-d_6, 100 MHz) aglycon δ 155.37 (C, C-1), 129.67 (CH, C-5), 129.15 (CH, C-3), 125.77 (C, C-2), 122.32 (CH, C-4), 115.46 (CH, C-6), 61.15 (CH_2, C-7), sugar δ 101.27 (CH, C-1`), 77.56 (CH, C-5`), 77.01 (CH, C-3`), 73.79 (CH, C-2`), 70.16 (CH, C-4`), 61.18 ($CH_2$, C-6`), caffoyl δ 166.99 (C, CO), 149.0 (C, C-4``), 146.06 (C, C-3``), 145.90 (CH, C-*β*), 125.95 (C, C-1``), 119.70 (CH, C-6``), 116.15 (CH, C-2``), 115.40 (CH, C-5``), 114.22 (CH, C-*α*); ESIMS *m\z* 471 $[M+Na]^+$.

Compound (7): White crystal needles; UV λ_{max} (MeOH) nm: 205, 319; IR υ_{max} (KBr) cm^{-1}: 3390 (OH); 2930 (CH); 1685 (C=O); 1610, 1587 (aromatic); ^{1}H NMR (DMSO-d_6, 400 MHz) aglycon δ 7.34 (1H. d, *J* = 7.3 Hz, H-3), 7.29 (1H, dd, *J* = 7.6, 1.4 Hz, H-5), 7.17 (1H, d, *J* = 8.1 Hz, H-6), 7.03 (1H, t, *J* = 7.5 Hz, H-4), 5.31 (1H, d, *J* = 13.0 Hz, H-7a), 5.26 (1H, d, *J* = 13.1 Hz, H-7b), sugar δ 4.86 (1H, d, *J* = 7.2 Hz, H-1`), 3.71 (1H, d, *J* = 10.5 Hz, H-6`a), 3.48 (1H, dd, *J* = 11.8, 5.6 Hz, H-6`b), 3.36 (1H, t, *J* = 9.5 Hz, H-3`), 3.33 (1H, dd, *J* = 9.7, 7.2 Hz, H-2`), 3.28 (1H, m, H-5`), 3.19 (1H, d, *J* = 9.1 Hz, H-4`), *p*-coumaroyl δ 7.61 (1H, d, *J* = 15.9 Hz, H-*β*), 7.57 (2H, d, *J* = 8.5 Hz, H-2``, 6``). 6.80 (2H, d, *J* = 8.4 Hz, H-3``, H-5``), 6.47 (1H, d, *J* = 15.9 Hz, H-*α*); ^{13}C NMR (DMSO-d_6, 100 MHz) aglycon δ 155.62 (C, C-1), 129.71 (CH, C-5), 129.12 (CH, C-3), 125.73 (C, C-2), 122.26 (CH, C-4), 115.44 (CH, C-6), 61.20 (CH_2, C-7), sugar δ 101.51 (CH, C-1`), 77.54 (CH, C-5`), 77.01 (CH, C-3`), 73.79 (CH, C-2`), 70.17 (CH, C-4`), 61.20 ($CH_2$, C-6`), *p*-coumaroyl δ 167.06 (C, CO), 160.37 (C, C-4``), 145.46 (CH, C-*β*), 130.86 (CH, C-2``, C-6``), 125.55 (C, C-1``), 116.24 (CH, C-3``, C-5``), 114.53 (CH, C-*α*); ESIMS *m\z* 433 $[M+H]^+$, 455 $[M+Na]^+$.

Compound (8): Colorless needles; IR (KBr) υ_{max} cm^{-1}: 3425 (OH), 1645 (C=C); ^{1}H NMR (*CDCl*$_3$, 400 MHz) δ 5.34 (1H, m, H-6), 3.51 (1H, m, H-3), 1.00 (3H, s, Me-18), 0.91 (3H, d, *J*=6.5 Hz, Me-21), 0.84 (3H, t, *J*=7.0 Hz, Me-29), 0.82 (3H, d, *J*=6.5 Hz, Me-26), 0.80 (3H, d, *J*=6.5 Hz, Me-27), 0.67 (3H, s, Me-19); ^{13}C NMR (*CDCl*$_3$,100.0 MHz) δ 140.58 (C, C-5), 121.70 (CH, C-6), 71.79 (CH, C-3), 56.74 (CH, C-14), 56.03 (CH, C-17), 50.11 (CH, C-9), 45.81 (CH, C-24), 42.26 (CH_2, C-4), 42.26 (C, C-13), 39.75 (CH_2, C-12), 37.23 (CH_2, C-1), 36.38 (C, C-10), 36.13 (CH, C-20), 33.92 (CH_2, C-22), 31.88 (CH_2, C-7), 31.88 (CH, C-8), 31.62 (CH_2, C-2), 29.12 (CH, C-25), 28.23 (CH_2, C-16), 26.04 (CH_2, C-23), 24.29 (CH_2, C-15), 23.05 (CH_2, C-28), 21.07 (CH_2, C-11), 19.81 (CH_3, C-26), 19.39 (CH_3, C-27), 19.02 (CH_3, C-21), 18.77 (CH_3, C-19), 11.97 (CH_3, C-29), 11.85 (CH_3, C-18); ESIMS m\z 415 $[M+H]^+$, 437 $[M+Na]^+$.

Acid hydrolysis of compound 1.

Compound 1 (5mg) was refluxed with 7% HCl (10mL) at 70^{0}C for 3h. After cooling, the reaction mixture was diluted with H_2O and then extracted with CH_2Cl_2 (2mL each) four times. The combined organic layers were evaporated to dryness to give residue, which was identified as chrysoeriol on the basis of TLC comparison with an authentic sample. The aqueous layer was neutralized with sodium carbonate and concentrated to 1ml under reduced pressure and compared with standard sugars using TLC [Si gel, ($CHCl_3$-MeOH-

H_2O:30:12:4), 9ml of lower layer and 1ml of HOAc], which indicated the sugar of **1** to be glucuronic acid.

Animals

White male albino rats weighing between (120 and 170gm) and white male albino mice weighing between (25 and 30gm) were selected for evaluation of the anti-inflammatory and analgesic activity, respectively. All animals were housed 6 per cage and kept at the laboratory animal home of the Faculty of Pharmacy, Al-Azhar University, Cairo, Egypt under standard environmental conditions of room temperature at 25 ± 2°C with 12hr light/dark cycle and had free access to standard diet and water. All animal procedures were performed in accordance with the Ethics Committee of the Faculty of Pharmacy, Al-Azhar University, Cairo, Egypt, and followed the recommendations for the proper care and use of laboratory animals (NIH publication No. 85-23, revised 1985).

Analgesic Activity

Hot plate method

The hot plate method,[11,12] was followed for the assessment of analgesic activity. Eddy's hot plate maintained at a temperature of 55 ± 1°C was used. Acetylsalicylic acid (ASPEGIC)® vial (AMRIYA For Pharmaceutical Industries; Egypt) was used as positive control.

Acetic acid induced writhing method

The analgesic activity of the samples was evaluated using acetic acid induced writhing method in mice.[13] Acetylsalicylic acid (ASPEGIC)® vial (AMRIYA For Pharmaceutical Industries; Egypt) was used as positive control.

Anti-inflammatory activity

Anti-inflammatory activity was measured using carrageenan induced rat paw edema method (1% w/v carrageenan (Winlab, England) suspension in 0.9% NaCl solution).[13,14] Vernier caliper, calibrated from 0.05 to 200 mm (Stecox Wuppertal-Germany) was used for linear paw circumference measurement. Acetylsalicylic acid (ASPEGIC)® vial (AMRIYA For Pharmaceutical Industries; Egypt) was used as positive control.

Statistical analysis

All values were presented as means ± standard error of the means (SEM). Statistical analysis was performed using Graph-Pad Prism version 5 (Graph-Pad, San Diego, CA). A comparison

between different groups was carried out using one-way analysis of variance (ANOVA), followed by Tukey–Kramer's multiple comparison tests. The difference is considered significant when $p < 0.05$.

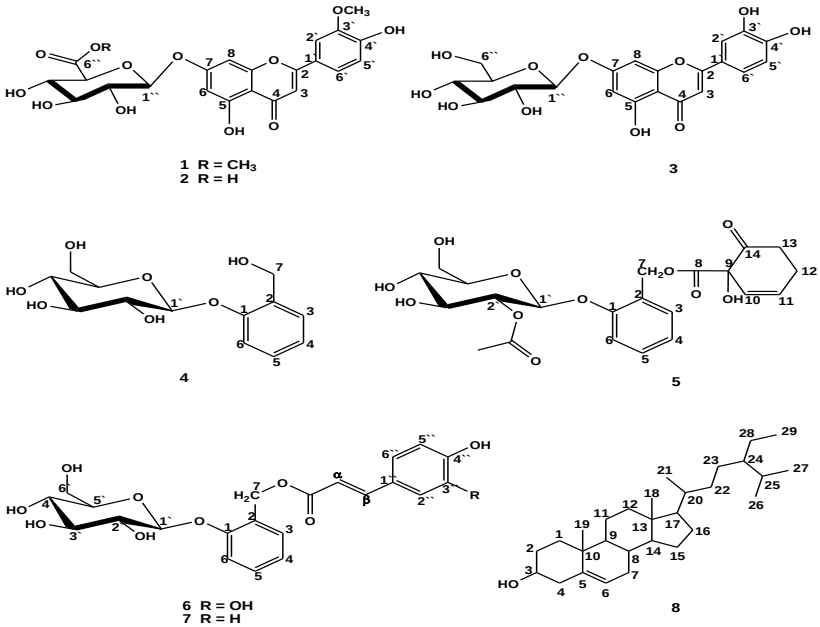


Fig. 1: Structures of compounds 1-8

RESULTS AND DISCUSSION

From EtOAc extract of *Ficus laurifolia*, after multi-stage column chromatography, one new flavonoid (**1**) was isolated and additionally seven known compounds (**2**-**8**) (Figure 1) were obtained from this plant for the first time and identified by comparison of their spectroscopic data with the corresponding literature values as chrysoeriol 7-*O*-glucuronoide (**2**),[15] luteolin 7-*O*-*β*-D-glucopyranoside (cinaroside) (**3**),[16] salicyl alcohol glucoside (salicin) (**4**),[17,18] 2`-acetylsalicortin (**5**),[19] 2-(*β*-D-glucopyranosyloxy)-benzyl (3,4-dihydroxy) cinnamoate (populoside) (**6**) and 2-(*β*-D-glucopyranosyloxy)-benzyl (4-hydroxy) cinnamoate (populoside B) (**7**),[20] and β-sitosterol (**8**).[21]

The new compound **1** was obtained as yellow crystals. Its molecular formula was deduced to be $C_{23}H_{22}O_{12}$ from ESIMS molecular ion peak at *m/z* 491 $[M+H]^+$. Its IR spectrum indicated the presence of hydroxyl groups (3360), ester carbonyl group (1727), γ-pyrone carbonyl group (1655) and aromatic ring (1610, 1578 and 1497). The UV spectrum of **1** showed absorption maxima at 266, 347 and 354 nm which are typical for flavones and flavonols.[22] The bathochromic shift with $AlCl_3$, which did not show any change on the addition of HCl, showed the presence of a chelated OH group at C-5. The addition of NaOMe caused a

bathochromic shift in band I suggesting that the C-4` phenolic hydroxy group is free. Complete analysis of the 1D and 2D NMR (HSQC and HMBC) data of compound **1** (see Supplementary Data **Table S1**) revealed chrysoeriol algycon and a *β*-D-glucouronic acid methyl ester moiety attached to C-7 of the flavonoid was identified. In the ^{1}H NMR spectrum of **1**, two broad singlets at δ 6.48 and 6.89, which correlated with their corresponding carbons at δ 99.73 and 95.0 in the HSQC spectrum, were assigned to H-6 and H-8 of the A ring, respectively. The ^{1}H NMR spectrum showed also signals of a 3`, 4`-substituted B ring at δ 7.58 (1H, brs, H-2`) and δ 7.60 (1H, d, $J$ = 7.8 Hz, H-6`). The broad singlet at δ 6.98 (integrating for two protons) which showed HSQC correlations with two carbons at δ 116.29 (C-5`) and δ 103.87 (C-3), was assigned to H-5` of B ring and H-3 of C ring. This further confirmed by HMBC correlations observed between the proton at δ 6.98 (H-3) and C-2 (164.37), C-4 (182.49) and C-10 (105.96) and between the proton at δ 6.98 (H-5`) and C-4` (151.50) and C-6` (121.01). In the HMBC spectrum, a singlet methoxyl proton signal at δ 3.90 ppm was correlated with the signal at δ 148.57, confirmed the C-3` methoxyl group. These data indicated that the aglycon of **1** was a 5, 7, 4` tri-oxygenated, 3`-methoxylated flavone derivative which is in good agreement with chrysoeriol.[23] Acid hydrolysis of **1** yielded glucuronic acid and chrysoeriol, as determined by the TLC comparison with standards. ^{13}C NMR spectrum of compound **1** also supported the structure assignment made above on the bases of comparison with those of chrysoeriol. It also revealed that sugar moiety consisted of one molecule of glucuronic acid methyl ester, based on the existence of one anomeric carbon signal at δ 99.54 ppm (C-1``), and the presence of signals at δ 169.70 ppm and $\delta$ 52.46 ppm typical of 6``-methyl ester (COOMe) of glucuronic acid.[24,25] Also in the ^{1}H NMR spectrum the presence of methyl ester group was evidenced by three-proton singlet at δ 3.67 and additionally one-proton doublet at δ 4.21 (J =9 Hz) characteristic of O-CH-COOMe (H-5``) and one-proton doublet at $\delta$ 5.33 ($J$ =6.9 Hz) corresponding to an anomeric proton of sugar (H-1``) which has *β*-D-configuration. Furthermore, an HMBC correlation between H-5 and a carboxylic carbon at δ 169.70 indicated an oxidized C-6, thus establishing a glucopyranoside uronic acid moiety in **1** with a $^{4}C_{1}$ conformation. The methoxy singlet proton signal at δ 3.67 was correlated with the carbonyl carbon signal at δ 169.70 in the HMBC spectrum, which confirmed the 6``-methyl ester of glucouronic acid. The pyranose form and the anomeric *β*-configuration of glucopyranoside uronic acid were deduced from its ^{13}C NMR data and from its chemical shift and $^{3}J_{H1,H2}$ coupling constant, respectively.[26] Substitution at position 7 with a glucopyranoside uronic acid unit was indicated by the UV spectra upon addition of diagnostic shift reagents and from correlations in the HMBC NMR

spectrum, which exhibited a cross-peak between the anomeric proton H-1`` at C-7. On the basis of above analysis the structure of Luteolin 3`-methyl ether-7-*O*-(6``-methyl glucuronoide) [chrysoeriol-7-*O*-(6``-methylglucuronoide)] was assigned to compound **1**.

Analgesic activity

Results of hot plate test are presented in Table 1 and Figure 2a. Both *n*-butanol fraction and alcoholic extract of the plant significantly increased the reaction time of heat sensation in mice at a dose of 150mg/kg body weight (P<0.05) when compared with the control. It is an established fact that any agent that causes a prolongation of the hotplate latency using this test must be acting centrally.[11] Therefore, the *n*-butanol fraction and the alcoholic extract might have a central activity. The administration of the test drugs at a dose of 150mg/kg body weight significantly (p<0.05) attenuated number of acetic acid induced abdominal writhes compared with the acetic acid group as shown in Table 1 and Figure 2b. The reduction of abdominal writhes may be due to inhibition of prostaglandin synthesis, a peripheral mechanism of pain inhibition.[11]

Table 1: Analgesic effect of *Ficus laurifolia* leaves.

Group	Effect on thermally induced pain	Effect on acetic acid-induced writhing	
	Mean latency period (sec.)	Mean no. of writhing/10 min.	Percent of inhibition
Control	5.79 ±0.4126	42.5 ±0.7638	0%
Alcoholic extract	9.18* ±0.2330	22.83* ±1.078	46%
Ethyl acetate fraction	7.3 ±0.3396	29.33* ±0.7149	31%
***n*- Butanol fraction**	12.12* ±0.5653	16.83* ±0.4773	60%
Acetyl salicylic acid	14.67* ±0.5678	15.67* ±0.3333	63%

*Data are represented as the mean ± SEM, (n=6); *P < 0.05, were considered significantly different in comparison with control*.

Anti-inflammatory activity

The anti-inflammatory effects are presented in Table 2 and Figure 3. In tested animals, the sub plantar injection of carrageenan produced a local edema that increased progressively to reach a maximal intensity 3 hours after injection. The administration of different extracts of *Ficus laurifolia* leaves significantly (p<0.05) inhibited inflammatory response induced by carrageenan in rats. The most prominent inhibition (79%) was observed by *n*-butanol fraction (150mg/kg), at the 3rd hour of the study in comparison with the carrageenan group. This

significant effect may be due to inhibition of the mediators of inflammation such as histamine, serotonin and prostaglandin.

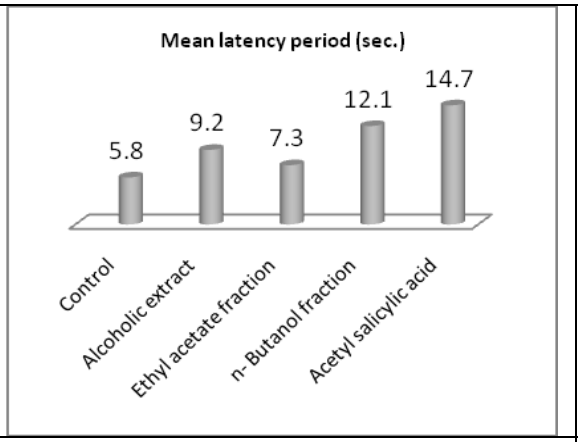


Fig. 2a: Effect on thermally induced pain

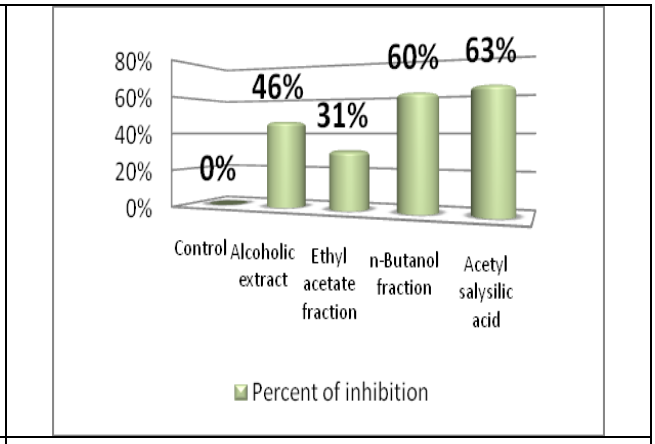


Fig. 2b: Effect on acetic acid-induced writhing

Table 2: Anti-inflammatory activity of *Ficus laurifolia* leaves.

	Linear paw circumference			Mean of change in paw size		Percent of inhibition	
Group	**0**	**3hrs**	**6hrs**	**After 3 hrs**	**After 6 hrs**	**After 3 hrs**	**After 6 hrs**
Placebo	0.3 ±0.010	0.3 ±0.010	0.3 ±0.010	0	0	---	---
Control	0.274 ±0.006	0.656 ±0.035	0.6 ±0.019	0.3803	0.32493	0%	0%
Alcoholic extract	0.242 ±0.008	0.418* ±0.012	0.383* ±0.009	0.17567	0.14117	54%	57%
Ethyl acetate fraction	0.267 ±0.007	0.448* ±0.009	0.398* ±0.007	0.1809	0.13108	52%	60%
***n*- Butanol fraction**	0.265 ±0.009	0.346* ±0.013	0.326* ±0.010	0.08045	0.06025	79%	81%
Acetyl salicylic acid	0.248 ±0.006	0.317* ±0.009	0.304* ±0.006	0.06842	0.05527	82%	83%

*Data are represented as the mean ± SEM, (n=6); *P < 0.05 were considered significantly different in comparison with Control group*

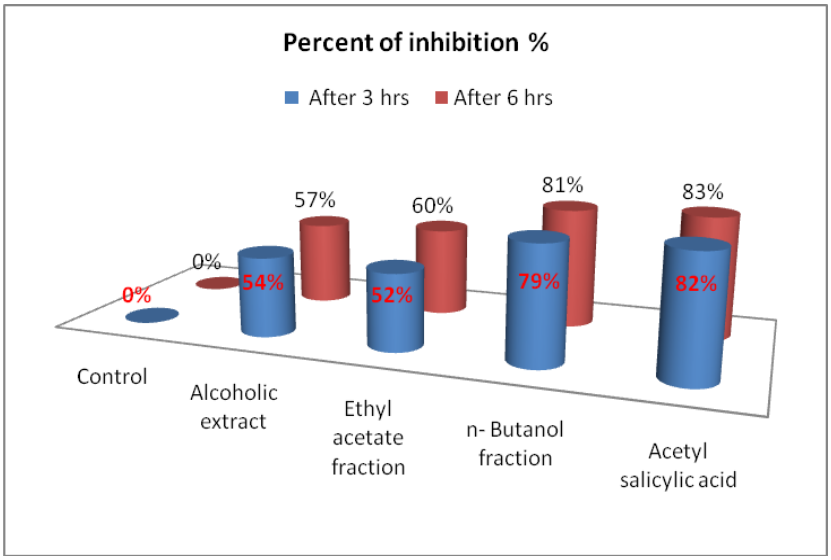


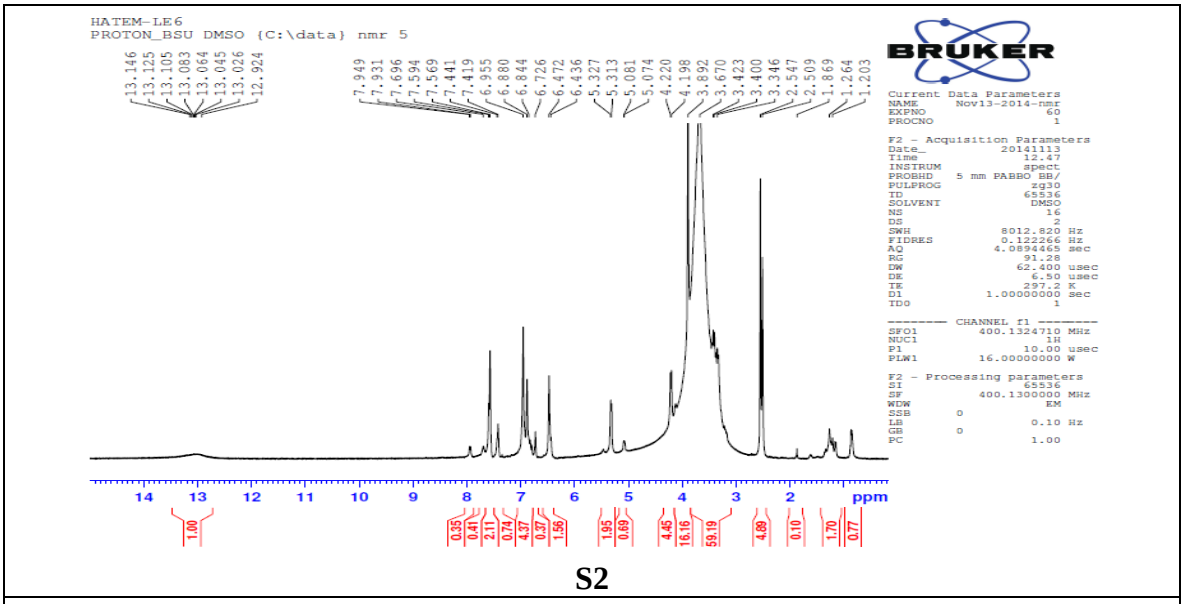
Fig. 3: Anti-inflammatory activity.

Supplementary data: Complete analysis of the 1D and 2D NMR (HSQC and HMBC) data and both ESIMS and UV of compound **1** represented by S1, S2, S3, S4, S5, S6, and S7.

Table S1: 1D and 2D-NMR spectral data of compound 1 (DMSO-d_6, 400 and 100 MHz)

Position	^{1}H (*J* in Hz)	^{13}C	HMBC
2	-	164.37	-
3	6.98, brs	103.87	C-2, 4, 10, 1`
4	-	182.49	-
5	-	161.66	-
6	6.48, brs	99.73	C-5, 7, 8, 10
7	-	162.88	-
8	6.89, brs	95.0	C-6, 7, 9, 10
9	-	157.42	-
10	-	105.96	-
1`	-	121.56	-
2`	7.58, brs	110.80	C-2, 1`, 3`, 4`, 6`
3`	-	148.57	-
4`	-	151.50*	-
5`	6.98, brs	116.29	C-4`, 6`
6`	7.60, d, 7.8	121.01	C-2, 1`, 2`
1``	5.33, d, 6.9	99.54	C-7
2``	3.33, d, 7.2	73.19	C-1``, 3``
3``	3.37, d, 8.6	75.91	C-2``, 4``
4``	3.40, t, 9.2	71.81	C-3``, 5``
5``	4.21, d, 9.0	75.58	C-1``, 3``, 4``, 6``
6``	-	169.70	-
3`-OCH_3	3.90, s	56.43	C-3``
6``-OCH_3	3.67, s	52.46	C-6``
5-OH	13.02, brs	-	-

** Obtained from HMBC correlations.*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BRUKER
ppm

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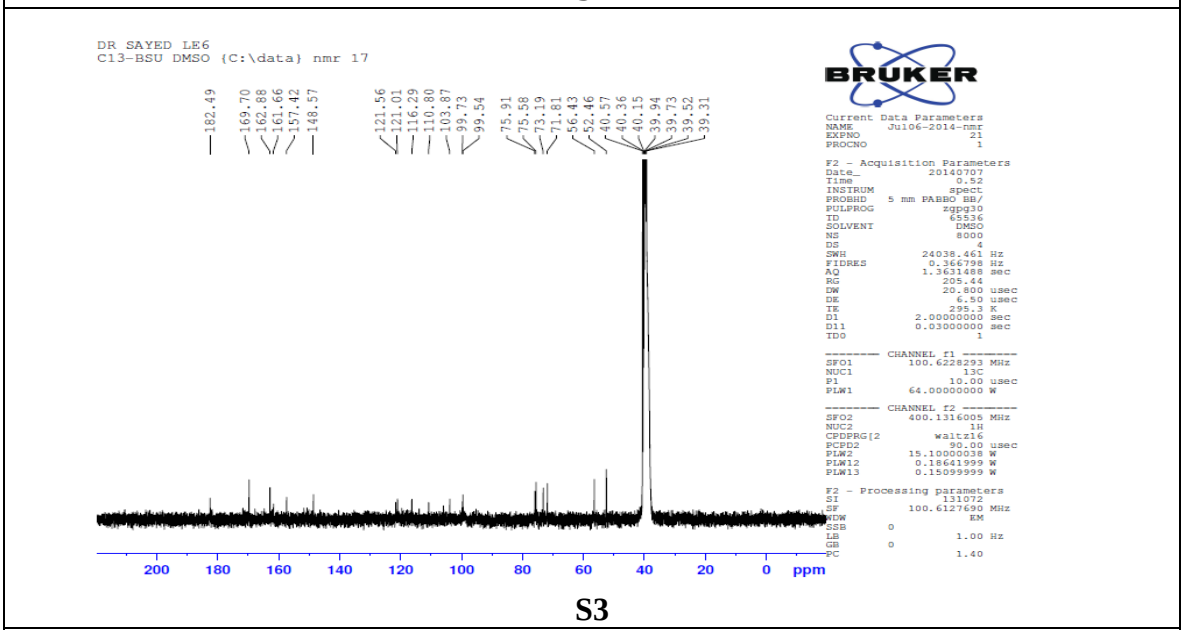
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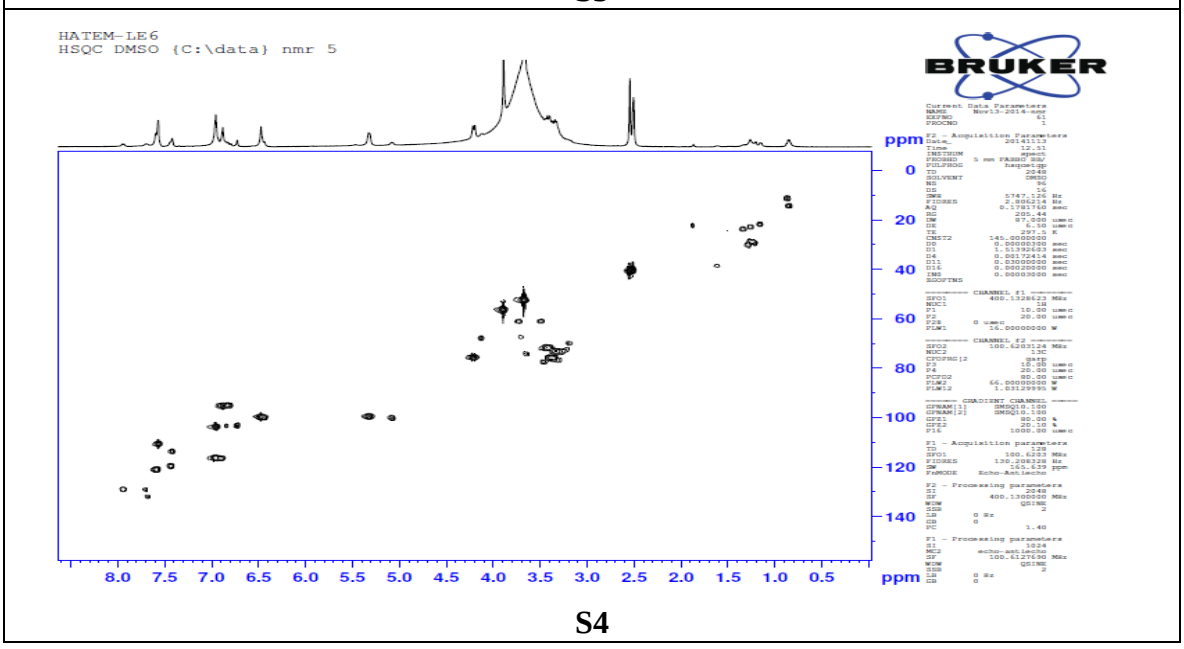
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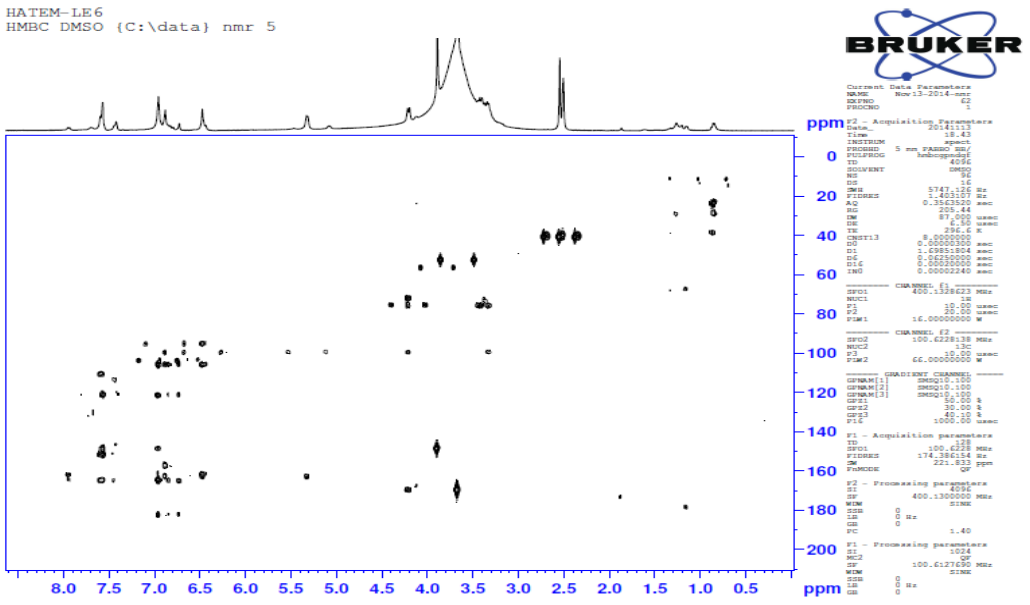
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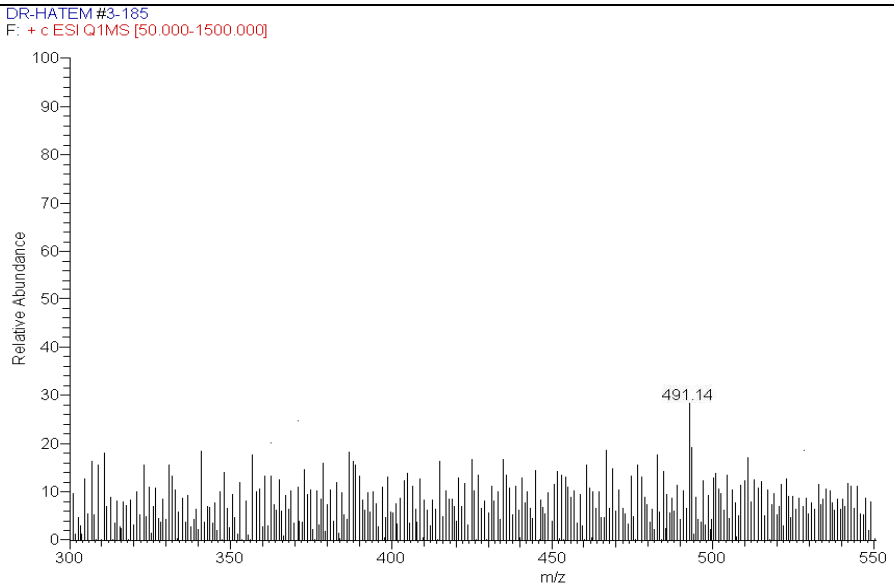
S5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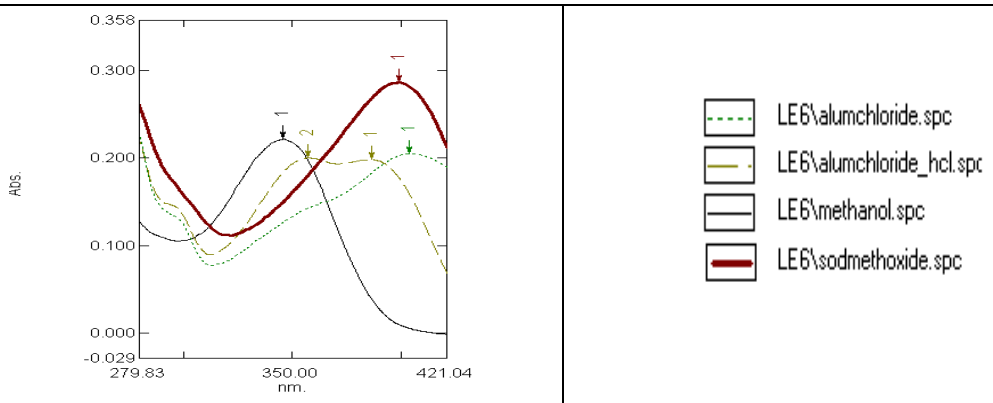
m/z

S6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nm.
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LE6\sodmethoxide.spc

S7

CONCLUSION

The present study has identified the isolation and characterization of a new flavone glucuronic acid methyl ester along with seven known compounds; two flavone glycosides, four salicin derivatives and one sterol for the first time from the leaves of *Ficus laurifolia*. The present study also indicates the efficacy of *Ficus laurifolia* extracts especially the *n*-butanol fraction as an efficient therapeutic agent in pain reduction and in acute anti-inflammatory conditions. This activity may be due to salicin and salicin derivatives.

Supplementary Data

Complete analysis of the 1D and 2D NMR (HSQC and HMBC) data, table of compound (**1)** along with the NMR and MS spectra are available in the online edition of this article.

ACKNOWLEDGMENTS

We are grateful to Dr. Ahmed Mansour, Assoc. Prof. of Pharmacology, Faculty of Pharmacy, Al-Azhar University, Cairo, Egypt, for his help in carrying out the analgesic and anti-inflammatory activity.

REFERENCES

1. Wagner WL, Herbst DR, Sohmer SH. Manual of the Flowering Plants of Hawaii, Vol. 2, University of Hawaii and Bishop Museum Press, Honolulu: 1999.
2. Ripu, MK, Rainer WB. Ficus (Fig) species in Nepal: A review of diversity and indigenous uses. Lyonia, 2006; 11: 85–97.
3. Annan K. Antibacterial and wound healing properties of some indigenous Ghanaian plants. Ph.D. Thesis, University of London, 2007; UK: 30–32.
4. Pandita R, Phadkeb A, Jagtapa A. Antidiabetic effect of Ficus religiosa extract in streptozotocin-induced diabetic rats. Journal of Ethnopharmacology, 2010; 128: 462–66.
5. Veberic R, Colaric M, Stampar F. Phenolic acids and flavonoids of fig fruit (Ficus carica L.) in the Northern Mediterranean region. Food Chemistry, 2008; 106: 153–57.
6. Li YC, Kuo YH. A monoterpenoids and two simple phenols from heartwood of Ficus microcarpa. Phytochemistry, 1998; 49(8): 2417-19.
7. Chang MS, Yang YC, Kuo YC, Kuo YH, Chang C, Chen, CM, Lee TH. Furocoumarin glycosides from the leaves of Ficus ruficaulis Merr. var. antaoensis. J. Nat. Prod., 2005; 68(1): 11–13. Erratum in J Nat Prod., 2005 Apr; 68(4): 634.

8. Rubnov S, Kashman Y, Rabinowitz R, Schlesinger M, Mechoulam R. Suppressors of cancer cell proliferation from Fig (Ficus carica) resin: Isolation and structure elucidation. J. Nat. Prod., 2001; 64: 993-96.
9. Chiang YM, Chang JY, Kuo CC, Chang CY, Kuo YH. Cytotoxic triterpenes from the aerial roots of Ficus microcarpa. Phytochemistry, 2005; 66: 495–501.
10. Baumgartner B, Erdelmeier CAJ, Wright AD, Rali T, Sticher O. An antimicrobial alkaloid from Ficus septica. Phytochemistry, 1990; 29: 3327–30.
11. Das B, Ferdous T, Mahmood QA, Hannan JMA, Bhattacharjee R, Das BK. Antinociceptive and anti-inflammatory activity of the bark extract of Plumeria rubra on laboratory animals. European Journal of Medicinal Plants, 2013; 3(1): 114-126.
12. Turner RA, Hebban P. Analgesics: Screening methods in pharmacology. New York; Academic Press: 1965; 100.
13. Onasanwo SA, Elegbe RA. Anti-nociceptive and anti-inflammatory properties of the leaf extracts of Hedranthera barteri in rats and mice. African Journal of Biomedical Research, 2006; 9: 109-117.
14. Ratheesh M, Helen A. Anti-inflammatory activity of Ruta graveolens Linn. on carrageenan induced paw edema in wistar male rats. African Journal of Biotechnology, 2007; 6(10): 1209-1211.
15. Kwak JH, Kim HJ, Lee KH, Kang SC, Zee OP. Antioxidative iridoid glycosides and phenolic compounds from Veronica peregrine Arch. Pharm Res., 2009; 32(2): 207-213.
16. Malikova VM, Yuldashev MP. Phenolic compounds of plants of the Scutellaria L. genus. Distribution, structure and properties. Chemistry of natural compounds, 2002; 38(4): 406.
17. Mizuno M, Kato M, Misu M, Inuma M, Anaka T. Chaenomeloidin A phenolic glucoside from leaves of Salix chaenomeloides. J. Nat. Prod., 1991; 54(5): 1447-1450.
18. Wei XH, Yang SJ, Liang N, Hu DY, Jin LH, Xue W, Yang S. Chemical constituents of Caesalpinia decapetala (Roth) Alston. Molecules, 2013; 18: 1325-1336.
19. Reichardt PB, Merken HM, Clausen TP. Phenolic glycosides from Salix lasiandra. J. Nat. Prod., 1992; 55(7): 970-973.
20. Zhang X, Thuong PT, Min B, Ngoc TM, Hung TM, Lee IS, Na M, Seong Y, Song K, Bae K. Phenolic glycosides with antioxidant activity from the stem bark of Populusda vidiana. J. Nat. Prod., 2006; 69: 1370-1373.
21. Chaurasia N, Wichtl M. Sterols and steryl glycosides from Urtica dioica. J. Nat. Prod., 1987; 50(5): 881-885.

22. Markham KR. Techniques of Flavonoid Identification. Academic Press, London: 1982; 251–262.
23. Awaad SA, Maitland DJ, Soliman GA. Hepatoprotective activity of Schouwia thebica webb. Bioorganic & Medicinal Chemistry Letters, 2006; 16: 4624–4628.
24. Nazaruk J, Jakoniuk P. Flavonoid composition and antimicrobial activity of Cirsiumri vulare (Jacq.) All. Flowers. Journal of Ethnopharmacology, 2005; 102: 208–212.
25. Du Q, Cui H. A new flavone glycoside from the fruits of Luffa cylindrical. Fitoterapia, 2007; 78: 609–610.
26. Gorin PAJ, Mazurek M. Further studies on the assignment of signals in ^{13}C magnetic resonance spectra of aldoses and derived methyl glycosides. Can. J. Chem., 1975; 53: 1212-1223.