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Isolation and elucidation of some chemical constituents of *Lavandula officinalis*

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ABSTRACT

Dry leaves powder of Lavandula officinalis chaix was extracted by different polarity solvents then the resulted extracts were purified by column chromatography. Four compounds were i solated and fully identified. The characterization was carried out using different spectrophotometric methods including ^{1}H, $^{13}CNMR$, IR and Mass spectra. The isolated compounds were pentacyclic triterpene alcohol uvaol, 7-methoxycoumarin, 3-epiursolic acid in addition to the flavonoid glycoside luteolin-3´-O-glucoside.

Keywords: *Lavandula Officinalis*, Extraction, Chemical constituents, Characterization

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INTRODUCTION

Lavandula officinalis (chaix.) (Figure 1) is a very aromatic; greyish leaves, branched under shrub growing to 30-60 cm. Long stemmed slender spikes of blue flowers, corolla twice as the calyx hairy outside, flowering from July to September. The natural habitat of this herb is Mediterranean region especially south and central Europe and north of Africa, but now cultivated in America and Russia [1].

Figure 1: *Lavandula Officinalis* plant

Pharmacological actions that have been documented for L. *officinalis*nare primarily associated with its volatile oil components. Lavender oils and extraction contain more than 100 compounds, with the two major constituents being linalool and linalyl acetate [2,3]. The distilled volatile oil has gained great importance in aromatherapy and in perfume, cosmetic and flavoring industries [4,5]. Furthermore, the volatile constituents of lavender are of special significance in pharmaceutical and food industries [5]. The essential oil has sedative properties when it tested on experimental animals (mice) [6], In other study it showed axiolytic effect when applied on albino rats [7], while

lavender straw decreased incidences and severity of travel sickness in pigs [8]. Inhalation of the vapor reduced cholesterol content in aorta [9] and increased mental capacity and blood content of vassels in human [10]. When lavender oil was added to bath water it showed reduced perennial discomfort [11] and antiseptic and healing properties to women following childbirth [12]. In addition other activities such as reduction of stress and tension when used as massage [13] and shows effective treatment of alopecia areata [14]. Wax from essential oil exhibited an anti-inflammatory [15], antifungal [16] and acaricidal properties [17].

EXPERIMENTAL SECTION

General

Melting points were determined on a hot stage Griffin apparatus and are uncorrected. Proton nuclear magnetic resonance (^{1}HNMR) spectra were recorded on Bruker 500 and 300 MHz. Carbon-13 nuclear magnetic resonance (^{13}CNMR) spectra were measured at 125 and 75 MHz. Unless otherwise, stated the spectra were measured in pyridin–d5. IR (λ_{max} in cm^{-1}) spectra were recorded in KBr discs using Philips PYE Unicam SP3-200 instrument. Mass spectra were measured at 70 ev on OC 220109 SSX spectrometer. Preparative TLC was conducted on glass plates (20 cm × 20 cm) coated with 0.1 cm silica gel GF 254. Wet column chromatography was carried out using MERCK silica gel 60 (0.063-0.200 mm).

Plant material

The plant material of *Lavandula officinalis* was collected near Amman (Jordan) and identified by botany department of Al-Albait university, Jordan.

Extraction

The dried powered leaves of *L. officinalis* (500g) was extracted by shaking with 80 % aqueous methanol (3 x 1.5 liter) for three days at room temperature. The crude methanol extract was filtered and concentrated using rotary evaporator apparatus at 45^{o}C. The aqueous concentrate was extracted with n-hexane, to remove chlorophylls, lipids, oils, waxes and other non-polar constituents, then with chloroform (3 x 200 ml) to extract semi-polar constituents and ethyl acetate to extract the polar constituents.

RESULTS AND DISCUSSION

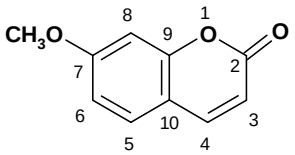
Isolation and identification of 7-methoxycoumarin (1)

This compoud was isolated from the early fractions of column chromatography purification of chloroform extract as white crystals, m.p 112-114 ^{o}C, which give bright blue color in UV light. EIMS spectrum showed molecular ion $[M]^+$ as base peak at m/z 176, suggesting a possible molecular formula of ($C_{10}H_8O_3$). ^{1}HNMR spectrum (Table 1) showed five aromatic and/or olefinic signals in the range δ 6.19-7.57, each integrating for one proton and a singlet at δ 3.80 (3H) typical of methoxy group, which confirmed by ^{13}CNMR spectrum at δ 55.7.

Table (1): 300 MHz ^{1}H-NMR spectral data (δ_H) of 7-methoxy coumarin (1) in $CDCl_3$

H	δ_H (ppm)	pattern	J (Hz)
3	6.19	d	9
4	7.57	d	9
5	7.31	d	8
6	6.77	dd	8, 2.5
8	6.79	d	2.5
OCH_3	3.80	s	-

The ^{13}CNMR spectrum (Table 2) confirmed the presence of 10 carbon atoms, 9 of them were sp^2 carbons, signal at δ 162.8 typical of carbonyl group of α,β-unsaturated esters or lactones, signal at δ 161.1 for deshilded sp^2 quaternary carbon carrying methoxyl group. From the above data and full analysis of ^{1}H and ^{13}C -NMR and MS spectra we can suggest to confirm the structure as 7-methoxycoumarin (Herniarin, 1). These data were in agreement with reported data for the same compound [18].



(1)

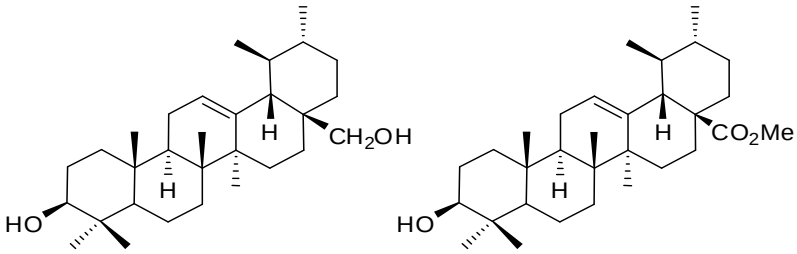
Table 2: ^{13}C-NMR spectral data (δ_C) of 7-methoxycoumarin (1)[a]

C	δ_C
2	162.8
3	112.5
4	143.3
5	128.7
6	113.1
7	161.1
8	100.8
9	155.9
10	112.5
OCH_3	55.7

[a]) ^{13}C-NMR spectral data measured at 75 MHz in $CDCl_3$

Isolation and identification of urs-12-ene-3β-28 diol (Uvaol, 2)

This compound was also obtained by using column chromatography of chloroform extract as white amorphous powder, m.p 192-197 ^oC. IR spectrum showed an absorption for hydroxyl group (3400 cm^{-1}). EI Mass spectrum showed a molecular ion peak, $[M].^+$ at m/z 442 (9), suggesting the possibility of triterpendiol of molecular formula ($C_{30}H_{50}O_2$). The ^{13}CNMR of unknown compound showed 30 carbon atom signals, this supporting the triterpenic molecular formula. ^{13}CNMR data analysis indicated a structural features of urs –12-ene which showed a trisubstituted double bonded carbon signals assignable to C-12 and C-13 at δ 125.0 and 138.7 respectively and carbon signal due to C-18 at δ 54.0 and C-19 at δ 39.4 [19,20]. The ^{13}CNMR spectra also showed the presence of hydroxy methine and hydroxy methylene carbon signals at δ 79.0 ppm and δ 69.9 ppm respectively. These structural features were confirmed by ^{1}HNMR spectrum which showed the olefinic proton H-12 at δ 5.12 (t, J = 3.5 Hz), the hydroxy methine proton at δ 3.19 as unresolved multiplet and the hydroxy methylene protons signal as an AB system at δ 3.50. From the above data and the comparison of ^{13}CNMR with those of methyl ursolate (3) (Table 3), the structure was elucidated as urs-12-ene-3β-28 diol (Uvaol, 2)



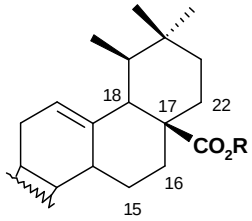
(2) (3)

Table 3: ^{13}CNMR spectral data (δ_C) of uvaol (2)[a] and methyl ursolate (3)[b] in $CDCl_3$

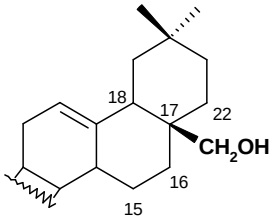
C	δ_C (2)	δ_C (3)	C	δ_C (2)	δ_C (3)
1	38.7	38.8	16	23.2	24.3
2	27.3	27.3	17	37.9	48.1
3	79.0	78.8	18	54.0	52.8
4	38.7	38.8	19	39.4	39.1
5	55.1	55.4	20	39.3	38.8
6	18.3	18.4	21	30.6	30.7
7	32.8	33.0	22	35.1	36.1
8	39.9	39.6	23	28.1	28.2
9	47.7	47.5	24	15.6	15.5
10	36.8	37.0	25	15.5	15.7
11	23.2	23.6	26	16.7	16.9
12	125.2	125.5	27	23.3	23.3
13	138.7	138.0	28	69.9	177.7
14	42.0	42.0	29	17.3	16.9
15	25.9	28.2	30	21.3	21.2
-	-	-	CO_2Me	-	51.4

[a]) ^{13}C-NMR spectral data measured at 75 MHz, [b]) Reported ^{13}C-NMR data measured at 15.09 MHz [19]

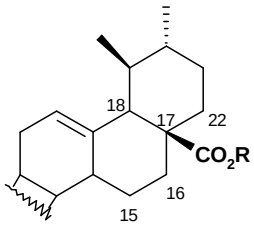
The full ^{13}CNMR chemical shifts (Table 3) showed very close agreement with those previously reported for methyl ursolate (3) [15], except for C-15 (Δ = -2.3), C-16 (Δ = -0.9), C-17 (Δ = -10.2), C-18 (Δ = +1.2), C-22 (Δ = -1.6) and C-28, due to change of C-28 function group from COOR to CH_2OH. This is in accordance with the shifts recorded for similar change in olean-12-ene system as shown in scheme (1), which also shows the calculated and found carbon resonances for uvaol (2) according to the shift obtained in olean-12-ene system [20].



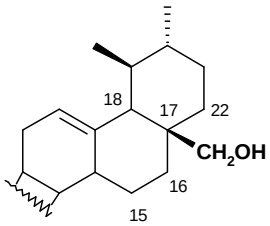
δ_{15}	27.7
δ_{16}	23.4
δ_{17}	46.6
δ_{18}	41.3
δ_{22}	32.3



	$\Delta\delta_C$
25.6	-2.1
21.9	-1.5
36.8	-9.8
42.5	+1.2
30.9	-1.4



δ_{15}	28.2
δ_{16}	24.3
δ_{17}	48.1
δ_{18}	52.8
δ_{22}	36.7



Uvaol	
Calculated	**Found**
26.1	25.9
22.8	23.2
38.3	37.9
54.0	54.0
35.3	35.1

Scheme (1): Effect of changing C-28 ($CO_2H \rightarrow CH_2OH$), $\Delta\delta_C$ in ppm

The EI Mass fragmentation pattern of uvaol (2) exhibited predominate fragment peak due to retro-Diels-Alder rupture of ring C, characteristic of Δ12-ursene carbon skeleton [21], giving peaks at m/z 234 (45), which indicate the lass of CH_2OH fragment at C-17 to give the base peak at m/z 203 (100). Other fragments which are also characteristic of Δ12-pentcyclic triterpenoid is the ion at m/z 207 (28), which arises from the cleavage of ring C with hydrogen atom transfer from Me-26 to C-11. This ion losses water molecule to give the ion at m/z 189 (12), which is typical of 3-hydroxy grouping of these skeletons. Uvaol (2) was previously isolated from *Lavandula* pedunculta [22], and other Labiatea species such as Nepeta argonesis [23].

Isolation and identification of 3-epi-ursolic acid (4)

This compound was obtained from the benzene insoluble part extracted from organic layer of chloroform extract, after crystallization from methyl acetate as white amorphous powder, m.p 242-245^{o}C. IR spectrum showed an absorption for hydroxyl group (3540 cm^{-1}) and carboxyl group (2700, 1700 cm^{-1}). EIMS showed $[M]^+$ at m/z 456 suggesting a triterpenoid carboxylic acid of molecular formula ($C_{30}H_{48}O_3$), which is supported by ^{13}CNMR spectrum which showed 30 carbon signals.

500 MHz ^{1}HNMR data (Table 4) indicated structural features of urs-12-en-28-oic as demonstrated by the appearance of signals assignable to the H-18 at δ 2.63 (d, J = 11.2 Hz) [23]. The olefinic H-12 at δ 5.48 (brs), two secondary methyl groups as doublets at δ 1.02 and δ 0.95 for Me-29 and Me-30 respectively, and five sharp singlets at δ 0.88-1.24 for five tertiary methyl groups. The proton NMR spectrum also displayed one hydroxy methine proton at δ 3.44 as broad singlet at 500 MHz, these data indicated on equatorial proton and axial hydroxyl group, this carbinylic carbon was absorbed at δ 78.1 in CMR

Table (4): 500 MHz ^{1}H-NMR (δ_H) spectral data of 3-epi-ursolic acid (4) and ursolic acid (5)

H	Type	(4)	Pattern	J_{Hz}	(5)	Pattern	J_{Hz}
1	CH_2	1.00 1.60	m m		0.88 1.43	m m	
2	CH_2	1.82	m		1.64	m	
3	CH	3.44	brs		3.25	dd	5.5;10.5
5	CH	0.90	m		0.68	d	11.0
6	CH_2	1.35 1.55	m m		1.24 1.44	m m	
7	CH_2	1.40 1.50	m m		1.20 1.42	m m	
9	CH	1.62	t		1.46	t	
11	CH_2	1.95	m		1.76	m	
12	CH	5.48	brs		5.27	t	3.5
15	CH_2	1.20 2.31	m brt	12.0,12.0	1.04 2.06	m dt	14.0,14.0,4.0
16	CH_2	2.06 2.50	m m		1.74 1.95	d dt	14.0,14.0,4.0
18	CH	2.63	d	11.2	2.36	d	11.0
19	CH	1.50	m		1.30	m	
20	CH	1.05	m		0.36	m	
21	CH_2	1.35 1.55	m m		1.24 1.35	m m	
22	CH_2	1.95	m		1.73	m	
23	CH_3	1.22	s		1.02	s	
24	CH_3	0.95	s		0.80	s	
25	CH_3	0.88	s		0.75	s	
26	CH_3	1.04	s		0.83	s	
27	CH_3	1.24	s		1.06	s	
29	CH_3	1.01	d		0.83	d	7.0
30	CH_3	0.98	d		0.84	d	7.0

The full structure of this compound was unambiguously determined as 3 α-hydroxy-urs-12-ene-28-oic acid (epi-ursolic acid, 4) from the above data and full analysis of 2D, APT NMR data and comparison with reported data of ursolic acid (5) mentioned in the last table [24].

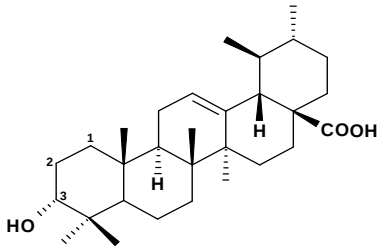
The ^{13}CNMR spectral data (Table 5) were very close to those reported for ursolic acid (5), which indicate unprofound changes in ^{13}CNMR of ring A carbons in changing configuration of 3-OH from equatorial (ursolic acid) to axial (epi-ursolic acid). This urs-12-ene skeleton was also confirmed by ^{13}CNMR data, which showed the trisubstituted double bond carbons C-12 and C-13 at δ 125.5 and δ139.2 respectively, carbon signal due to C-18 at δ 53.5 and C-19 at δ 39.3 [19].

Table (5): ^{13}CNMR spectral data (δ_C) of 3-epi-ursolic acid (4)[a] and ursolic acid (5)[b] in pyridine-d_5

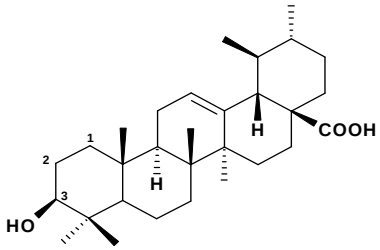
C	δ_C (4)	δ_C (5)	C	δ_C (4)	δ_C (5)
1	38.98	38.6	16	24.89	24.3
2	28.11	27.2	17	48.10	47.6
3	78.16	77.9	18	53.52	53.1
4	39.00	38.8	19	39.35	39.1
5	55.80	55.4	20	39.55	39.0
6	18.76	18.3	21	31.05	30.6
7	33.56	33.1	22	37.42	36.9
8	39.95	39.5	23	29.29	28.1
9	48.00	47.6	24	16.26	15.8
10	37.26	36.8	25	15.64	15.1
11	23.61	23.1	26	17.64	16.9
12	125.62	125.3	27	23.61	23.3
13	139.24	138.7	28	179.85	179.6
14	42.47	42.0	29	17.64	16.9
15	28.66	28.1	30	21.79	20.8

a) ^{13}C-NMR spectral data measured at 125 MHz, b) ^{13}C-NMR spectral data measured at 125 MHz [20]

The full ^{1}H-NMR data were assigned from direct ^{1}H-^{1}H COSY and ^{1}H-^{13}C COSY (HMQC) and are incomplete agreement with the structure.



(4)



(5)

The EIMS fragmentation pattern of this compound showed the retro-Diels-Alder rupture of ring C for the Δ^{12} – ursene skeleton to produce the ion at m/z 248 (100) as a base peak, which further fragments to m/z 203 (43), by loss of carboxyl group at C-17 [17]. Other fragments were at m/z 207 (15), due to other cleavage of ring C, and at m/z 189 (6) produced by loss of water molecule from this fragment. This compound has been previously isolated from *Lavandula officinalis* [25].

Isolation and identification of luteolin-3´-O-glucoside (6)

This compound was isolated from the concentrated ethyl acetate extract as yellow needles m.p 240-24^{o}C. IR spectrum showed absorption bands for hydroxyl group (3400 cm^{-1}), carbonyl group (1680 cm^{-1}) and aromatic double bond (1600 cm^{-1}). ^{1}HNMR spectrum exhibited signals for six sp^{2} aromatic and/or olefinic protons (δ_H 6.86-7.91) and seven sp^{3} proton, their pattern indicating a glycosidic flavone in nature. The ^{1}HNMR spectrum (Table 6) showed two meta-coupled protons at δ 6.86 (d, J = 2 Hz) and at δ 7.01 (d, J = 2 Hz) and singlet at δ 6.95. These data implied a flavone with 5,7 dioxygenated ring A, and these signals are due to H-6, H-8 and H-3 respectively [26].

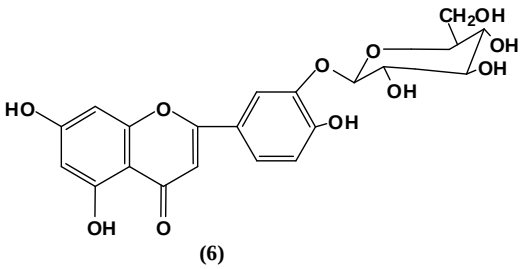
The ^{1}H-NMR spectrum also displayed signals due to three AMX type protons at δ 7.29 (1H, d, J = 9.0 Hz), δ 7.52 (1H,dd, J = 9.0 and 2.0 Hz) and δ 7.91 (1H, d, J= 2.0 Hz). These data indicated a flavone with 3', 4' dioxygenated ring B and from their pattern the signals are due to H-5', H-6' and H-2' respectively.

The above data suggested that, the isolated compound is a derivative of luteoline (7) (5,7,3',4' -tetrahydroxy flavone). ^{1}HNMR also exhibited typical signals of seven sugar protons (δ_H 4.20-5.84), the anomeric sugar proton (H-1) being displayed at δ 5.84 (d, J = 7.2 Hz) indicated β-linked of the sugar [26] and confirming the glycosidic nature of this compound.

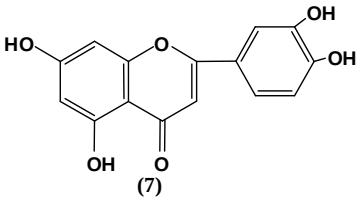
Table (6): 300 MHz ^{1}HNMR spectral data of luteolin-3'-O-glucoside (6) in pyridine-d5

H	δ_H	Pattern	J $_{(Hz)}$
3	6.95	s	-
6	6.86	d	2.0
8	7.01	d	2.0
´2	7.91	d	2.0
´5	7.29	d	9.0, 2.0
´6	7.52	dd	9.0, 2.0
˝1	5.84	d	7.2
˝2	4.41	m	-
˝3	4.41	m	-
˝4	4.41	m	-
˝5	4.20	m	-
˝6	4.41	m	-
	4.59	dd	9.0, 2.0

The position of glycosylation was assigned at the 3'-position due to lower field resonance of H-2' proton (δ 7.91) and it was found in accordance with the reported effect of O-glycosylation on the ^{1}HNMR spectrum of flavonoid aglycones which result in downfield shifts of the signals of the ortho-protons to the site of glycosylation [26].The full analysis of ^{13}CNMR confirmed the unknown structure as luteolin-3´-O-β-D-glucoside (6).



(6)



(7)

The ^{13}CNMR signals values (Table 7) supported the suggested structure showing twenty one carbon, fifteen of them sp^2 carbon due to the flavonoid aglycone, and six due to the sugar carbons. The main features of the ^{13}CNMR is the signal at δ 187.2 ppm due to C-4 carbonyl group and C-1 carbon of the sugar at δ 102.2 which indicated the sugar being β-D-glycoside [27]. The rest of ^{13}CNMR data are in complete agreement with structure and was further confirmed by comparison of the data with the literature values of luteoline (7) [28], which shows close agreement and the slight shift in resonances may be due to solvent effect.

Table (7): ^{13}CNMR spectral data of isolated and reported luteolin-3´-O-glucoside (6) and luteolin (7)

C	δ_C (6[a])	δ_C (6[b])	δ_C (7[b])	C	δ_C (6 [a])	δ_C (6 [b])	δ_C (7 [b])
2	165.7	164.6	164.5	´3	148.2	145.7	147.2
3	104.5	103.3	103.3	´4	152.3	150.9	150.1
4	183.2	181.7	182.2	´5	117.3	116.6	116.4
5	163.0	161.5	162.1	´6	120.1	122.1	119.3
6	101.0	99.0	99.2	˝1	102.2	102.4	-
7	164.4	163.5	164.7	˝2	75.2	73.5	-
8	95.7	94.2	94.2	˝3	78.9	76.3	-
9	158.3	157.5	157.9	˝4	71.5	70.3	-
10	107.0	103.9	104.2	˝5	79.7	77.3	-
´1	123.0	122.1	122.1	˝6	62.7	61.7	-
´2	115.1	115.1	113.8				

[a]) ^{13}C-NMR spectral data measured in pyridine-d_5 at 75 MHz.
[b]) Reported ^{13}C-NMR spectral data measured in DMSO-d_6 at 25.15 MHz [24]

In addition to clear NMR data, Positive FAB mass spectrum showed a prominent peak at m/z 540 attributed to [M+glycerol,448+92]$^{+}$ confirming the moleculer weight of 448. This flavonoid is very rare, however, it has been isolated from Dracocephalum thymiflorum [29] and from Luteola resede [30].

CONCLUSION

Pentacyclic triterpene alcohol uvaol, 7-methoxycoumarin, 3-epiursolic acid and the glycoside luteolin-3´-O-glucoside were isolated from *Lavandula officinalis* leaves powder after it was partitioned between different solvents and carefully purified using flash column chromatography. Isolated compounds were completely characterized by ^{1}H, ^{13}CNMR, IR and Mass spectra.

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