Hany Ezzat Khalil et al /J. Pharm. Sci. & Res. Vol. 7(8), 2015, 509-513

ISSN:0975-1459
Journal of Pharmaceutical Sciences and Research
www.jpsr.pharmainfo.in

Phytochemical and Biological Studies of *Cichorium endivia* L. Leaves

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Abstract

Phytochemical investigation of the air dried leaves of *Cichorium endivia* using various chromatographic separation techniques, led to the isolation of ten compounds of which four coumarin derivatives (aesculetin, scopolin, cichoriin and hymexelsin), two flavonoid derivatives (quercetin and kaempferol-3-O-β-D-glucoside), three phenolic acids (4-hydroxy-phenyl acetic acid , ferulic acid and caffeic acid) and z-3-hexenyl-β-D-glucoside. The structures of these compounds were determined by interpretation of their physical and spectroscopic data and comparison to those reported in literature. All isolated compounds were evaluated for their antileishmanial, multidrug resistance and 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activities. Compounds **3**, **6** and **7** showed marked scavenging activities, while compound **9** showed moderate activity on the other hand compounds **1**, **5** and **8** demonstrated much weaker effects, comparable with the standard trolox. The rest of the tested compounds did not exhibit DPPH radical scavenging activity. None of the tested compounds (**1**–**10**) showed anti-leishmanial or multidrug resistant activities.

Key words: *Cichorium endivia*, Asteraceae, coumarins, anti-leishmanial, DPPH.

1. Introduction

Family Asteraceae is one of the largest families of vascular plants. It contains more than 22,750 species, spread across 1620 genera of trees, shrubs and herbaceous [1]. Many economically important remedies come from members of composites, including cooking oils, lettuce, artichokes, sunflower seeds, sweetening agents, and teas [2-7].

Cichorium endivia L. is one of the six species of the genus *Cichorium,* which is a nutritionally valuable member of the sunflower family (Asteraceae). *C. endivia* L. is a popular salad vegetable of Mediterranean plants that indigenous to Europe, Western Asia and North America and is now widely cultivated [8-10].

C. endivia L. contains wide range of compounds; Sesquiterpenes and their glycosides [9, 11-13], flavonoid and phenolic compounds [10, 14-19] and nitrogenous compounds [20]. The various components of this vegetable also have been tested for some biological activities; such as, hepatoprotective [21], antioxidant [22-24] and cytotoxic activities [12, 20, 25]. In spite of the worldwide distribution of *C. endivia* L. and the fact that the plant material collected from different geographical regions has different contents of biologically active compounds, most of the phytochemical investigations described in the literature refer to *C. endivia* L. of Chinese, Polish, Japanese, Italian, Saudi and Brazilian origins. Careful search on the available literature data revealed that there is a single report on *C. endivia* L. of Egyptian origin with the isolation of the flavonoid (luteolin) only [9]. As one of targets of this study is to widen the screening of biological activities and by checking the various biological activities reported in literature [12, 20-25]. The findings prompted us to pursue the current study with carrying out some other biological activities like assessing the effect of isolated pure compounds from *C. endivia* L. as multidrug resistance and anti-leishmanial activities.

2. Materials And Methods

2.1. Instruments and material

^{1}H- and ^{13}C-NMR spectra were recorded on JEOL JNM α-400 spectrometer with tetramethylsilane (TMS) as an external standard. Highly porous synthetic resin Diaion HP-20 was purchased from Mitsubishi Chemical Co., Ltd. (Tokyo, Japan). Silica gel column chromatography (CC) was performed on silica gel 60 [(E. Merck, Darmstadt, Germany) 70-230 mesh]. Reversed-phase [octadecylsilanized silica gel (ODS)] open CC (RPCC) was performed on Cosmosil 75C18-OPN (Nacalai Tesque, Kyoto,Japan). The droplet counter-current chromatography (DCCC) (Tokyo Rikakikai, Tokyo, Japan) was equipped with 500 glass columns (Φ 2 mm, L=40 cm), and the lower and upper layers of a solvent mixture of $CHCl_3$–MeOH–H_2O–1-PrOH (9:12: 8:2) were used as the mobile and stationary phases, respectively. Five-gram fractions were collected and numbered according to their order of elution with the mobile phase. High-performance liquid chromatography (HPLC) was performed on an ODS column [Inertsil ODS-3; GL Science, Tokyo, Japan; (Φ =6 mm, L=25 cm, flow rate: 1.5 ml/min) using a refractive index refractometer and UV detector. Pre-coated Reversed-phase [octadecylsilanized silica gel (ODS)] F_{254} plates (E. Merck; 0.25 mm in thickness) were used for preparative TLC process .Pre-coated silica gel 60 F_{254} plates (E. Merck; 0.25 mm in thickness) were used for TLC analyses, visualized by spraying with a 10% H_2SO_4 solution in ethanol and heating to 150 °C on a hotplate. VERSA max tunable UV-micro plate reader (Molecular Devices, USA). Asahi, 4020, CO2 incubator (Japan). Kubota, KR/702, centrifuge (Japan). Fluorescence-activated cell sorter (FACS) (Japan). Human Adriamycin resistant cell line K562/Adr was obtained from the RIKEN Cell Bank, Japan. M-199 medium (Sigma-Aldrich Co., USA), supplemented with 10% heat-inactivated fetal bovine serum (FBS) and seeded with Leishmania promastigotes at 2x105

cells/100µl of medium (L. major: MHOM/SU/73/5ASKH strain) From (National Bio-Resource Project, Japan). Kanamycin (Wako, Japan). Amphotericin B (Sigma-Aldrich Co, USA). 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT Solution) (Nacalai, Japan). S(−)-6-hydroxy-2,5,7,8-tetramethylchlroman-2-carboxylic acid (Trolox) (Aldrich Chemical Co., Japan). 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Aldrich Chemical Co., Japan). Folin-Ciocalteu reagent (Sigma-Aldrich Co, USA). Gallic acid (Wako, Japan). Verapamil (Wako, Japan). RPMI-1640 medium and Fetal Calf Serum (FCS) (Nacalai, Japan).

2.2. Plant Material

The leaves of *Cichorium endivia* L. *were* collected from Assuit Botanical Garden, Assuit, Egypt (April 2007)**.** A voucher specimen of the plant is deposited in the Herbarium of Department of pharmacognosy, Faculty of Pharmacy, Minia University, Minia, Egypt (Minia-07-July-CE).

2.3. Extraction and Isolation

The air-dried powdered leaves of *C. endivia* L (2.0 kg) were exhaustively extracted with 70% MeOH, (10 L X 3) at 25 °C for 1 week and then the combined extracts were concentrated under vacuum yielded 213.0 g viscous extract. The extract was partitioned between n-hexane (6 L) and H_2O to give n-hexane derived residue (35.0 g), and then the aqueous layer was further fractionated with ethyl acetate (6 L) and 1-butanol (6 L) to give an ethyl acetate soluble fraction (20.0 g) and 1-butanol-soluble fraction (40.0 g), respectively. The remaining aqueous fraction was of 110.0 g.

The ethyl acetate fraction (19.0 g) was fractionated by normal phase silica gel (NP-Silica) (*Φ* =5.0, L=100 cm, 1000 g), employing gradient elution from n-hexane to EtOAc, yielded six fraction (I-VIII), monitored by TLC. Fraction V (606.0mg) was purified using NP-Silica (Φ = 3 cm x 100 cm, 200.0 g, 100 ml each) employing gradient elution from n-hexane to EtOAc, to yield five fractions (5.1 to 5.5). Fraction 5.5 (190.0 mg) was subjected to ODS (Φ =3 cm, L= 25 cm), employing 70 % MeOH in water as eluent to yield six fractions (5.5.1 to 5.5.6). Fraction 5.5.1(28.0 mg) was subjected to HPLC (65% MeOH) to produce compound **1** (3.0 mg). Fraction 5.5.2 (46.0 mg) was subjected to HPLC (65% MeOH) to produce compound **2** (2.5 mg). Fraction VI (1.5 g) was purified using NP-Silica (Φ = 5 cm x 100 cm, 400 g, 100 ml each) employing gradient elution from n-hexane to EtOAc, to yield six fractions (6.1 to 66). Fraction 6.2(198.0 mg) was subjected to ODS (Φ =3 cm, L= 25 cm), employing 50-100 % MeOH in water gradient as eluent to yield compound **3** (20.0 mg). Fraction VII (5.5 g) was purified using NP-Silica (Φ = 5 cm x 100 cm, 800.0 g, 100 ml each) employing gradient elution from CH_3Cl to MeOH to yield five fractions (7.1 to 7.5). Fraction 7.1(145.0 mg) was subjected to ODS (Φ =3 cm, L= 25 cm), employing 30 % MeOH in water as eluent to yield compound **4** (5.0 mg). Fraction 7.2(1.0g) was subjected to NP-Silica (Φ = 5 cm x 100 cm, 400.0 g, 50 ml each) employing gradient elution from CH_3Cl to MeOH, to yield six fractions (7.2.1 to 7.2.6). Fraction 7.2.2(300.0 mg) was subjected to ODS (Φ =3 cm, L= 25 cm), employing 30 % MeOH in water as eluent to yield compound **5** (50.0 mg). Fraction 7.2.4(220.0 mg) was subjected to DCCC to yield compound **5** (40.0 mg) and compound **6** (5.6 mg). Fraction 7.2.6(110.0 mg) was subjected to DCCC to yield compound **7** (6.0 mg). Fraction 7.3(870.0 mg) was subjected to NP-Silica (Φ = 5 cm x 100 cm, 200.0 g, 50 ml each) employing gradient elution from CH_3Cl to MeOH, to yield six fractions (7.3.1 to 7.3.6). Fraction 7.3.5(120.0 mg) was subjected to DCCC to yield compound **8** (4.3mg).

The 1-BuOH fraction (40.0 g) was chromatographed over Diaion-HP-20 CC (1.5kg), (Φ =10 cm, L =100 cm), using MeOH-H_2O gradient system, 1000ml fractions being collected and the similar fractions were combined to yield four fractions, the B.10% MeOH, B.30% MeOH, B.80% MeOH and B.100%MeOH. Fraction B.30% MeOH (5.0 g) was purified using NP-Silica (Φ = 5 cm x 100 cm, 800.0 g, 100 ml each) employing gradient elution from CH_3Cl to MeOH to yield six fractions (B.30.1to B.30.6). Fraction B.30.1 (160.0 mg) was subjected to DCCC to yield 5 fractions (B.30.1.1to B.30.1.5). Fraction B.30.1.5 (30.0 mg) was subjected to preparative Reversed-phase TLC using 30% MeOH as developer to yield compound **9** (7.0 mg). Fraction B.80% MeOH (7.0 g) was purified using NP-Silica (Φ = 5 cm x 100 cm, 1000 g, 100 ml each) employing gradient elution from CH_3Cl to MeOH to yield six fractions (B.80.1to B.80.6). Fraction B.80.3 (230.0 mg) was subjected to DCCC to yield compound **10** (10.0 mg).

2.4. Determination of total phenolic contents

The total phenolic content was measured using the Folin-Ciocalteu index method according to (Artemio et al., 2012) [26]. Stock solutions (1mg/ml) of different extracts were prepared in methanol. Six ml of water and 0.5 ml of Folin-Ciocalteu reagent were successively added to 0.1 ml of stock solution of each extracts. In addition, 1.5 ml of a 20% sodium carbonate solution and water were added to obtain 10 ml. A reaction took place within 120 min. at room temperature. Absorbance was measured at 760 nm. Calibration was done using Gallic acid serial dilution as a standard (0.5, 0.4, 0.3, 0.2 and 0.1 mg/ml in distilled water, y = 0.016x - 0.001, R2 = 0.9856,). The concentration of phenolic components was expressed as the equivalent to milligrams of Gallic acid per gram of dry extract (mg GAE/g).

2.5. Biological Activities

2.5.1. Multidrug resistance activity

P-glycoprotein (P-gp) is a plasma membrane transporter that excludes therapeutically administrated anti-cancer drugs from cancer cells. High expression of this glycoprotein yields multidrug resistance and this is a serious chemotherapeutic issue. Thus, inhibition of the activity of this protein can maintain the effects of the therapeutic anti-cancer drugs and the co-administration of the chemicals having such activity is clinically useful. A P-gp over expressing adriamycin resistant cell line, K562/Adr cells, was used to assay the inhibitory activity of the

isolated compounds by monitoring the accumulation of calcein in the cells. The amounts of the accumulated calcein were determined by flow cytometey using a fluorescence-activated cell sorter (FACS). K562/Adr cells were inoculated at density of $1X10^5$ cells per well in 150 µl of RPMI-1640 medium containing 10% Fetal Calf Serum (FCS), supplemented with 100 mg/ml of kanamycin sulphate and 0.5µg/ml of amphotericin B, on the 96-well plates and then incubated in 5% CO_2 incubator at 37 °C for 15 min. Prior to inoculation, 2µl aliquots of sample solution in DMSO were added. At 15 min after the addition of 50 µl of 1µM calcein-AM in the medium, the fluorescence of individual cells was measured with FACS. Verapamil was used as a positive control [27].

2.5.2. Anti-leishmanial assay

The leishmanicidal activities of isolated compounds were performed using the colorimetric MTT assay. "Medium-199" medium supplemented with 10% heat-inactivated fetal bovine serum (FBS) and 100 µg/ml of kanamycin was used as the cell culture medium. The test compounds were dissolved in DMSO and added to the each well of the 96-well micro-titration plates at 1% as final concentration. *Leishmania major* cells (2×10^5 cells /well) were cultured in a CO_2 incubator at 25 °C for 72 h. then, MTT solution was added to each well and the plates were incubated overnight at 25 °C. The yellow MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] penetrates the healthy living cells and in the presence of the mitochondrial dehydrogenases, MTT is transformed to its blue formazan product. After the incubation and liberation of formazan product, the absorbance was measured at 540 nm using a microplate reader. Amphotericin B was used as a positive control [28].

The inhibition % was calculated using the following equation:

% Inhibition= [1- (A sample - A blank) / (A control - A blank)] × 100

Where A control is the absorbance of the control reaction mixture (containing DMSO and all reagents except for the test compounds). IC_{50} was determined as the concentration of sample required to inhibit the formation of MTT formazan by 50%.

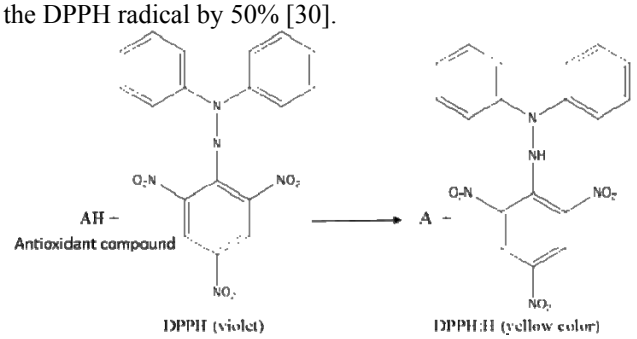
2.5.3. DPPH radical scavenging activity

The method is based on the reduction of methanolic 2,2-diphenyl-1-picrylhydrazyl (DPPH) solution in the presence of a hydrogen donating antioxidant, due to the formation of the non-radical form DPPH-H by the reaction. The reduction in DPPH radical was determined by the decrease in its absorbance at 515 nm induced by antioxidants, resulting in a color change from violet to yellow. This change in colour is a simple yet effective qualitative and quantitative parameter for the presence of an antioxidant. Hence, DPPH radical is used as a substrate to evaluate the antioxidative action of antioxidants [29].

The absorbance with various concentrations of the test extracts and compounds dissolved in MeOH (100 µl) in a 96-well micro-titration plate was measured at 515 nm at zero time as A blank. Then, 200 µM DPPH solution (100µl) was add to each well, followed by incubation at room temperature for 30 min. The absorbance was measured again as A sample. The % inhibition was calculated using the following equation:

% inhibition= [1 - (A sample - A blank) / (A control - A blank)] ×100

Where A control is the absorbance of the control reaction mixture (containing DMSO and all reagents except for the test extracts and compounds). IC_{50} was determined as the concentration of sample required to inhibit the formation of the DPPH radical by 50% [30].



3. RESTLTS AND DISCUSSION

Air-dried leaves of *C. endivia* L. were extracted with 70% MeOH three times and the concentrated MeOH extract was partitioned with solvents of increasing polarity. The EtOAc and 1-BuOH-soluble fraction were separated by means of various chromatographic procedures including column chromatography (CC) on a highly-porous synthetic resin (Diaion HP-20), normal silica gel CC and reversed-phase octadecyl silica gel (ODS) CC, The droplet counter-current chromatography (DCCC) and high-performance liquid chromatography (HPLC), to afford ten compounds (**1-10**). The structures of the isolated compounds were determined to be 4-hydroxy-phenyl acetic acid (**1**) [31], z-3-hexenyl-β-D-glucoside (**2**) [32], aesculetin (**3**) [33-34], scopolin (**4**) [35-36], cichoriin (**5**) [35, 37], quercetin (**6**) [38], Astragalin [kaempferol-3-O-β-D-glucoside](**7**) [39], ferulic acid (**8**) [40-41], caffeic acid (**9**) [34] and hymexelsin [xeroboside] (**10**) [36, 42] by comparing their spectroscopic data with those reported in literature (Figure 1).

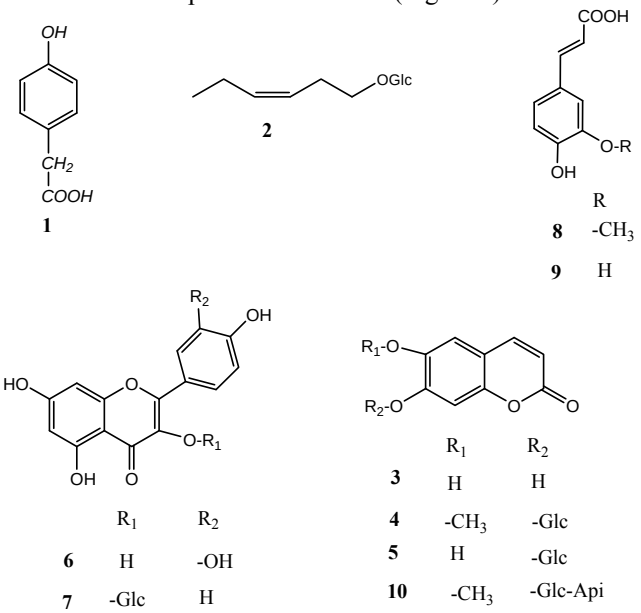


Figure 1: Structure of the isolated compounds (**1-10**)

4-Hydroxy phenyl acetic acid (1)

^{1}HNMR (400 MHz, DMSO-d6): δ_H 6.8 (2H, d, J=8.4 Hz, H-3 & H-5), 7.1 (2H, d, J=8.4 Hz, H-2 &H-6). ^{13}C NMR (100 MHz, DMSO-d6): δ_c 39.99 (CH2-), 114.92 (C-3 & C-5), 125.21 (C-1), 130.10 (C-2 & C-6), 155.93 (C-4), 173.07 (-COOH).

z-3-Hexenyl-β-D-glucoside (2)

^{1}HNMR (400 MHz, CD_3OD): δ_H 5.44 (IH, m, H-4), 5.39 (IH, m, H-43), 4.26 (IH, d, J=7.9 Hz, H`-1), 2.39 (2H. q, J= 8 Hz, H-2), 2.07 (2H, q, J=7.1 Hz, H-5), 0.96 (3H, t, J=7.5 Hz, H-6). $^{13}$C NMR (100 MHz, $CD_3OD$): $\delta_c$ 70.5 (C-1), 28.7 (C2), 134.6 (C-3), 125.9 (C-4), 21.6 (C-5), 14.6 (C-6), 104.4 (C-1`),75.2 (C-2`), 78.0 (C-3`), 71.7 (C-4`), 78.2 (C-5`), 62.9 (C-6`).

Aesculetin (3)

^{1}H-NMR (400 MHz, CD_3OD): δ_H 7.76 (1H, d, J = 9.5 Hz, H-3), 6.93 (1H, s, H-5), 6.75 (1H, s, H-8), 6.17 (1H, d, J = 9.5 Hz, H-4). ^{13}C-NMR (100 MHz, CD_3OD): δ_c 164.3 (C-2), 152.0 (C-7), 150.6 (C-9), 146.1 (C-4), 144.6 (C-6), 113.1 (C-5), 112.9 (C-3), 112.6 (C-10), 103.7 (C-8).

Scopolin (4)

^{1}H-NMR (400 MHz, CD_3OD): δ_H 7.91 (1H, d, J = 9.5 Hz, H-3), 7.21 (1H, s, H-5), 7.18 (1H, s, H-8), 6.30 (1H, d, J = 9.5 Hz, H-4), 5.06 (IH, d, J=7.3 Hz, H`-1), 3.90 (3H, s, O-Me). $^{13}$C-NMR (100 MHz, $CD_3OD$): $\delta_c$ 163.7 (C-2), 150.5 (C-7), 149.7 (C-9), 145.7 (C-4), 145.5 (C-6), 115.4 (C-10), 114.8 (C-3), 114.0 (C-5), 57.1 (O-Me), 105.6 (C-8), 103.3 (C-1`),74.8 (C-2`), 78.6 (C-3`), 71.4 (C-4`), 77.6 (C-5`), 62.5 (C-6`).

Cichoriin (5)

^{1}H-NMR (400 MHz, CD_3OD): δ_H 7.81 (1H, d, J = 9.5 Hz, H-3), 7.20 (1H, s, H-5), 7.03 (1H, s, H-8), 6.28 (1H, d, J = 9.5 Hz, H-4), 4.97 (IH, d, J=7.5 Hz, H`-1). $^{13}$C-NMR (100 MHz, $CD_3OD$): $\delta_c$ 163.7 (C-2), 150.5 (C-7), 149.7 (C-9), 145.7 (C-4), 145.5 (C-6), 115.4 (C-10), 114.8 (C-3), 114.0 (C-5), 105.6 (C-8), 103.3 (C-1`),74.8 (C-2`), 78.6 (C-3`), 71.4 (C-4`), 77.6 (C-5`), 62.5 (C-6`).

Quercetin (6)

^{1}H-NMR (400 MHz, DMSO-d6): δ_H, 7.66 (1H, d, J=2.2 Hz, H-2`), 7.54 (1H, dd, J=8.6, 2.2 Hz, H-6`), 6.88 (1H, d, J=8.6 Hz, H-5`), 6.40 (1H, d, $J$= 2.2 Hz, H-8), 6.19 (1H, d, $J$= 2.2 Hz, H- 6). $^{13}$C NMR (100 MHz, DMSO-d6): $\delta_c$ 175.8 (C-4), 163.8 (C-7), 160.6 (C-5), 156.1 (C-9), 147.6 (C-4`), 146.8(C-2), 145.0 (C-3`), 135.6 (C-3), 121.9 (C-1`) 120.0 (C-6`), 115.5 (C-5`), 115.0 (C-2`), 103.0 (C-10), 98.1 (C-6), 93.3 (C-8).

Astragalin [kaempferol-3-O-β-D-glucoside] (7)

^{1}H-NMR (400 MHz, CD3OD): δ_H 8.05 (2H, d, J= 9.0 Hz, H-2`, 6`), 6.88 (2H, d, J= 9.0 Hz, H- 3`, 5`), 6.40 (1H, d, J= 2.2 Hz, H-8), 6.20 (1H, d, J= 2.1 Hz, H-6), 5.22 (1H, d, J= 7.5 Hz, H-1``), 3.10- 3.80 (6H, m, H-2`` to H-6``). $^{13}$C NMR (100 MHz, $CD_3OD$): $\delta_c$ 179.6 (C-4), 166.1 (C-7), 163.2 (C-5), 161.6 (C-4`), 159.2 (C-9), 158.6(C-2), 135.6 (C-3), 132.3 (C-2`, 6`), 122.9 (C-1`), 116.2 (C-3`, 5`), 105.8 (C-10), 100.0 (C-6), 94.9 (C-8), 104.3 (C-1``),75.8 (C-2``), 78.5 (C-3``), 71.5 (C-4``), 78.2 (C-5``), 62.8 (C-6``).

Ferulic acid (8)

^{1}H-NMR (400 MHz, CD_3OD): δ_H 7.59 (1H, d, J=15.9 Hz, H-3), 7.16 (1H, br.s, H-2`), 7.05 (1H, d, J=8.2Hz, H-6`), 6.83 (1H, d, J=8.2Hz, H-5`), 6.29 (1H, d, J=15.9 Hz, H-2), 3.89 (3H, s, O-Me). $^{13}$C-NMR ($CD_3OD$, 100 MHz): $\delta_c$ $\delta_c$ 171.0 (C-1), 149.5 (C-3`), 149.3 (C-4`), 146.9 (C-3), 127.9 (C-1`), 124.0 (C-6`), 116.5 (C-5`), 116.0 (C-2), 111.9 (C-2`), 56.5 (O-Me).

Caffeic acid (9)

^{1}H-NMR (400 MHz, CD_3OD): δ_H 7.52 (1H, d, J=15.9 Hz, H-3), 7.03 (1H, d, J=2.0 Hz, H-2`), 6.93 (1H, dd, J=8.2, 2.0 Hz, H-6`), 6.77 (1H, d, J=8.2 Hz, H-5`), 6.22 (1H, d, J=15.9 Hz, H-2). $^{13}$C-NMR ($CD_3OD$, 100 MHz): $\delta_c$ 171.1 (C-1), 149.5 (C-4`), 147.1 (C-3), 146.8 (C-3`), 127.9 (C-1`), 122.9 (C-6`), 116.6 (C-5`), 115.7 (C-2), 115.2 (C-2`).

Hymexelsin [xeroboside] (10)

^{1}H-NMR (400 MHz, DMSO-d6): δ_H 7.96 (1H, d, J = 9.5 Hz, H-4), 7.29 (1H, s, H-5), 7.15 (1H, s, H-8), 6.32 (1H, d, J = 9.5 Hz, H-3), 5.05 (IH, d, J=7.3 Hz, H`-1), 4.78 (IH, d, J=3.1 Hz, H``-1) 3.82 (3H, s, O-Me). $^{13}$C-NMR (100 MHz, DMSO-d6): $\delta_c$ 163.7 (C-2), 150.5 (C-7), 149.7 (C-9), 145.7 (C-4), 145.5 (C-6), 115.4 (C-10), 114.8 (C-3), 114.0 (C-5), 56.0 (O-Me), 105.6 (C-8), 103.3 (C-1`),74.8 (C-2`), 78.6 (C-3`), 71.4 (C-4`), 77.6 (C-5`), 67.4 (C-6`), 109.2 (C-1``),75.4(C-2``), 78.7 (C-3``), 73.4 (C-4``), 63.4 (C-5``)

Determination of total phenolic contents showed that the quantity of total phenolic components varies from 0.17 to 26.54 mg GAE/gm of dry extract. Ethyl acetate fraction contains the highest percentage of total phenolic components (26.54 mg GAE/gm of dry extract), followed by butanol fraction (21.83 mg GAE/gm of dry extract) while n. hexane extract hardly contains any (0.17 mg GAE/gm of dry extract)

From the biological point of view, none of the tested compounds (**1**–**10**) showed any activity toward anti-leishmanial or multidrug resistant assays.

On the other hand, compounds **1**-**10** were examined for their radical scavenger activity using the DPPH free radical scavenging assay. Compounds **3**, **6** and **7** showed marked scavenging activities (IC_{50}: 18.1, 27.1 and 33.7 µM, respectively), compound **9** showed moderate activity (IC_{50}: 54.9 µM) and compounds **1**, **5** and **8** demonstrated much weaker effects with IC_{50} above 100 µM, comparable with the standard trolox (IC_{50}:17.9 µM). The rest of the tested compounds did not exhibit any DPPH radical scavenging activity.

4. CONCLUSION

The current study discusses the isolation and structural elucidation of ten compounds (**1**-**10**). Compound **7** is reported previously in *C. endivia* L, while the rest of compounds are obtained for the first time from leaves of *C. endivia* L. These compounds include; four coumarin derivatives, two flavonoid derivatives, three phenolic acids and z-3-hexenyl-β-D-glucoside. The present study highlights the importance of this plant as a good source of phenolic compounds, which are responsible for its antioxidant activity.

ACKNOWLEDGEMENTS

The authors are grateful for access to the superconducting NMR instrument, UV and ESI-MS at the Analytical Center of Molecular Medicine, the Analysis Center of Life Science and the Natural Science Center for Basic Research and Development of the Graduate School of Biomedical and Health Sciences, Hiroshima University, Japan.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

[1] Quattrocchi, U., CRC World Dictionary of Plant Name. CRC press, Boca Raton, London, New York, Washington, D. C., Vol. II 2000.
[2] Bailey, L. H., The Standard Cyclopedia of Horticulture. The Mc Millan Co., New York 1953.
[3] Bedolla, P.C., Campos, M.G., Sanchez, A.V., Ponce, M.H., *Phytotherapy Resear*. 1997, 11, 11-16.
[4] Zhou, N.B., Bai, N.S., Cordell G.A., *Phytochemistry* 1993, 34, 249-252.
[5] Oshima, Y., Geoffery, A., Fong, H.S., *J. Nat. Prod*. 1986, 49, 552-553.
[6] Segal, R., Eden, L., Danin, A., Kaiser, M., Duddeck, H., *Phytochemistry* 1985, 24, 1381-1382.
[7] Quijano, L., Calderon, J. S., Gomez, F., Rios, T., *Phytochemistry* 1982, 21, 2041-2044.
[8] Adele, P., Maria, D., Camilla, A., Barbara, S., Valentina, S., Chiara, C., Gabriella, G*., Journal of Pharmaceutical and Biomedical Analysis* 2008, 48, 472–476.
[9] Kiers, A. M., Mes, T.H. M., Van der Meijden, R., Bachmann, K., *Genome* 2000, 43(3), 470-476.
[10] Wanda, K., Klaudia, M., *Fitoterapia* 2006, 77, 354–357.
[11] Wanda, K., Klaudia, Z., *Phytochemistry* 2001, 57, 523–527.
[12] Mamoru, S., Toshio, M., Kaoru, U., Akira, U., Yutake, H., Nprio, O., *Chem. Pharm. Bull.* 1988, 36(7), 2423-2429.
[13] Tsutomu, W., Toshio, M*., Chem. Pharm. Bull.* 2008, 56(10), 1445-1451.
[14] Woldecke, M., Herrmann, K., *Zeitschrift fur Naturforschung* 1974, 29(7-8), 355-61.
[15] Pascale, M., Varoquaux, P.J.A., Nicolas, J.J., Macheix, J.J., *Journal of Agricultural and Food Chemistry* 1990, 38(12), 2116-2121.
[16] Dembitsky, V.M., Goldshlag, P., Srebnik, M., Journal *of Chemical Ecology* 2002, 28(8), 1623-1628.
[17] Llorach, R., Martinez, S.A., Tomas, B.F.A., Gil, M.I., Ferreres, F., *Food Chemistry* 2008, 108(3), 1028-1038.
[18] Susan, D.M., Zofia, M., Gary, W., Keith, R.P., *J. Agric. Food Chem.* 2000, 48 (9), 3957–3964.
[19] Nadia M.E., Hamada A., *Acta Physiol Plant* 2012, 34, 2165–2177.
[20] Fu-Xin, W., An-Jun, D., Ming, L., Jin-Feng, W., Hai-Lin, Q., Ai-Ping, W., *Molecules* 2013, 18, 418-429.
[21] Chao, J.C., An-Jun, D., Chang, L., Rui, S., Hai-Lin, Q., Ai-Ping, W., *Molecules* 2011, 16, 9049-9066.
[22] Ana, P.T., Priscilla, S.M., Keityane, B.B., Thais, M.F.S.V., Marisa, A.B.R., Severino, M.A., *Int. J. Mol. Sci.* 2012, 13, 8943-8957.
[23] Hyun, W.K., *J. Korean Soc. Food Sci. Nutr.* 2012, 41(11), 1487-1492.
[24] Papetti, A., Daglia, M., Gazzani, G., *Journal of Agricultural and Food Chemistry* 2002, 50(16), 4696-4704.
[25] Ali, A., Hafez, E.E., *Journal of Biotechnology and Pharmaceutical Research* 2012, 3(4), 74-82.
[26] Artemio, C.P., Martha, R., Christian, W., Rahim, F., Humberto, G., Fortunato, G., *Tropical and Subtropical Agroecosystems* 2012 15, 621-628.
[27] Tamaki, N., Matsunami, K., Otsuka, H., Shinzato, T., Aramoto, M., Takeda, Y., *J. Nat. Med.* 2008, 62, 314-320.
[28] Takahashi, M., Fuchino. H., Satake, M., Agatsuma, Y., Sekita, S., *Biol. Pharm. Bull*. 2004, 27, 921-925.
[29] Lopes, T.I.B., Coelho, R.G., Yoshida, N.C., Honda, N.K., *Chem. Pharm. Bull.* 2008, 56(11), 1551-1554.
[30] Matsunami, K., Otsuka, H., Takeda, Y., *Chem. Pharm. Bull.* 2010, 58(3), 438-441.
[31] Saraswathy, A., Vidhya, B., *Journal of Pharmacognosy and Phytochemistry*, 2013, 1(5), 52-56.
[32] Kenji, M., Masamichi, Y., Osamu, T., Yuh-ichirou, S., Tohru, F., Ming-Ru, J., Yi-Kul, L., Xui-Feng P., *Chem. Pharm. Bull.* 1988, 36(7), 2689-441.
[33] Renmin, L., Qinghua, S., Ailing, S., Jichun, C., *Journal of Chromatography A* 2005, 1072, 195-199.
[34] Ryun, K., Qinglong, J., Hong, G.J., Hae J.K., Eun, R.W., *Arch. Pharm. Res.* 2014, 37, 845-851.
[35] Johannes, R., Stefan, H.A., *Phytochemistry* 1992, 31(12), 4376-4377.
[36] Sibanda, S., Ndengu, B., Multari, G., Pompi, V., Galeffi, C., *Phytochemistry* 1989, 28(5), 1550-1552.
[37] Hirushi, K., Masami, M., Kiyokazu, T., Kenichroi N., Tetsuro F., Zheng, D. H., Chung, R. Y., *Phytochemistry* 1992, 31(4), 1277-1280.
[38] ujuan, Z., Dongmei, W., Lina, Y., Dan, Z., Jingfang, Z., *PLOS ONE* 2014, 9(8), 1-11.
[39] Lee, D., Lyu, H., Kwak, H., Jung, L., Lee, L., Kim, D., Chung, I., Kim, S., Baek, N., *J. Appl. Biol. Chem.* 2007, 50, 144-147.
[40] Jean, J.M., Jean, C.S., Annie, F., Leec, C.Y., *Critical Reviews in Food Science and Nutrition* 1991, 30(4), 441-486.
[41] Beate, B., Peter, W., *J. Agric. Food Chem.* 2001, 49, 2788-2798.
[42] Rao, P.S., Asheervadam, Y., Khaleelullah, M.D., Subba N.R., Murray R.D.H., *J. Nat. Prod*. 1988, 51(5), 959-961.