A NEW BIFLAVONOID FROM LONICERA SEMPERVIRENS

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ABSTRACT

Phytochemical investigation of both the chloroform and ethyl nortate estincts of Londorn semperatures led to the including of seven compounds identified as betalin I, a new flavonoid compound named (londsemperin) scutcharein-(6-O-I)-accordin 2, luteolin 3, 8-sitosterol 3-O-β-D-glucoside 4, from the chloroform extract and accordin-7-O-β-D-glucoside 5, lutenin-7-O-β-D-glucoside 6 and apigenin-7-O-β-D-neohesperidoside 7, from the ethyl acctate extract. The isolated compounds were identified according to their chemical and spectral data. The antifungal activity of the extracts and some of the isolated compounds were performed using Aspergillus parasiticum which secrets aflatosines

INTRODUCTION

Lonicera sempervirens (trumpet Honeysuckle) is a wild plant, belongs to family Caprifoliaceae. The genus Lonicera (Honeysuckle) comprises 200 species of evergreen and deciduous flowering shrubs and woody climbers. The flowers are basically tubular with diverging lips. Those of the climber are often fragrant⁽¹⁾. It is native to south-eastern USA, but widely cultivated and escaping elsewhere.

The herbage of Honeysuckles is a favourate food of goats, therefore, the latin name Caprifolium (goat's leaf). The French chevrefeuille, German geißblatt and Italian capri-foglio, all signifying the same. The berries have been used as food for chicken. Some species of Lonicera are used in Chinese and Korean herbal medicine for their antipyretic, detoxicant and anti-inflammatory actions (2.3) and for treatment of hepatitis and stomatitis (4). Others were used as anti-bacterial and anti-viral to treat fever, dysentry, enteritis, pneumonia, encephalitis, and influenza (5).

Genus Lonicera is characterized by its contents of flavonoids, irridoids, triterpenoids^(2,44-5). On reviewing the appropriate literature, it was apparent that there are no previous scientific reports on Lonicera sempervirens, therefore it was considered to be of interest to carry out the present study on this plant.

EXPERIMENTAL

Plant material:

The aerial parts of *L. sempervirens* were collected at the flowering stage from the wild plants growing at Mansoura area during the period from April to May, 2002. The plant material was identified by Dr. I. Mashaly, Department of Botany, Faculty of Science, Mansoura University. A voucher specimen documenting this collection has been deposited at the Pharmacognosy Department, Faculty of Pharmacy, Mansoura University.

General experimental procedure:

CC silica gel Merk, 70-230 mesh. TLC silica gel 60 F₂₅₄ precoated plates (E-Merk, Germany). UV spectra: Beckmann DU-7 spectrophotometer. IR spectra: Nicolet, Mx-1 FT-IR spectrophotometer, USA. H-, ¹³C-, and COSY NMR spectra: JEOL spectrometer, 400 and 500 MHz

Extraction and isolation:

The air-dried powdered leaves of Lonicara sempervirens (1.7 kg) were extracted with methanol till exhaustion. The methanolic extract (200 gm) was concentrated in a rotary evaporator at 40°C to 200 ml. diluted with distilled water (100 ml) and successively extracted with petroleum other, chloroform, ethylacetate and n-butanol. The chloroform extract (20 g) was loaded on a silica gel packed column (5×106 cm. 300 g) then gradiently eluted with CHCl₁ containing increasing propotions of MeOH. Fractions of 100 ml were collected, concentrated and munitioned by TLC on scilia gel G plates using CHCl-McOH (9.5.0.5). (9:1) or (8:2) as solvents systems and vanillin-H-SO₂ and KOH as spray reagents. Similar fractions were pooled together and concentrated to give four fractions A, B, C and D. Each fraction was purified on a another column of silica gel using the same elating system to affored pure compounds 1 (40 mg), 2 (30 mg), 3 (30 mg) and 4 (25 mg). The ethyl accetate extract was applied as a band on a silica get column Elution was performed using CHCl3-MeOH-H3O with increasing polarity (90:10:1), (80:20:2) and (70:30.3). Fractions of 100 ml were collected together and concentrated to affored four fractions. Each one was purified on a silica gel column using EtOAc-MeOH-H2O with increasing proportions to affored four compounds 5 (8 mg), 6 (7 mg), 7 (9 mg).

Antifungal activity:

The phytopathogenic fungal strain used was Aspergillus parasiticum NRRL 2999 obtained from the National Research Center at El-Doki, Giza, Egypt. The fungus strain was maintained at 5°C until used The antifungal activity of the chloroform and ethylacetate extracts of L. sempervirens leaves, together with the isolated compounds 1, 2, 3, 4, and 5 was assessed using methods described by Farag et. al (1986)[9]and Chkhikvishvili and Gogiya (1995)[16] Ten-mi portions of Potato Dextrose Agar medium (PDA) were placed in Petri dishes. The spores suspension of the fungus was poured in the center of solid agar surface (control). Different quantities of each extract or tested compounds were mixed thoroughly with 10 ml of melted PDA mediam to give final concentrations of 500, 1000, and 2000 ppm (0.5 mg, I mg and 2 mg / 10 ml melted PDA medium) then poured into Petri dishes. All plates were incubated at 28°C for 7 days after which the fungal growth diameter was measured (mm) for each plate and compared with control. The percentage of inhibition was calculated as the difference between the growth diameter of control and that of the tested extract or compound divided by the growth diameter of control and multiplied by 100.

Characterization of the isolated compounds:

Compound 1 (betulin) occurred as a white powder It gave a deep pink-violet color with vanillin-H2SO4 spray reagents and heating for one minute. It gave a positive Liebermann- Burchard's test. M.p. 236-238 °C. R_F: 0.34 [silica gel, CH₂Cl_{2*} MeOH, 9:1] UV \(\)_max (CHCl₃):241 nm. ¹H-NMR spectral data (400 MHz, CDCl₃) δ 4.68 and 4.58 (2H, 2 br.s, CH₂-29), 3.8 and 3.33 (2H, 2 d, J=11 Hz, CH₂OH-28), 3.19 (m, H-3), 2.38 (m, H-19), 1.68 (3H, s, CH₂-30) and five singlet each equal to 3 H at δ 1.02, 0.98, 0.97, 0.82 and 0.76 ppm assigned for CH3- 24, 25, 23, 27 and 26, respectively. ¹³C-NMR data (67.5 MHz, CDCl₂) at δ 38.75 (C-1), 27.45 (C-2), 78.98 (C-3), 38.91 (C-4), 55.30 (C-5), 18.39 (C-6), 34.29 (C-7), 40.96 (C-8), 50.42 (C-9), 37.21 (C-10), 20.91 (C-11), 25.28 (C-12), 37.36 (C-13), 42.75 (C-14), 27.12 (C-15), 29.24 (C-16), 47.82 (C-17), 48.79 (C-18), 47.82 (C-19), 150.32 (C-20), 29.82 (C-21), 34.04 (C-22), 28.05 (C-23), 15.46 (C-24), 16.08 (C-25), 16.21 (C-26), 14.86 (C-27), 60.56 (C-28), 109.61 (C-29), 19.17 (C-30).

Compound 2 (lonisemperin) occurred as a yellow powder (MeOH). It also gave a yellow color with NaOH and vanillin-H₂SO₄ spray reagents R_F: 0.51 [silica gel, CH₂Cl₂-MeOH, 9.5:0.5]. UV λ_{max} (MeOH): 327 and 271 nm; +NaOMe: 368, 295 (sh) and 278 nm; +AlCl₃: 339, 293 and 281 nm, +AlCl₃/HCl:339, 293 and 281 nm; +NaOAc; 363, 277 and 224 nm; +NaOAc/H₃BO₃: 327, 306, 271 and 228 nm. Positive FAB-MS gave molecular ion peaks at m/z: 553 [M+1]⁺, 552 [M]⁺, 307, 289, 273, 154 (base peak) and 136.

¹H-NMR spectral data (500 MHz, DMSO-d6) and ¹³C-NMR spectral data (125 MHz, DMSO-d6) Table (1).

Compound 3 (luteolin) occurred also as a yellow powder (MeOH). It gave a yellow color with NaOH and vanillin- H_2SO_4 spray reagents. R_F : 0.38 [silica gel, $CH_2Cl_2 \sim$ MeOH, 9:1]. UV λ_{mea} (MeOH): 349, 255 nm; +NaOMe: 401, 335, 257 nm; +AlCl₃ 425, 330, 360, 272 um; +AlCl₃/HCl: 385, 358 and 295, 272 nm; + NaOAc: 382, 267 nm; +NaOAc/ H_3BO_3 : 373, 267 nm. ¹H-NMR spectral data (500 MHz, DMSO-d6): 5.7.38 (dd, J= 2. 2, 8.4 Hz, H-6'), 7.36 (d, J= 2. 2 Hz, H-2'), 6.85 (d, J=8.4 Hz, H-5'), 6.65 (s, H-3), 6.42 (d, J=2.3 Hz, H-8) and 6.15 ppm (d, J=2.3 Hz, H-6). ¹³C-NMR data (125 MHz, DMSO-d6), Table (2)

Conspound 4 (B-situaterol glucoside) occurred as a white precipitate. It gave a deep violet color with vanilin-H₂SO₄ spray reagents and hunting for our minute. It gave positive Liebermann-Burchard's and Molische's tests. its mp 237-258 °C. R₄ 0.63 failes gel, CH₂Cl₂-MeOH, 9:1] UV (CHCl₃) λ_{max} 342 and 272 sh nm. El-MS m/2 576 [M], 414 [M-glc] 'R. NMR (500 MHz, pyridine-d₃), 8 5.38 (1H, m. H-6), 5.08 (1H, d, J = 7.8 Hz, H-1'), 4.58 (1H, dd, J = 1.0 and 11.9 Hz, H-6'0), 4.42 (1H, dd, J = 1.0 and 6.3 Hz, H-6'B), 4.06-4.33 (H-2', 3', 4' and 5'), 4.92 (1H, m, H-3), 0.68 (3H, s, CH₂-18), and 0.90 ppm (3H, d, J = 6.6 Hz, CH₂-21).

Compounds 5, 6, 7 were obtained as pellow precipitate (MeOH). They gave a pellow color with NaOH and vanillin-H₂SO_A spray reagants and positive Molische's test.

Compound 5 (acacetin-7-O-β-D-glucomide): ETV λ_{max} (MeOH): 325, 268 nm, +NaOMe: 336, 287-362 nm; +AlCl₃: 381, 345, 300, 277 nm; +AlCl₃: 381, 381, 399, 278 nm; + NaOAc: 325, 268 nm; +NaOAc/H₃BO₃: 328, 268 nm; R_c 0.60 [stitica gni; EtOAc-MeOH-H₂O, 100 16.5 13.5] 'H-NMR spectral data (500 MHz, DMSO-do): 5.7.93 (2H, d.)-8.4 Hz, H-2"/6"), 6.91 (2H, d.)-8.4 Hz, H-3" (2H, d.)-8.4 Hz, H-2"/6"), 6.80 (s, H-8), 6.42 (s, H-6), 5.40 (d.)-5.4 Hz, H-4"), 5.13 (d.)-4.6 Hz, H-3" (2. 5.06 (d.)-5.4 Hz, H-2"), 5.03 (d.)-7.7 Hz, H-1" (2. 4.6) (d.)-6.1 Hz, H-5"), 4.38 and 3.68 (dd.)-1.9, 10.9 Hz, H-6" and 3.86 (s, OCH₃). ¹³C-NMR dams (125 MHz, DMSO-d6), Table (2).

Compound 6 (luteolin-7-*O*- β-D-gincoside): UV λ_{max} (MeOH): 348, 255 nm, +NaOMe 402, 362 mm, +AlCl₃: 419, 273 nm; +AlCl₃:HCl 386, 361, 270 mm, + NaOAc: 399, 261 nm; +NaOAc:H₃BO₁ 372, 261 nm. R₆: 0.55 [silica gel, ErOAc-MeOH-H₃O, 100.16.5:13.5]. H-NMR (500 MHz, DMSO-d6): δ 7.42 (dd, , *J*= 2, 2, 8.4 Hz H-6"), 7.39 (d, *J*= 2, 2 Hz, H-2"), 6.87 (d, *J*= 7.6 Hz, H-5"), 6.76 (s, H-3), 6.72 (s, H-8), 6.41(s, H-6), 5.05 (d, *J*= 6.9 Hz, H-1") and 3.69-3.13 (H-2"-H-6"). C-NMR data (125 MHz, DMSO-d6), Table (2)

Compound 7 (apigenm-7-t)- β-D-neohesperidoside): UV λ_{max} (MeOH): 341, 262 nm. +NaOMe: 394, 259 nm; +AlCl₁: 388, 349, 297, 274 nm; +AlCl₂/HCl: 388, 346, 297, 272 nm, + NaOAc 394, 266 nm; +NaOAc/H₁BO₃: 344, 266 nm; R₁: 0.53 [silica gel, EtOAc-MeOH-H₂O, 100:16.5:13.5]. 'H-NMR (500 MHz, DMSO-d6): δ 7.90 (2H, d, J= 8.4 Hz, H-2'/H-6'), 6.90 (2H, d, J= 8.4 Hz, H-3'/5'), 6.80 (s, H-3), 6.76 (s, H-8), 6.33 (s, H-6), 5.20 (s, H-1'''), 5.19 (d, J= 6.8 Hz, H-1''). ¹³C-NMR data (£25 MHz, DMSO-d6), Table (2).

Table (1): 13C- and 1H-NMR spectral data of

| compound 2. | | | | | | | |
|------------------|--------|----------------------|-----------|--------|----------------------|--|--|
| Atom | NMR | H-NMR (J) | Atom | NMR | ¹H-NMR (J) | | |
| | Moiety | | Moiety B: | | | | |
| 2. | 163.92 | | 2 | 163.92 | | | |
| - 3 | 103.54 | 6.95, s | 3 | 103.54 | 6.86, s | | |
| 4 | 181.34 | | 4 | 181.34 | | | |
| 5 | 161.04 | | 5 | 161.08 | | | |
| 6 | 98.68 | 6.20, br. s | 6 | 103.82 | | | |
| 7 | 161.70 | | 7 | 162.53 | | | |
| . 8 | 93.80 | 6.50, br. s | 8 | 93.91 | 6.48, s | | |
| 9 | 156.98 | | 9 | 156.88 | - | | |
| 10 | 103.98 | | 10 | 103.98 | | | |
| ľ | 123,09 | | 1' | 124.24 | | | |
| 2' | 120.44 | 7.94, br. s | 2' | 128,16 | 8.03, d (8.91 Hz) | | |
| 3' | 142.07 | | 3' | 115.53 | 7.02, d (8.37 Hz) | | |
| 4' | 154.13 | | 4' | 160,33 | | | |
| 5' | 113.90 | 7.39, d (8.91 Hz) | 5' | 115.53 | 7.02, d (8.37 Hz) | | |
| 6' | 125.13 | 8,03, unres. dd | 6' | 128.16 | 8.03, d (8.91 Hz) | | |
| OCH ₃ | 56.44 | 3.84, s | | | | | |

Table (2): ¹³C-NMR spectral data of compounds 3, 5, 6, 7 and 8.

Carbons Compound Compound Compound Compound 7 163.47 164.87 164.62 163.46 2 103.46 105.85 103.68 103.59 3 182.43 182.51 182.55 182.34 4 5 161.43 161.89 161.66 161.61 100.02 100.05 98.26 99.49 6 7 164.46 164.76 164.99 163.01 8 94.96 95.37 95.24 95.01 9 157.23 157.47 157.47 157.51 10 104.85 103.63 105.86 105.93 1' 121.80 121.55 121.88 121.30 2' 113.89 129.16 114.07 129.14 3' 145.78 116.53 146.31 116.63 4 149.93 161.89 150.48 162.93 5' 116.45 119.53 116.50 116.63 6' 119.36 129.16 119.72 129.14 1" 100.40 100.38 100.99 2" 73,62 73.63 77.69 3" 77.69 77.67 77.50 4" 70.00 70.04 70.13 5" 76.94 76.90 76.79 6" 61.10 61.12 60.97 OCH₃ 56.49 1"" 100.99 70.96 3" 70.90 72.35 5111 68.88 18.61

Table (3): Antifungal activity of the extracts and some isolated compounds.

| Extracts / | | Growth of A. Parasiticus (mm) according to concentration | | | | | |
|--------------------------|-----------------|--|-----------------|----------|-----------------|----------|-----------------|
| compounds | Control (mm) | 500 ppm | % inhibition | 1000 ppm | % inhibition | 2000 ppm | % inhibition |
| Chloroform extract | 50 | 16 | 68% | 7 | 86% | 6 | 88% |
| Ethyl acetate extract | 50 | 19 | 62% | 9 | 82% | 7 | 86% |
| Compound 1 | 50 | 32 | 36% | 28 | 44% | 24 | 52% |
| Compound 2 | 50 | 30 | 40% | 28 | 44% | 23 | 54% |
| Compound 3 | 50 | 30 | 40% | 27 | 46% | 23 | 54% |
| Compound 4 | 50 | 26 | 48% | 24 | 52% | 20 | 60% |
| Compound 5 | 50 | 36 | 28% | 31 | 38% | 26 | 48% |

RO OH O

| | 011 | 1- | r |
|----------|--------------|----|-----------------|
| Compound | R | R | R ₂ |
| 3 | Н | ОН | Н |
| 5 | glc | Н | CH ₃ |
| 6 | gle | OH | Н |
| 7 | Rham(1-2)glc | Н | H |

DISCUSSION

gave positive Liebermann-Compounds I Burchard's test indicating its steroidal or triterpenoidal nature. It was identified as betulin. 13 C-NMR spectrum revealed the presence of 30 carbon atoms. EI-MS gave a molecular ion peak at m/z 442 calculated for C30 H50 O2. H- NMR spectrum showed two broad singlets at 8 4.68 and 4.58 ppm assigned to the two protons of exocyclic methylene group (H-29) which were confirmed in 13C-NMR spectrum by two signals in the downfield region at 8 150.32 and 109.61 assigned for C-20 and C-29, respectively. The two doublets at δ 3.80 and 3.33 ppm (J=11 Hz) were assigned for the two protons of C-28 and resonated at δ 60.56 ppm in ¹³C-NMR. The multiplet at δ 3.19 assigned for H-3 and resonated at 5 78.98 in 13C-NMR was downfield shifted due to the presence of OH group at this position. Comparing these spectral data with the published literature [11] confirm the structure of compound I as Betulin.

Compound 2 gave a yellow colour with AlCl3 and NaOH test solutions indicating its flavonoidal nature. 13C-NMR spectrum revealed the presence of 31 earbon atoms. Positive FAB-MS displayed a molecular ion peak at m/z 552 [M] * consistant with the molecular formula C31 H20 O10. Both H- and 13C-NMR spectra indicated the presence of a biflavonoide compound consisting of two flavone nuclei as indicated from the UV spectrum in MeOH at λ_{max} 327 nm and was confirmed from the signal at δ 181.34 ppm assigned for C-4 for both parts. Part A showed a meta- coupling between H-2' (& 7.94, s)and H-6' (& 8.03, unres.dd) and ortho-coupling between H-6' and H-5' (δ 7.39, d, J= 8.91 Hz) while part B showed two doublets at & 8.03 and 7.02 ppm, respectively, assigned for H-2' / H-6' and H-3' / H-5', respectively. The bathochromic shift with NaOMe (41nm) indicated the presence of a free OH group at C-4 on part b which was confirmed from the bathochromic shift in band I (36 nm). The absence of any shift after the addition of Alcl3 relative to Alcl3 /HC1 indicated the absence of free ortho-dihydroxy group in any part of the compound which was confirmed by the absence of any shift in band I by the addition of NaOAc/H1BO3. The bathochromic shift in band II (16 mm) by the addition of NaOAc indicated the presence of free OH group at C-7. H-NMR spectrum showed a singlet at δ 6.50 assigned for H-8 in part B and no signal for H-6 indicated that this position is occupied. Comparing the data of part B by the published ones about scutellarein and comparing the data of part A by the published ones for acacetin [12-13], it was found that the signal assigned for C-3' in part A in 13 C-NMR spectraum was shifted to the dowenfield region at & 142.07 ppm (c.f. acacetin at 114.8 ppm) and the signal assigned for C-6 in part B was shifted to the upfield region at δ 103.82 ppm (c.f. scutellarein at 130.4 ppm) indicated that the linkage occured at these two positions. The interperetation of these data as well as COSY, HMBC and HMQC-NMR spectra confirm the structure of compound 2 to be scutellarein-(6-O-3')acacetin. It is considered as a new compound and have never been isolated from any natural source and hence, it was named as Lonisemperin.

Compound 4 was identified as β -sitosterol glucoside. EI-MS gave a molecular ion peak at m/z 576 [M]*. Acid hydrolysis and TLC alongside authentic sugars revealed the presence of D-glucose which indicated in the ¹H-NMR spectrum by the anomeric proton signal at δ 5.08 (d, J= 7.8 Hz) as indicated in the EI-MS by the cleavage of 162 amu to give a molecular ion peak at m/z 414 [M-glc]*. The coupling constant of the anomeric proton indicating β -configuration of the glucose moiety. TLC comparison with authentic samples as well as comparison of these spectral data with the published ones⁽¹⁴⁻¹⁵⁾ confirmed the identity of compound 4 as β -sitosterol glucoside.

Compounds 3, 5, 6 and 7 gave a yellow colour with AlCl₃ and NaOH test solutions indicating their flavonoidal nature. Their UV spectrum in MeOH indicating the presence of a flavone nucleus in these compounds which was confirmed from the ¹³C-NMR spectral data, Table (1) from the signals at ca δ 182 assigned to C-4. Compounds 5, 6 and 7 gave a positive Molische's test indicated their glycosidic nature.

Compound 3 was identified as luteolin. A significant bathochromic shift in band I with increasing intensity with NaOMe (52 nm) indicating the presence of free OH group at C-4' which was confirmed from the bathochromic shift in band 1 (33 nm) after the addition of NaOAc while the bathochromic shift in band II (12 nm) after the addition of NaOAc indicated the presence of a free OH group at C-7. The characteristic bathochromic shift (76 nm) in band I with AlCl3 indicated the presence of a free OH group at C-5 and the shift decrease to (36 nm) on adding HCl indicated the presence of a free ortho-dihydroxy group in ring B. The bathochromic shift in band I (24 nm) with NaOAc/H₃BO₃ confirmed the presence of orthodihydroxy group in ring B. 13C-NMR spectrum showed two signals at δ 94.96 and 99.49 ppm assigned to C-8 and C-6, respectively, indicated that these positions are unsubstituted which was confirmed from H-NMR spectrum where the doublet at 8 6.15 (1H, d, J = 2.3 Hz, H-6) showed a meta-coupling with the doublet at δ 6.42 (1H, d, J = 2.3 Hz, H-8). Comparing these data with the puplished ones⁽¹²⁻¹³⁾, confirm the structure of compound 3 to be luteolin.

Compound 6 showed spectral data similar to that of compound 3 except the presence of an anomeric proton signal at δ 5.05 (d, J= 6.9 Hz) assigned to H-1" of sugar moiety and indicated by the anomeric carbon signal at δ 100.38 ppm. The other values of the carbons indicated that the sugar moiety was glucose and the high J value indicated the β -configuration of the sugar which was attached to position 7 of the aglycone moiety. This was confirmed by the absence of a real shift in band II after the addition of NaOAc indicated the absence of free OH group at this position which was occupied by the glucose moiety. Comparing these data with the published ones (12-13), confirm the identity of compound 6 to be luteolin-7-O- β -D-glucoside.

The spectral data of compounds 5 and 7 were similar to that of apigenin-7-O-β-D-glucoside(12-13) except in compound 5, there was an extra OCH3 group which appeared in the H-NMR spectrum δ 3.86 and ¹³C-NMR spectrum at δ 56.49 ppm. The bathochromic shift in band I by the addition of NaOCH, with decrease in intensity indicated the absence of a free OH group at C-4' which was confirmed by the absence of any shift in band I after the addition of NaOAc indicated the presence of the OCH3 group at this position. The analysis of these data and by comparing it with published ones(13) confirmed the structure of compound 5 to be acacetin-7-O-β-D-glucoside. But in case of compound 7, there was an additional sugar moiety more than that of apigenin-7-O-β-D-glucoside as indicated by the anomeric proton signal at δ 5.20 and the anomeric carbon signal at 8 100.99 ppm. The other assignment of carbons atoms indicated that the sugar moiety is rhamnose. The dowenfield shift of C-2" of glucosyl moiety indicated that the rhamnosyl moiety linked at these position(16) By comparing the spectral data with the published ones (14), compound was confirmed to be apigenin-7-O-β-D-neohesperidoside.

Antifungal activity:

The chloroform and ethyl acetate extracts of L sempervirens leaves, together with the isolated compounds 1, 2, 3, 4, and 5 showed variable degrees of dose dependant antifungal activity. At different concentrations (500, 1000, and 2000 ppm), the chloroform extract has the most potent anti-fungal activity compared to ethyl acetate extract. Similarly, among the tested compounds, compound 4 has the most potent anti-fungal activity at different concentrations compared to other tested compounds.

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REFERENCE

- Reader's Digest Encyclopedia of garden plants and flowers, 417- (1975).
- Kakuda, R., Imai, M., Yaoita, Y., Machida, K. and Kikuchi, M., Phytochemistry, 55, 879-(2000).
- Dictionary of natural products (seven-volume set), Chapman and Hall, London (1994). Through Lee, J.S., Kim, H.J., Woo, E.R. and Park, H., Planta Med., 67, 99- (2001).
- Flamini, G., Braca, A., Cioni, P.L. and Morelli,
 J. Nat. Prod., 60, 449 (1997).
- Xiang, T., Xiong, Q., Ketut, A., Tezuka, Y., Nagaoka, T., Wu, L. and Kadota, S., Planta Med., 67, 322- (2001).
- Son, K.H., Jung, k.y., Chang, H.W., Kim, H.P. and Kang, S.S., Phytochemistry, 35 (4), 1005-(1994).
- Kita, M., Kigoshi, H. And Uemura, D., J. Nat. Prod., 64, 1090- (2001).
- Kumar, S., Sati, O.P., Semwal, V.D., Nautiyal, M., Sati, S. and Tkeda, Y., Phytochemistry, 53, 499- (2000).
- Farag, S.A., Madkour, M.A. and Shehata, M.R. J. Agric. Sci. Mansoura University, II: 578-584 (1986).
- Collins, C.H. and Lyne, P.M. Microbiological methods. 5th Ed. Butter Worth & Co Pub. Ltd., London, Toronto, 167- (1985).
- Tinto, w.f., Blair, L.C., Alli, A., Renolds, W.F. and McLean, S., J. Nat. Prod., 55 (3), 395-(1992).
- Mabry, J.J., Makham, K.R. and Thomas, M.B., "The systematic identification of flavonoids", Springer-Verlag, New York (1970).
- Harborne, J.B. and Williams, T.J., "The flavonoids: Advances in Research", 1st ed., Chapman and Hall LTD, London and New York (1982).
- Rubinstein, I., Goad, L.J., Clague, A.D.H. and Mulheirn, L.J., Phytochemistry, 15, 195- (1976).
- Buckingham, J., Dictionary of organic compounds, 5th ed., 5, 5009 (1982).
- Nakano, A., Murakami, K. Nohara, T., Tomimatsu, T. and Kawasaki, T., Chem. Pharm. Bull., 29(5) 1445 (1981).

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مركب بايغلافونويدى جديدا من لونيسيرا ممبرفيرنس

منی جوده زغلول

قسم العقاقير - كلية الصيدلية - جامعة المنصورة - المنصورة - مصر

أنت الدراسة الكيميائية لخلاصة خلات الاثير لنبات لونيسيرا سمبرفيرنس اليفصل سبعه مركبات وهي بتيولين ومركب فلافونيدي جديد وهو لونيسمبرين ولتيولين وبيتاسيتوستيرول جليكوزيد من خلاصة الكلوروفورم، وكذلك أكاسيتين-٧-أ- جليكوزيد و لتيولين-٧-أ- جليكوزيد وأبيجينين-٧-أ- نيوهيسبريدوزيد من خلاصة خلات الاثير، وقد تم التعرف عليهم بواسطة خواصهم الطبيعية والطيفية، وتم دراسه النشاط المضاد للفطريات لهذه الخلاصات وكذلك لبعض المواد المفصوله باستخدام فطر أسبار اجالاس باراستيكم الذي يفرز الأفلاتوكسين، وقد أظهرت خلاصة الكلوروفورم وكذلك بعضيتوستيرول جليكوزيد أقوى تأثير مضاد للفطريات،