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Chemical Constituents and Biological Activities of *Cichorium intybus* L.

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Abstract – Continuation of a phytochemical study of *Cichorium intybus* L. (Astraceae) growing in Egypt, resulted in the isolation and identification of a new sesquiterpene lactone 3, 4-dihydrolactucin, in addition to the eight known compounds; kaempferol, isoscutellarin, cichoriin, umbelliferone, lupeol, lupeol acetate, β-sitosterol, and β-sitosterol-3-*O*-glucoside. Chemical structures of the isolated compounds were assigned based on different physical, chemical, and spectroscopic techniques including IR, UV, MS, 1D- and 2D-NMR spectra. Furthermore, the antimicrobial, and spasmogenic activities of some fractions and isolates were also assessed.

Key words – *Cichorium intybus* L.; Lactuaceae; Asteraceae; chemical constituents; new sesquiterpene lactone; antimicrobial, spasmogenic activities.

Introduction

Lactuaceae (Asteraceae) is a well-known tribe belonging to subfamily liguliflorae. Plants of the tribe were reported to possess diverse pharmacological actions, such as antidiabetic, anticarcinogenic, and hepatoprotective activities (Steven and Tyler, 1992; Hertog *et al.*, 1992). Flavonoids, coumarins, and sesquiterpene lactones are among the most common secondary metabolites of this tribe. *Cichorium intybus* L. (Lactuaceae) is a perennial herb with historical use as laxative, diuretic, and in the treatment of gallstones, hepatic disorders, and indigestion (Tyler *et al.*, 1988; Crellin and Philpatt, 1990). Previous work on this plant has lead to the isolation of isoquercetrin (Amer, 1974), 10-hydroxyguaia-4,11(13)-diene-6,12-olide, its 11,13-dihydro derivative (El-Masry *et al.*, 1984), desacetylmatricarin, aloe-emodin, esculetin, 3,4-dihydroxymethylbenzoate, caffeic acid, and quercetin (Aboul-Ela, *et al.*, 2002).

In this communication, we report on the isolation and identification of the new sesquiterpene lactone, 3,4-dihydrolactucin, and the known compounds, kaempferol, isoscutellarin, cichoriin, umbelliferone, lupeol, lupeol acetate, β-sitosterol, and β-sitosterol-3-*O*-glucoside. Antibacterial and antifungal screening for some pure isolates was conducted. Furthermore, the spasmogenic activity of some fractions and isolates were performed using rabbit intestine preparation.

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Experimental

Cichorium intybus – L. used in this study was collected in April, 2001 from the flowering plants growing wildly in Abyss province, South of Alexandria. The identity of the plant was confirmed at the Faculty of Science, University of Alexandria, Alexandria, Egypt.

General – Melting points were determined on a Griffin melting point apparatus and were uncorrected. UV spectra were recorded on a Perkin Elmer double beam spectrophotometer Model 550S, attached to a Hitachi recorder Model 561. IR spectra were determined on a Jasco infrared spectrophotometer, Model (FT/IR-300E), in KBr pellets. NMR analyses were recorded on a Bruker Avance 300 MHz instrument. MS spectra were recorded on a GC coupled with a Shimadzu 8080A Mass Spectrometer. Solvents used were of analytical grade. Silica gel for CC and silica gel GF_{254} for TLC (Merck) were used. Shift reagents for UV spectral analysis are: 5% CH_3ONa, 5% $AlCl_3$, conc. HCl, anhydrous NaOAc and CH_3OH. Pharmacological effects were recorded on a chymograph.

Extraction and isolation – A. Air-dried and powdered roots of *C. intybus* (2.5 kg) were macerated in 90% EtOH. The concentrated hydroalcoholic extract was successively extracted with petroleum ether, $CHCl_3$, EtOAc and *n*-BuOH. The EtOAc fraction (6 g) was subjected to CC using $CHCl_3$ and MeOH mixtures. Crystallization of fractions obtained using 8-10% MeOH in $CHCl_3$ afforded 10 mg of compound **1**. PTLC (Solvent, EtOAc: $CHCl_3$: MeOH, 5:2:1) of fractions eluted using 14-16% MeOH in $CHCl_3$ afforded 100 mg of

compound **2**.

B. Air-dried and powdered aerial parts of *C. intybus* (21 Kg) were macerated in 90% alcohol. The concentrated hydroalcoholic extract was successively extracted with petroleum ether, $CHCl_3$, EtOAc, and *n*-BuOH. The $CHCl_3$ fraction (250 g) was subjected to CC, using petroleum ether. The polarity was increased by gradual addition of $CHCl_3$, then by CH_3OH. PTLC (Solvent, $CHCl_3$: MeOH, 9:1) of fractions obtained using 11-19% MeOH in $CHCl_3$ afforded 23 mg of compound **3** and 10 mg of compound **4.** In addition, lupeol, lupeol acetate, β-sitosterol and β-sitosterol-3-*O*-glucoside were also isolated and identified. The EtOAc fraction (15 g) was subjected to CC using $CHCl_3$, EtOAc and MeOH mixtures. Crystallization of fractions obtained using 60-90% EtOAc in $CHCl_3$ from MeOH afforded 0.8 g of compound **5**.

Compound (**1**): White amorphous powder. UV λ_{max} (MeOH): 246 nm. IR ν_{max} (KBr): 1782, 1678, 1635, 1620 and 1215 cm^{-1}. EIMS *m/z* (rel. abund. %): 278 (2) [M^+, $C_{15}H_{18}O_5$], 262 (2.9), 247 (3.1), 233 (9.15), 219 (3.6), 203 (3.4), 177 (9.5), 167 (8), 149 (32), 133 (19), 111 (17), 57 (100). ^1H NMR (δ, CD_3OD): 2.46 (3H, s, H-14), 2.49 (1H, m, H-9β), 2.54 (2H, d, *J* = 7.4 Hz, $C\underline{H}_2$-3), 2.52 (1H, m, H-4), 2.74 (H, t, *J* = 11.0 Hz, H-9α), 3.02 (1H, t.t, *J* = 10.1, 3.0 Hz, H-7), 3.11 (1H, t, *J* = 2.9 Hz, H-5), 3.63 (1H, dd, *J* = 10.9, 5.6 Hz, H-15a), 3.75 (1H, dd, *J* = 10.1, 4 Hz, H-6), 3.77 (1H, dd, *J* = 10.9, 4.0 Hz, H-15b), 3.79 (1H, dt, *J* = 4.2, 6.6 Hz, H-8), 6.16 (1H, dd, *J* = 3.1, 1.2 Hz, H-13a), 6.23 (1H, dd, *J* = 3.1, 1.2 Hz, H-13b). ^{13}C NMR (δ, CD_3OD): 153.3 (C-1), 208.5 (C-2), 43.7 (C-3), 38.4 (C-4), 48.0 (C-5), 83.6 (C-6), 59.2 (C-7), 68.9 (C-8), 51.0 (C-9), 135.6 (C-10), 140.2 (C-11), 176.4 (C-12), 123.4 (C-13), 23.6 (C-14), 66.1 (C-15).

Compound (**2**): Colorless needles, m.p. 228°C. It showed intense blue fluorescence under UV lamp and gave a negative Molisch's test. EIMS *m/z* (rel. abund. %): 162 (8) [M^+, $C_9H_6O_3$], 149 (25), 138 (13), 121 (7), 110 (100), 94 (90), 81 (41), 66 (75), 55 (89). ^1H NMR (δ, $CDCl_3$ + CD_3OD): 7.59 (1H, d, *J* = 15.8 Hz, H-4), 7.07 (1H, br.s, H-8), 6.97 (1H, br.d, *J* = 8.1 Hz, H-6), 6.80 (1H, d, *J* = 8.1 Hz, H-5), 6.30 (1H, d, *J* = 15.8 Hz, H-3). ^{13}C NMR (δ, $CDCl_3$ + CD_3OD): 167.3 (C-2), 114.5 (C-3), 144.8 (C-4), 147.7 (C-4a), 114.7 (C-5), 121.2 (C-6), 144.2 (C-7), 113.4 (C-8), 145.3 (C-8a).

Compound (**3**): Yellow crystals, m.p. 304-306°C. UV λ_{max} (MeOH): 370, 266 nm, (MeOH + NaOMe): 408, 277 nm, (MeOH + $AlCl_3$): 400, 278 nm, (MeOH + $AlCl_3$ + HCl): 400, 278 nm, (MeOH + NaOAc): 397, 277 nm. EIMS *m/z* (rel. abund. %): 286 (3) [M^+, $C_{15}H_{10}O_6$], 256 (7), 128 (100), 118 (33), 113 (67), 97 (95). ^1H NMR (δ, CD_3OD): 7.09 (2H, d, *J* = 11.4 Hz, H-2', H-6'), 6.74 (2H, d, *J* = 11.4 Hz, H-3', H-4'), 5.95 (1H, d, *J* = 3.0 Hz, H-6), 5.14 (1H, d, *J* = 3.0 Hz, H-8). ^{13}C NMR (δ, CD_3OD): 144.3 (C-2), 136.8 (C-3), 170.9 (C-4), 164.4 (C-5), 99.5 (C-6), 168.0 (C-7), 92.3 (C-8), 149.5 (C-9), 107.8 (C-10), 125.3 (C-1'), 130.9 (C-2'), 116.1 (C-3'), 158.6 (C-4'), 116.1 (C-5'), 130.9 (C-6').

Compound (**4**)**:** Dark yellow crystals, m.p. 300-301°C. UV λ_{max} (MeOH): 282, 332 nm. Degradation occurs with all shift reagents. EIMS *m/z* (rel. abund. %): 286 (100) [M^+, $C_{15}H_{10}O_6$], 258 (47), 257 (9), 168 (80), 140 (52), 118 (38), 112 (5.4). ^1H NMR (δ, CD_3OD): 7.06 (2H, d, *J* = 8.5 Hz, H-2', H-6'), 6.77 (2H, d, *J* = 8.5 Hz, H-3', H-5'), 6.08 (1H, s, H-3), 5.59 (1H, s, H-6).

Compound (**5**)**:** Transparent prisms, m.p. 215°C. It showed an intense blue fluorescence under UV lamp and gave a positive Molisch's test. UV λ_{max} (MeOH): 234, 289, 347 nm, (MeOH + NaOMe): 249, 306, 390 nm, (MeOH +$AlCl_3$): 234, 289, 347 nm, (MeOH + $AlCl_3$ + HCl): 234, 289, 347 nm, (MeOH + NaOAc): 252, 282, 352 nm. EIMS *m/z* (rel. abund. %): 340 (2) [M^+, $C_{15}H_{16}O_9$], 320 (2), 293 (72), 179 (90), 178 (97), 167 (90), 149 (100), 127 (68), 97 (56). ^1H NMR (δ, $CDCl_3$ + CD_3OD): 7.72 (1H, d, *J* = 9.5 Hz, H-4), 7.11 (1H, s, H-8), 6.94 (1H, s, H-5), 6.19 (1H, d, *J* = 9.4 Hz, H-3), 4.88 (1H, d, *J* = 7.3Hz, H-1'), 3.31-3.85 (m, 6H, H-2'-H-6'). ^{13}C NMR (δ, $CDCl_3$ + CD_3OD): 163.0 (C-2), 114.1 (C-3), 144.9 (C-4), 141.3 (C-4a), 113.3 (C-5), 148.9 (C-6), 149.8 (C-7), 104.7 (C-8), 149.99 (C-8a), 102.4 (C-1'), 76.0 (C-2'), 77.8 (C-3'), 74.0 (C-4'), 78.0 (C-5'), 61.7 (C-6').

Antimicrobial activity – Antibacterial and antifungal assays were carried out using the agar diffusion technique (Jian and Kar, 1971) against one Gram-positive bacterium; *Staphylococcus aureus*, two Gram-negative bacteria; *Escherichia coli* and *Pseudomonas aeroginosea*, and the Fungus; *Candida albicans*. These organisms were local isolates provided from Department of Microbiology, Faculty of Pharmacy, University of Alexandria.

Procedure. 1 ml of 24 hours broth culture of each of the tested organisms was separately inoculated into 100 ml of sterile molten nutrient agar maintained at 45°C. The inoculated medium is well mixed and poured into sterile 10 cm diameter Petri dishes, receiving 15 ml. After setting, ten cups, each 8 mm in diameter, were cut in the agar medium (Oxoid). Five milligrams of each fraction or isolate, accurately weighed, were dissolved in 1 ml DMF. The solutions were inserted in the cups and incubated at 37°C for 24 hours. The sensitivities were observed by measuring the Inhibited Zones (IZ) in mm of some isolates with those of the control (Table 1). Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of some isolates were also conducted (Table 2).

Spasmogenic activities: A preliminary pharmacological study was carried out on some isolates and fractions of *C.*

Table 1. Antibacterial and antifungal activity of some isolates of *C. intybus* L.

Isolate	Inhibition Zones (IZ) in mm			
	Bacteria			Fungi
	G-Positive	G-Negative		
	S. aureus	*E. coli*	*P. aeroginosa*	*C. albicans*
Desacetyl matricarin*	14	18	12	17
Caffeic acid*	15	19	12	17
Quercetin*	19	18	16	17
Aloe-emodin*	17	18	17	20
Esculetin	14	18	16	17
Isoquercetrin*	15	18	14	20
Cichoriin	14	20	14	20
Control (DMF)	14	18	12	17

Weak activity : Control<Observed value by 2 units.
Moderate activity : Control+2<Observed value>Control+5.
Strong activity : Observed value>Control+5.
*Previously isolated (Aboul-Ela *et al.*, 2002).

intybus L. using rabbit intestine (Berry, 1970).

Materials and reagents: Rabbit intestine, Tyroid solution, 50 ml organ bath, chymograph, DMSO, BuOH, quercetin, esculetin and cichoriin. The response of the rabbit intestine to different isolates and fractions from the aerial parts is shown in Table 3.

Results and discussion

^{13}C-NMR and MS analyses of compound **1** established the molecular formula to be $C_{15}H_{18}O_5$. MS showed the molecular ion peak at *m/z* 278, while ^{13}C-NMR indicated the presence of 15 carbons. DEPT experiment indicated these carbons to be: $1CH_3$, $4CH_2$, 5CH, and 5 quaternary carbons. IR spectrum showed the presence of absorption bands characteristic for α, β-unsaturated carbonyl (1678 cm^{-1}) and a carbonyl for γ-lactone ring (1782 cm^{-1}). The guaianolide nucleus was inferred from the ^{1}H-NMR spectrum where the chemical shifts and coupling constants of the observed signals supported this suggestion (El-Masry *et al.*, 1984). ^{1}H-NMR spectrum showed two doublets of doublets (J = 3.1, 1.2Hz) at δ 6.16 and δ 6.23, both corresponding to a single vinylic carbon appearing at δ 140.2; as indicated by DEPT-135° and HMQC experiments; characteristic for the exocyclic α-methylene γ-lactone moiety. Furthermore, the spectrum revealed the presence of a vinylic methyl signal at δ 2.46 due to $C\underline{H}_3$-14 and a hydroxymethyl group $C\underline{H}_2OH$-15, appearing as two doublets of doublets at δ 3.63 (J = 10.9, 5.6 Hz) and δ 3.77 (J = 10.9, 4.0 Hz). Furthermore, H-6 appeared as a doublet of doublet at δ 3.75 (J = 10.1, 4.0 Hz). The large coupling constant (J = 10.1 Hz) between H-6 and H-7 supported the presence of 6,7-*trans*-lactone ring (Williams and Fleming, 1980; Kisiel *et al.*, 1997). ^{13}C-NMR spectrum indicated the occurrence of two carbonyl carbons at δ 208.5 and δ 176.4 assigned to C-2 and C-12 of the lactone moiety, respectively. Three oxygenated carbon signals were resonating at δ 83.6, 68.9 and 66.1, were due to C-6, C-8 and C-15, respectively. Comparison of the observed data of compound **1** with

Table 2. Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of some isolates from *C. intybus* L.

Isolate Number.	MIC (μg/ml)				MBC (μg/ml)			
	S. aureus	*E. coli*	*P. aeroginosea*	*C. albicans*	*S. aureus*	*E. coli*	*P. aeroginosea*	*C. albicans*
Quercetin*	62.5	62.5	–	–	12.5	62.5	–	–
Aloe-emodin*	–	–	31.5	500	–	–	>500	31.5
Isoquercetrin*	125	–	–	500	125	–	–	>500
Cichoriin	–	–	62.5	62.5	–	–	62.5	>500

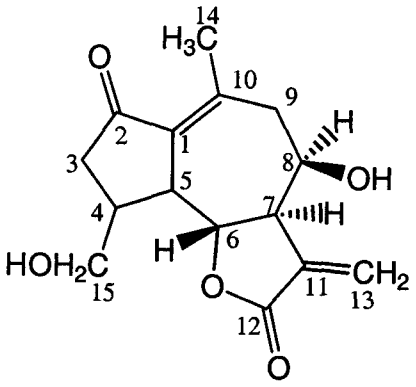
No activity: >125 Moderate activity: 62.5.
Weak activity: 125 Strong Activity: 31.5.
*Previously isolated (Aboul-Ela *et al.*, 2002).

Table 3. Response of the rabbit intestine

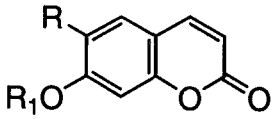
Isolates and Fractions	Dose / ml	Response
DMSO	0.5 ml and 1 ml	No response
Quercetin*	10 mg / 1 ml	40% enhancement followed by 60% relaxation
Esculetin	10 mg / 0.5 ml	33% enhancement
Cichoriin	10 mg / 0.5 ml	83% enhancement
$CHCl_3$ Fraction	10 mg / 0.5 ml	40% relaxation followed by 110% enhancement
	5 mg / 0.25 ml	33% relaxation followed by 40% enhancement
BuOH	1 ml	Relaxation 100 %
BuOH Fraction	10 mg / 1 ml BuOH	Relaxation 100%

*Previously isolated (Aboul-Ela *et al.*, 2002).

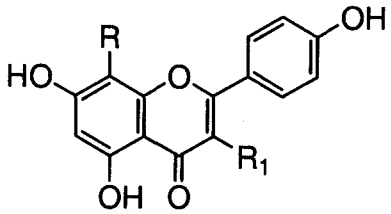
those reported for lactucin (Khalil *et al.*, 1991), indicated their basic structural similarity. The appearance of the molecular ion peak at *m/z* 278, i.e. 2 mass units higher than that of lactucin, suggested that compound **1** is a dihydroderivative of lactucin. However, the absence of the characteristic H-3 signal, which was recorded at δ 6.15 in ^{1}H-NMR spectrum of lactucin, and the appearance of a doublet (J = 7.4 Hz), integrated for 2H, at δ 2.52, suggested that compound **1** could be a 3,4-dihydroderivative. HMBC experiment (Fig. 1) allowed the confirmation of the final structure to be the new sesquiterpene lactone; 3,4-dihydrolactucin.



3, 4-Dihydrolactucin (**1**)



Umbelliferone (**2**): R=H, R_1=H
Cichoriin (**5**): R=OH, R_1=Glc.



Kaempferol (**3**): R=H, R_1=OH
Isoscutellarin (**4**):R=OH, R_1=H

Isolated compounds

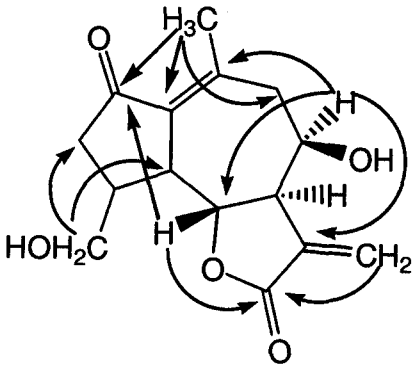


Fig. 1. The most important long-range 1H, 13C-relation of compound 1, as observed from HMBC.

Compounds **2**, **3**, **4**, and **5** were identified as umbelliferone (Yamoguchi, 1970), kaempferol (Markham *et al.*, 1978), isoscutellarin (Jay and Gonnet, 1973), and cichoriin (Abdel-Salam *et al.*, 1986, respectively) by comparing their spectral data with those previously published.

Results of the antimicrobial screening indicated that, quercetin, aloe-emodin showed moderate activity against *S. aureus*, while caffeic acid and isoquercetrin showed weak activity. Cichoriin and caffeic acid exhibited weak activity against *E. Coli*. Isoquercetrin and cichoriin showed weak activity against *P. aeroginosea*, while quercetin, aloe-emodin, esculetin showed moderate activity. This points to the stonger activity of aglycones over their glycosides. Aloe-emodin, isoquercetrin, and cichoriin showed moderate antifungal activity (Table 1). MIC and MBC determinations showed that, aloe-emodin is the only compound having strong fungicidal activity and the strongest bacteriostatic activities against *P. aeroginosea*. Quercetin showed moderate bacteriostatic activity against *S. aureus* and *E. Coli*. Cichoriin showed moderate bacteriostatstic and bactericidal action against *P. aeroginosea* (Table 2).

Only quercetin, showed relaxation effect on the rabbit intestine, while the other fractions and isolates showed spasmogenic activity. This founding concedes with the laxative effect of *Chicory* found in the literature (Fetrow and Avila, 1999; Crellin and philpatt, 1990; Tyler *et al.*, 1988).

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