Available online on www.ijppr.com
International Journal of Pharmacognosy and Phytochemical Research 2017; 9(12); 1446-1451
doi: 10.25258/phyto.v9i11.11190
ISSN: 0975-4873

Research Article

Phytochemical Investigation and Cytotoxic Activity of *Rosmarinus officinalis* L. *Fam. labiateae*

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Received: 19th Jul, 17; Revised 5th Dec, 17, Accepted: 8th Dec, 17; Available Online:25th Dec, 17

ABSTRACT

A new active rolyeanone-type diterpenoid p-quinone (Moquinone) against hepatocellular carcinoma cell line Hep G2 together with four known compounds were isolated from the aerial parts of *Rosmarinus officinalis* L. The structure of the new compound was determined by extensive spectroscopic analysis including IR, EIMS, 1D and 2D 400MHz NMR data (^{1}H, ^{13}C NMR, DEPT 135 and ^{1}H-^{1}H COSY), whereas the structure of the known compounds were identified by comparison of their IR, MS, ^{1}H and ^{13}C NMR analysis with those reported in the literature data.

Keywords: *Rosmarinus officinalis*; Phytochemicals; Moquinone; Hepatocellular carcinoma.

INTRODUCTION

Rosmarinus officinalis L. is a well-known and highly valued herb that is native to mediterranean region and belongs to family Labiateae (Laminaceae)[1] . Previous phytochemical studies on genus *Rosmarinus* have shown the presence of diterpenes and triterpenes and flavonoids[2]. Rosemary is characterized by its biological importance such as digestive, tonic, astringent, diuretic, diaphoritic, and for urinary ailments[3-6]. In the present study, we reported the isolation and structure elucidation of a new rolyeanone-type diterpenoid p-quinone (Moquinone) together with four known compounds, carnosol, 4′- Methoxytectochrysin, ursolic acid and micromeric acid. The structure of the new compound was determined by extensive spectroscopic analysis including IR, EIMS, 1D and 2D 400MHz NMR data (^{1}H, ^{13}C NMR, DEPT 135 and ^{1}H-^{1}H COSY), whereas the structure of the known compounds were identified by comparison of their IR, MS, ^{1}H and ^{13}C NMR analysis with those reported in the literature data. Moreover, the organic solvent extracts of *R. officinalis* as well as the isolated compounds were phytochemically analyzed and tested for hepatocellular carcinoma cell line Hep-G2 activity.

MATERIALS AND METHODS

Plant material

R. officinalis was collected in January 2014 from the plant cultivated in the vicinity of Zagazig University and from the experimental garden of Faculty of pharmacy, Zagazig University, Egypt. The systematic identification of the plant was kindly verified by Dr. Hussein M.Abdelbaset, Assistant Professor of plant taxonomy, Department of botany, Faculty of Science, Zagazig University, Zagazig, Egypt. The plant was shade dried and ground to moderately fine powder.

Extraction and chromatography

The air dried powdered aerial part of *R. officinalis* (5 kg) was extracted by cold maceration with 70% ethanol (4×20L) till complete exhaustion. The combined alcoholic extract was evaporated under reduced pressure at 50°C to give a dark syrupy extract (1126 g) which was suspended in water and extracted with petroleum ether (pet.ether) several times. The pet.ether soluble fraction was distilled under reduced pressure (60g). The aqueous layer remaining after fractionation with pet.ether was then fractionated with methylene chloride ethyl acetate and methanol to afford (900g), (60g) and (12g) respectively.

Apparatus and Equipment

Melting points were carried out on melting point apparatus Gallen Kamp (United Kingdom). U. V. lamp for TLC visualization UVGL-55 (λ_{max} 254 and 365 nm). Infrared spectra were carried out by Jasco FT/IR 4100 spectrophotometer. Mass spectra were carried out by Mass Spectrometer Model: ISQ™ LT and operate at 70 ev. ^{1}H-and ^{13}C-NMR spectra were recorded using Bruker at 400 and 100 MHz, respectively. TLC analyses were carried out on silica gel GF254precoated plates silica gel and silica gel (70-230 Mesh, Fluka) was used for column chromatography.

Isolation of compounds 1,2 and 3 from column chromatography of petroleum ether fraction of *R. officinalis*

About 60 gm of pet. ether fraction of *R. officinalis* was dissolved in 20 ml of methylene chloride and adsorbed on 60 gm silica gel and the dried mixed initial zone was placed on the top of a silica gel column (350 gm silica gel 70-230 Mesh, Fluka, 80×5 cm) packed by the wet method using petroleum ether. The elution was carried out starting with petroleum ether then the polarity increased gradually with methylene chloride then methanol. Fractions (250ml each) were collected, concentrated and monitored by TLC. The similar

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Table 1: IC_{50} values of different plant extracts and the isolated compounds.

Tested sample	IC_{50} against Hep G2 cell line
Total hydroalcoholic extract of stem and leaves	5.5 µg/ml
Petroleum ether fraction	0.71 µg/ml
Ethyl acetate fraction	16.2 µg/ml
Methanolic fraction	5.48 µg/ml
Compound 1	4.73 µg/ml
Compound 2	›50 µg/ml
Compound 3	5.08 µg/ml
Compound 4	6.72 µg/ml
Compound 5	6.72 µg/ml

Fractions less than 20 µg/ml are considered to be biologically active [9].

fractions were pooled together and subjected to further chromatographic processes. The fraction eluted with 60% methylene chloride in pet. ether gave a red spot Rf 0.63 (TLC, pet.ether : methylene chloride 0.5 : 9.5, *P*-anisaldehyde – sulphuric acid as a spraying reagent). Repeated crystallization by acetone from methanol gave reddish crystals with m.p.190 °C ; IR ν cm^{-1} : 3349, 3059, 2954, 1747, 1667 and 1634; EIMS m/z (M+) (rel.int.): 346(zero), 300 (47.09),244 (8.19) and 57.08 (100);^{1}H-NMR ($CDCl_3$, δ ppm): 0.883(s), 0.897(s), 1.210(d, J=7.2Hz, 400Hz), 1.228(d, J=7.2Hz, 400Hz), 3.218 (sept., J=7.2, 400Hz) and 5.8 (dd, J=1.6, 400Hz);^{13}C-NMR ($CDCl_3$, δ ppm): table (2); DEPT-135 ($CDCl_3$, δ ppm) showed carbons of this compound as following: $4CH_3$, $4CH_2$, 4 CH and 8 quaternary carbons **(Compound 1).**

The fraction eluted with polarity 80% methylene chloride in pet.ether gave one major yellow spot with Rf 0.7 (TLC, methylene chloride pure, *P*-anisaldehyde – sulphuric acid as a spraying reagent). Repeated crystallization by methanol gave yellowish crystals with m**.** p. 179-180°C**;** IR spectra revealed peaks at different ν cm^{-1}; 3432, 3073, 2923, 1668, 1440, 1349, 1026, 830 and 668; EIMS m/z : M+ (rel.int.) at 298.02 (100), 166.02 (16.51), 132.05 (30.85); ^{1}H-NMR ($CDCl_3$, δ ppm): 3.872(s), 6.996(s), 6.342(s), 6.454(s), 6.55(s) and 7.811(s); ^{13}C-NMR ($CDCl_3$, δ ppm): showed peaks at 55.65, 55.92, 123.59, 162.23, 165.56, 162.73, 164.14 and 182.46 **(Compound 2).**

The fraction eluted with polarity 100% methylene chloride gave a violet spot with R_f 0.68 (TLC, methylene chloride : methanol 9.5 : 0.5, *P*-anisaldehyde – sulphuric acid as a spraying reagent).Repeated crystallization by methanol gave colourless crystals with m. p. 234-236°C; IR ν cm^{-1} at 3490, 3289, 2959, 1713, 1455 and 1354 ; EIMS m/z (M+) (rel.int.)at 330.19(32.22), 286 (100), 215 (31.19); ^{1}H-NMR ($CDCl_3$, δ ppm): 6.638(s), 5.371(d. J=2.4Hz, 400Hz), 3.083(sept., J=6.8 Hz, 400Hz), 2.929(d, J=12.4Hz, 400Hz), 2.401(dt, J=4.4,400Hz), 1.237(s), 0.903(s), 0.860(s) and other several peaks from 1.5ppm to 2.3ppm; ^{13}C-NMR ($CDCl_3$, δ ppm): table (2) **(Compound 3).**

Isolation of compounds 4 and 5 from column chromatography of methanol fraction of *R. officinalis*

About 6 gm of methanol fraction of *R. officinalis* were adsorbed on 6 gm silica gel and the dried mixed initial zone was inserted on the top of a silica gel column (150 gm, 100×2.5 cm) packed by the wet method using pet.ether. The elution was carried out starting with petroleum ether then the polarity increased gradually with methylene chloride then methanol. Fractions (250ml each) were collected, concentrated and monitored by TLC. Similar fractions were pooled together and subjected to further purification processes. Fractions eluted by methylene chloride: methanol (98: 2) gave violet spot with R_f (TLC, methylene chloride : methanol 9.5 : 0.5, *P*-anisaldehyde – sulphuric acid as a spraying reagent) and was subjected to repeated crystallization by acetone to give white crystals with m**.**p. 285°C **;** IR ν cm^{-1} : 4331, 2932, 2872,1694, 1456,1382, 1313 and 1033; EIMS m/z M+: (rel.int.)at 456.44(0.61),248.19 (100), 246.16 (10.16), 207.17 (36.27), 133.12 (67.41); ^{1}H-NMR ($CDCl_3$, δ ppm): 0.675, 0.747, 0.818, 0.865, 0.913, 1.038, 1.28, 1.453, 1.479, 1.544, 1.824, 2.119, 3.167 and 5.126; ^{13}C-NMR ($CDCl_3$, δ ppm): table (3); DEPT-135 reveals the presence of 6 quaternary carbons, 7 CH, 10 CH_2 and 7 CH_3 **(Compound 4).**

Fractions eluted by methylene chloride: methanol (96:4) gave violet spot with R_f (TLC, methylene chloride : methanol 9.5 : 0.5, *P*-anisaldehyde – sulphuric acid as a spraying reagent) and was subjected to repeated crystallization by acetone to give white crystals ; IR spectra ν cm^{-1} at 4331, 2932, 2872,1694, 1456and 1382, 1313and 1033cm^{-1}; EIMS m/z (M+) (rel.int.)at 454.38 (4.59), 246.16 (15.1), 20.18 (29.65), 133.12 (100); ^{1}H-NMR ($CDCl_3$, δ ppm): showed peaks at 0.675, 0.747, 0.818, 0.865, 0.913, 1.038, 1.28, 1.453, 1.479, 1.544, 1.824, 2.119, 3.167 and 5.126; ^{13}C-NMR ($CDCl_3$, δ ppm): table (3); DEPT-135 reveals the presence of 7 quaternary carbons, 7 CH, 10 CH_2 and 6 CH_3 **(Compound 5).**

Biological screening

Cytotoxic activity

The total hydroalcoholic extracts *R. officinalis* L. and the isolated compounds were tested for their *in-vitro* cytotoxic activity against human hepatocarcinoma cell line (Hep G2) using the MTT Cell Viability Assay [7-8]. The tested extracts and isolated five compounds were added into 96-well plates containing the 24 hours incubated tumor cell lines at different concentrations to achieve six concentrations for each of them. Six vehicle controls with media or 0.5 % DMSO were run for each 96-well plate as a control. After 24 hours, 12 mM MTT stock solution (5 mg of MTT in 1 ml of PBS) was added to each well including the untreated controls and incubated at 37°C and 5% CO_2 for 4 hours. Then, 50 µl of DMSO was added to each well and mixed thoroughly with the pipette and incubated at 37°C for 10 minutes. Then, the optical density was measured at 590 nm using a microplate ELISA reader (SunRise, TECAN, Inc, USA). Triplicate repeats were performed for each concentration and the average was calculated. The percentage of viability was calculated and the percentage of cell viability was plotted against the tested sample concentrations and the IC_{50} was estimated from graphic

Table 2: ^{13}C-NMR comparison of our compound with the available data.

Carbon no	Compound 1 ($CDCl_3$)	Royleanonic acid ($(CD_3)_2CO$) [11]	Compound 3 ($CDCl_3$)	Carnosol ($(CD_3)_2CO$)[15]
C-1	24.27	27	29.32	29.8
C-2	18.36	18	19.01	19.7
C-3	40.67	41.9	41.13	41.9
C-4	34.79	34.5	34.66	35.1
C-5	48.92	53.4	45.57	46.4
C-6	27.28	20.8	29.87	30.6
C-7	70.38	35.1	78.02	78.2
C-8	147.42	147.5	132.24	133.6
C-9	151.12	143.8	121.75	123.1
C-10	45.21	47.5	48.53	49.2
C-11	173.59	176	141.18	143.8
C-12	124.07	153.2	141.89	143.4
C-13	139.18	125.3	132.91	135.1
C-14	180.23	184.1	112.43	112.4
C-15	27.92	25.1	27.44	27.6
C-16	19.91	20.6	22.59	23
C-17	19.57	20.5	22.65	23.1
C-18	34.79	33.1	31.84	32
C-19	19.92	20.2	19.84	20
C-20	182.25	188.4	175.99	175.9

plots of the dose response curve for each concentration using Graphpad Prism software (San Diego, CA, USA). The IC_{50} of tested samples were measured (µg/ml) table (1).

RESULTS AND DISCUSION

On fractionation and repeated chromatographic purification, the hydroalcoholic extract of *R. officinalis* L. growing in Egypt yielded five compounds (three compounds from the petroleum ether fraction and two compounds from methanol fraction).

Compound 1: obtained as reddish crystals with m.p.190 °C, R_f 0.26 (TLC, pet. ether : methylene chloride 0.5 : 9.5).The molecular formula was determined to be $C_{20}H_{42}O_4$ by EIMS (m/z 346 g/mol), ^{13} C NMR and DEPT analysis. The oxygen-containing functionalities could be deduced from the IR, ^{1}H and ^{13}C NMR spectral data which showed the presence of free carbonyl group [C=O (IR: ν =1747 cm-1; ^{13}CNMR: δ=173.59] and *p*-quinone carbonyl groups [2×C=O (IR: ν =; 1667 and 1634 cm-1; ^{13}C NMR δ= 182.25 and 180.23]. The ^{13}C NMR spectral data (Table 2) revealed 20 carbon atoms while their multiplicity essential were assigned by DEPT 135 analysis. The carbons were assigned as quaternary carbons, 4 CH, 4 CH_2 and 4CH_3. The ^{1}H NMR spectrum showed characteristic signals of an isopropyl group attached to an aromatic ring. EIMS (*m/z*): 346(M^+, zero), 302(3.74), 301(9.98), 300(47.09), 285(20.84), 257(18.44), 245(8.19), 230(45.15), 219(8.20), 69(92.81) and 57.08(100). The IR (KBr) absorption ν1667 and 1634 were characteristic for *para*-benzoquinone. The absorption in IR spectra at ν 3059 cm^{-1} and 1747 cm^{-1} suggested the existence of a carboxyl group. This was confirmed by ions *m/z* 300 and 302 in EIMS. The molecular ion lost CO_2 to form *m/z* 302 , lost carboxyl group and a hydrogen atom to form *m/z* 300. It was possible for the carboxyl group to locate at positions 18, 19 or 20. Since the ion *m/z* of 69 in EIMS was highly characteristic for royleanone type diterpenoids without modification on ring A[10], the carboxyl group was assigned to position 20. The ^{1}H NMR spectrum showed the presence of four methyl signals at δ 0.883, 0.897, 1.210, 1.228 and 5.8 (dd, J=4 Hz). Signal at δ 5.8 (dd, J=4 Hz) reveals the presence of a benzylic proton at C7 and its carbon's value in ^{13}C NMR is δ= 70.38. Signal at δ 7.134 (s) reveals the presence of a single aromatic proton at position C12.The presence of this signal indicating 5 substituted quinine moiety and excluding the known hydroxyl paraquinone moiety [11-12].The sec-methyl signals at δ 1.210 and 1.288 (each *d, J* = 7.2 Hz), which showed coupling with a septet proton at δ 3.218 (H-15) in ^{1}H-^{1}H COSY spectrum, indicated the presence of an isopropyl group, while the down-field shift (δ 3.218) indicated the attachment with an aromatic ring. Therefore, the compound was identified and named as Moquinone.

This represents the first report for the isolation of this compound from genus *Rosmarinus* and from nature.

Compound 2:was obtained as yellowish crystals with m.p. 179-180°C, R_f 0.7(TLC, methylene chloride pure).

The IR spectrum showed absorption peaks at (KBr) ν cm^{-1} : 3432(OH), 3073(CH aromatic), 2923(CH stretching), 1668(C=C), 1440, 1349 (CH_3, CH_2-bending), 1026 (C-O), 830 and 668 (out of plane bending of C=C) .

The mass spectrum showed the following mass fragments: m/z (relative int., %) 298.02 (100), 166.02 (16.51), 132.05 (30.85).

^{1}H-NMR spectra data showed methoxy groups at δ 3.872. It also showed signals of protons at position 2',6' and 3',5' at δ 6.996 and 7.811. Signals of protons at positions C3, C6 and C8 appear as single peaks at δ 6.342, 6.454 and 6.55.

^{13}C-NMR spectra data showed two signals of the

Table 3: ^{13}C-NMR of compound 4 , 5, the reported ursolic acid and micromeric acid.

Carbon no	Compound 4 (DMSO-d_6)	Ursolic acid (CD_3OD) [17]	Compound 5 (DMSO-d_6)	Micromeric acid ($(CD_3)_2CO$)[12]
C-1	38.23	39	38.37	39.9
C-2	28.25	28.3	27.53	28.5
C-3	76.82	79.1	76.82	80.3
C-4	38.43	39.2	38.49	39.9
C-5	54.48	55.5	54.78	56.7
C-6	17.99	18.6	16.9	18.3
C-7	32.7	33.3	32.7	34.2
C-8	38.37	38.9	38.23	40.7
C-9	47.01	47.8	46.48	48.8
C-10	36.31	37.1	36.54	38.2
C-11	23.26	23.6	23.12	24.6
C-12	124.57	125.8	124.95	127.7
C-13	138.18	138.5	137.87	138
C-14	41.7	42.3	41.63	43.3
C-15	26.98	27	26.98	29.1
C-16	23.8	24.5	23.98	25.6
C-17	47.04	48.1	47.04	48
C-18	52.3	53.1	52.3	56.1
C-19	38.49	39.4	38.43	38.7
C-20	41.63	39.7	152.94	152.8
C-21	30.18	30.9	30.18	33.5
C-22	36.52	37.2	36.31	40.1
C-23	27.53	28.3	28.25	29.4
C-24	16.9	17.1	16.9	16.9
C-25	15.22	15.7	15.22	16.6
C-26	16.07	15.9	16.07	18.3
C-27	23.12	23.8	23.26	24.6
C-28	178.26	181.1	177.63	177.8
C-29	17	17.3	17	17.3
C-30	21.07	21.5	104.71	106.5

methoxy groups at δ 55.65 and 55.92. The carbonyl signal appears at δ 182.46. Carbon carrying hydroxy group at positions C7 appears at δ 165.56 , C4' appears at δ 162.73 and C5 appears at δ 162.23. C1' appears at δ 123.59 while C2 appears at δ 164.14 [13-14]. From the above mentioned spectral characteristics beside the comparison with the available published data [13-14], it is concluded that compound 2 is identical with **4′-Methoxytectochrysin.**

Compound 3: was isolated as colorless crystals with m.p. 234-236°C, R_f 0.68 (TLC, petroleum ether : methylene chloride 0.5 : 9.5).

IR spectra showed peaks at 3490 which confirm presence of OH group, 3289 which confirm presence of CH aromatic, 2959 which confirm presence of CH_2CL_2 aliphatic, 1713 which confirm presence of carbonyl group, 1455 which confirm presence of CH_2, and 1354 which confirm presence of CH_3.

Mass spectrum illustrates the molecular weight at 330 and base peak at 286.

^{13}C-NMR showed 20 signals which illustrate presence of diterpene from the biosynthesis. Signal at 175.99 confirm presence of carbonyl group, signal at 78.02 reveals presence of oxygenated carbon.

^{1}H-NMR showed signals at singlet peak at 6.638 (s), 5.371 (d, J=2.4Hz), 3.118 (sept.,J=4 Hz), 2.929 (ddd, J=12.4Hz), 2.434 (dt, J=4.4Hz), 2.238, 0.903 (s) and 0.860 (s).

From the previous spectral data, it was found to be a carnosol upon comparison to previous literature as shown at table (2).

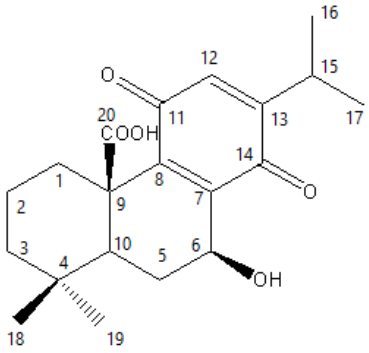
Compound 4:was crystallized from methanol fraction as white crystals.

IR spectra revealed peaks at different ν at 4331, 2932, 2872, 1694, 1456 and 1382, 1313 and 1033cm^{-1}. IR spectra shows the presence of strong absorption at 1694.16 cm^{-1} and broad absorption at 3431.71 cm^{-1} are characteristic of a carboxylic moiety. The IR absorption frequencies of Ros-54 matches with the reported data of ursolic acid [16].

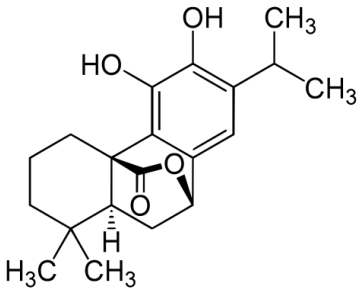
Mass spectra obtained m/z (M+) (rel.int.)at 456.44(0.61), 248.19 (100), 203.19(58.72), 133.12 (67.41) [16].

^{1}H-NMR ($CDCl_3$, δ ppm): showed peaks at 0.675, 0.747, 0.818, 0.865, 0.913, 1.038, 1.28, 1.453, 1.479, 1.544, 1.824, 2.119, 3.167 and 5.126.

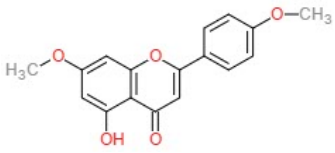
^{1}H-NMR showed signals at 0.675 (CH-5), 0.747 (CH_3-25), 0.818 (CH_3-30), 0.865 (CH_3-26), 0.913 (CH_3-29), 1.038 (CH_3-27), 1.28 (CH_2-7), 1.453 (m, 4H, CH_2(Hβ)-6, CH_2 (Hβ)-7, CH_2 (Hα)-9, CH_2 (Hα)-21)), 1.544 (m, 4H, CH_2 (Hβ)-1, CH_2 (Hα)-15, CH_2 (Hβ)-16, CH_2 (Hβ)-21)), 1.824 (CH_2-22), 2.119 (CH-18), 3.167(CH-3), 5.126



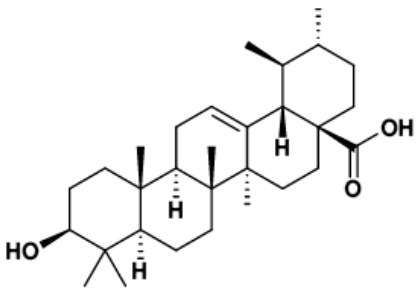
Moquinone (1)



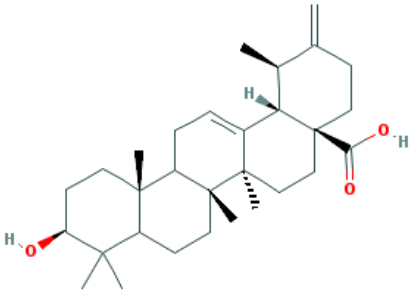
Carnosol (3)



4′- Methoxytectochrysin (2)



Ursolic acid



Micromeric acid (5)

(CH-12) which are compatibility with the reported data of ursolic acid [17-18].

^{13}C-NMR ($CDCl_3$, δ ppm): showed peaks at 15.22, 16.07, 16.9, 17, 17.99, 21.07, 23.26, 23.12, 23.8, 26.98, 28.25, 27.53, 30.18, 32.7, 36.31, 36.52, 38.37, 38.23, 38.43, 38.49, 41.63, 41.7, 47.01, 47.04, 52.3, 54.48, 76.82, 124.57, 138.18, 178.26.

^{13}C-NMR illustrates presence of triterpene from the biosynthesis. Signal at 178.26 confirms presence of carbonyl group, signal at 76.82 reveals presence of oxygenated carbon. Signals of methyl groups at positions C23, C24, C25, C26, C27, C29 and C30 appears at δ 27.53, 16.9, 15.22, 16.07, 23.12, 17 and 21.07 respectively. C12 presents at δ 124.57 and C13 presents at δ 138.18 which confirm the presence of the double bond between these two atoms. The previous ^{13} C-NMR spectral data is matched with the published one of ursolic acid [17] table (3).

DEPT-135 reveals the presence of 6 quaternary carbons, 7 CH, 10 CH_2 and 7 CH_3.

From the above mentioned spectral characteristics beside the comparison with the available data, compound 4 is concluded to be ursolic acid**.**

Compound 5: was crystallized from methanol fraction as white crystals.

IR spectra revealed peaks at different ν at 4331, 2932, 2872,1694, 1456 and 1382, 1313 and 1033cm^{-1} like the IR spectrum of compound 4.

Mass spectra obtained m/z (M+) (rel.int.)at 454.38 (4.59), 246.16 (15.1), 20.18 (29.65), 133.12 (100).

^{1}H-NMR ($CDCl_3$, δ ppm): showed peaks at 0.675, 0.747, 0.818, 0.865, 0.913, 1.038, 1.28, 1.453, 1.479, 1.544, 1.824, 2.119, 3.167 and 5.126.

^{1}H-NMR showed signals at 0.675 (CH-5), 0.747 (CH_3-25), 0.818 (CH_3-30), 0.865 (CH_3-26), 0.913 (CH_3-29), 1.038 (CH_3-27), 1.28 (CH_2-7), 1.453 (m, 4H, CH_2(Hβ)-6, CH_2 (Hβ)-7, CH_2 (Hα)-9, CH_2 (Hα)-21)), 1.544 (m, 4H, CH_2 (Hβ)-1, CH_2 (Hα)-15, CH_2 (Hβ)-16, CH_2 (Hβ)-21)), 1.824 (CH_2-22), 2.119 (CH-18), 3.167(CH-3), 5.126 (CH-12) .DEPT-135 reveals the presence of 7 quaternary carbons, 7 CH, 10 CH_2 and 6 CH_3.

^{13}C-NMR ($CDCl_3$, δ ppm) in table (3) illustrates presence of 30 carbons suggesting a triterpene skeleton. Signal at 177.63 confirms presence of carboxyl group, signal at 104.71 reveals the presence of exocyclic sp2 hybridized methylene for C30 and 152.94 for sp2 hybridized quaternary carbon at C20 , signal at 76.82 reveals presence of oxygenated carbon. Signals of methyl groups at positions C23, C24, C25, C26, C27, C29 and C30 appears at δ 27.53, 16.9, 15.22, 16.07, 23.12, 17 and 21.07 respectively. C12 presents at δ 124.57 and C13 presents at δ 137.87 which confirm the presence of the double bond between these two atoms. The previous spectral data show the presence of micromeric acid upon comparison with literature.

Biological studies

Cytotoxic activity

As shown in table 2 the total hydroalcoholic extracts of *Rosmarinus officinalis* possessed a promising cytotoxic activity against hepatocellular carcinoma.

CONCLUSION

A new active rolyeanone-type diterpenoid p-quinone (Moquinone) against hepatocellular carcinoma cell line Hep G2 with IC_{50} = 4.73 µg/ml together with four known compounds were isolated from the aerial parts of *Rosmarinus officinalis* L. According to the promising *in-vitro* result of the cytotoxicity of the new compound, we recommend further *in-vivo* cytotoxicity analysis for it.

ACKNOWLEDGMENTS

The authors are grateful to the Department of Pharmacognosy, faculty of pharmacy , Zagazig University, Zagazig, Egypt where this work was achieved.

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