Jordan Journal of Pharmaceutical Sciences, Volume 10, No. 3, 2017

Phytochemical Screening and Pharmacological Activities of Echium judaeum Lacaita Extracts Growing Wild in Jordan

Khawla D. Al-Hamaideh[1]*, Tamam El-Elimat[2], Fatma U. Afifi[3], Violet Kasabri[3]

[1] Faculty of Medicine, Al-Balqa' Applied University, Al-Salt, Amman, Jordan.
[2] Faculty of Pharmacy, Jordan University of Science and Technology, Irbid, Jordan.
[3] School of Pharmacy, The University of Jordan, Amman, Jordan.

ABSTRACT

Echium judaeum Lac. (Boraginaceae) growing wild in Jordan has not been studied before phytochemically and pharmacologically. In this study the hydro-alcoholic extract was analyzed using Ultra Performance Liquid Chromatography-Mass Spectrometry (UPLC-MS). Total phenols and flavonoids were determined colorimetrically. Using SRB assay, the anti-proliferative effect against a panel of colorectal cancer cell lines (HT29, HCT116, SW620 and CACO2) was determined. Moreover anti- lipedimic and anti-obesity effects were examined, in addition to the determination of median lethal dose (LD_{50}) of the aqueous extract. UPLC-MS analysis resulted in the identification of 6 compounds; luteolin, kaempferol, esculin, rosmarinic acid, echiumine and echimidine. LD_{50} was more than 3000mg/kg body weight. Neither anti-obesity nor anti-proliferative effects were detected. Since *E. judaeum* is a safe species and contains phenolic compounds, further pharmacological screening is recommended.

Keywords: *Echium judaeum* Lacaita, Boraginaceae, Obesity, starch digestion, $LD_{50,}$ Colorectal cancer, UPLC-MS.

1. INTRODUCTION

Obesity prevalence is alarmed globally and is associated with the prevalence of chronic diseases such as cardiovascular diseases, diabetes mellitus, and cancer (colon, breast, and endometrial).[1-4] Inhibition of human pancreatic lipase and amylase enzymes can be considered as a successful and relatively safe target of obesity treatment by using orlistat and acarbose which are the only approved synthetic drugs.[1] Unfortunately both drugs are accompanied by different side effects.[3-8] Therefore, many effective alternative natural preparations with fewer side effects have been proposed for the treatment of obesity[3-7]. In Jordan, many previous studies reported effective plant extracts with promising pancreatic lipase and amylase inhibitory potential.[2, 5, 8]

Jordan is known with its various typography and climate that locates it at the junction of four biogeographical regions: the Sudanian or tropical, the Saharo-Arabian, the Irano-Turanean and the Mediterranean.[9-12] Consequently, Jordan is rich and bio-diverse in its flora and fauna; where 2,500 plant species of about 900 genera and 140 families were documented. It is estimated that approximately 20% of the overall flora are used in tradition medicine.[13]

The family Boraginaceae covers about 120 genera with reported 200 species.[9,14] The genus *Echium* comprises about 40 species of annual, biennial or perennial herbs, mainly distributed in the Mediterranean region.[15] In

* kawladamen@bau.edu.jo
Received on 27/2/2017 and Accepted for Publication on 18/6/2017.

Jordan, the genus *Echium* is represented by four species, namely: *E. glomeratum* Poir., *E. plantagineum* L., *E. rauwolfii* Delile. and *E. judaeum* Lac.[12,15, 16]

Echium judaeum Lacaita (Judean Viper's-bugloss) is commonly known as "Hemem Al-Ghor" in Arabic.[17] It is an annual, erect, green showy plant which grows up to 20-50 cm and covered with dense whitish hairs. The leaves are oblong, sessile and hairy. The flowers are blue-violet, trumpet-like and 2-3 cm in diameter. It is mainly distributed in Upper and Lower Jordan Valley. Flowering occurs from March to April.[16] Earlier, the toxicity of different *Echium* species was determined and reported for *E. glomeratum*, *E. rauwolfii* and *E. plantagineum* L.[9,18,19] The toxicity in these studied species was mostly correlated to different pyrrolizidine alkaloids.[20-24] To the best of our knowledge, there are no previous reports found in the literature regarding the safety issue of *E. judaeum*. In a survey on less-frequently used medicinal herbs in Jordan, some of its traditional uses, such as in hyperactivity, nervosity, general weakness, eczema and dermatological ailments were reported.[25] Furthermore, Qasem (2015) has described other traditional uses for this species such as, analgesic, aphrodisiac and diaphoretic activity, as well as its possible beneficial use in snake bites.[26] Furthermore *E. judaeum* was reported to be a significant Honeybee polleniferous and nectariferous plant.[27]

The objective of the present study was to screen *E. judaeum*, grown wild in Jordan phytochemically and biologically in determining the median lethal dose (LD_{50}) of its aqueous extract and evaluating the possible anti-proliferative activity against colorectal cell lines as well as α-amylase and pancreatic lipase inhibitory potentials of its extracts.

MATERIALS AND METHODS

Chemicals, biochemicals and instruments

Unless stated otherwise, all reagents and chemicals were obtained from Sigma (Dorset, UK). Glucose GOD-PAP kit was obtained from BioLabo Reagents, France. In UV determinations; UV-VIS spectrophotometer from SpectroScan 80D (UK) was used. Sonicator (Bandelin Sonorex, Bandelin electronics, Germany), RPMI 1640, PAA Laboratories GmbH, Austria and rotary evaporator (Laborota 4000-efficient, Heidolph, Germany) were also used. From different companies aluminum chloride ($AlCl_3$) anhydrous, (Merck-Schuchardt, Germany), rutin, (Alpha-acer, Germany) and sodium carbonate (Na_2CO_3) anhydrous, (Merck-Schuchardt,Germany) were purchased.

Plant Collection

E. judaeum was collected from Al-Salt region, about 30 km northwest of Amman, during the period extending from March to April, 2016. The plant was identified by Prof. Barakat E. Abu-Irmaileh at the Department of Plant Protection, School of Agriculture, The University of Jordan. Voucher specimens (FMJ-BORA 2) were deposited at the School of Pharmacy, The University of Jordan and Al- Balqa Applied University. The whole plant was air dried in the shade and purified from extraneous material at room temperature (RT) without direct exposure to sunshine until constant weight, and later used for extraction.

Preparation of the *E. judaeum* extracts

Aqueous extract was prepared by refluxing 10 g of the dried coarsely powdered plant material with 100 mL tap water for 15 min. The overnight kept extract was filtered twice through filter paper and the volume of the filtered solution was increased to 100 mL with tap water to obtain 10% (equivalent to 100 mg/ mL) crude aqueous solutions.[28] Sonication of stock crude extract or testing concentrations was performed before application of examinations. For pancreatic lipase (PL) experiment; water was evaporated under vacuum at 40 ^{o}C using a rotary evaporator. The solid residue was collected and stored in

dry conditions until analysis. Hydro-alcoholic extract was prepared using 10 g of the dried and coarsely powdered plant material and was gently refluxed for 30 min using 70% ethanol, kept overnight, filtered and solvents were evaporated. For cytotoxicity assay, 100 mg of the hydro-alcoholic extract was dissolved in 10 mL DMSO (stock solution).

Ultraperformance liquid chromatography high-resolution mass spectrometric (UPLC- HR ESIMS) evaluation of the *E. judaeum* hydro-alcoholic extract

Phytochemical screening of *E. judaeum* was based on the method published earlier using the same chromatographic parameters.[29] The HRESIMS data were obtained using a Thermo QExactive Plus mass spectrometer (ThermoFisher, San Jose, CA, USA) paired with an electrospray ionization source. The QExactive Plus was adjusted to collect data from 150 to 2000 m/z at a resolution of 70 000.

Evaluation of Total Phenolic Content

The total phenolic content was determined as described earlier.[30] Briefly, a stock solution of 1 mg/ml in 95% v/v methanol (MeOH) was prepared using the solid hydro-alcoholic extract. Total phenolic content was estimated using the FCR Reagent method and gallic acid as standard. Ten milliliters of Folin–Ciocalteu reagent were dissolved in water (90 mL). Three grams of sodium carbonate (Na_2CO_3) was diluted in water (50 mL). Then 200 µL of the extract was added to 1.5 mL FCR in a test tube and kept for 5 min at room temperature in a dark place, followed by addition of 1.5 mL of Na_2CO_3 solution. The blue solution was mixed well by hand. The same procedure was followed for gallic acid standard using eight different concentrations for the calibration curve (0, 1.4, 4.3, 13, 40, 100, 300 and 1000 µg/mL in 95% v/v methanol). All the test tubes were kept for 120 min in a dark place at room temperature. The absorbance of the tested extract was measured at 750 nm and methanol 95% v/v as blank. All the measurements were prepared in triplicate. Total phenolic content is estimated as gallic acid equivalents (GAE) in mg/g for *E. judaeum* extract.

Evaluation of Total Flavonoids

The total flavonoid content was determined by spectrophotometric method as described earlier using rutin as reference compound.[31] *E. judaeum* hydro-alcoholic extract stock solution (1 mg/ml in 95% methanol) was mixed in (1:1) ratio with 2% solution of aluminum trichloride ($AlCl_3$) in 95% methanol. The absorption at 415 nm was measured after 1hr at RT. Quantitative determinations were done based on standard calibration curve of six different concentrations (0, 10, 20, 40, 80 and 100 µg/mL of rutin in 95% MeOH). The absorption of rutin solutions were measured under the same conditions. All the measurements were prepared in triplicate. The amount of flavonoids in *E. judaeum* extract expressed as rutin equivalents (RE) and was calculated by the following formula:

$$X= A - 0.0154/0.0037.$$

Where: X is the flavonoid content in (mg RE/g plant extract); A is the absorption of plant extract solution.

Determination of oral acute toxicity

Animals

BALB/C male mice (25-30g) were used for acute toxicity estimation in the Experimental Animal Laboratory of the School of Medicine, The University of Jordan. All animals were pathogen free housed, fed and treated in accordance with the in-house ethical guidelines for animal protection.[32,33] The animals were kept in groups of six and sustained on 12 h light-dark cycle under controlled temperature (25 °C) with standard pellet diet and water ad libitum. Animals were acclimated before starting the experiment. Before the administration of the oral

solutions, animals were retained without food for 12 h but free access to water was permitted. The experiment was performed following the OECD standards for these studies.[33]

Determination of LD_{50}

E. judaeum aqueous extract was administered orally by gavage in stepwise graded doses of (250, 500, 1000, 1500, 2000, 2500, 3000 mg/kg) to the animals in the test groups. The animals were randomly divided in eight groups, each group consisting of six animals with an average body weight of 25 grams. Each of the mice in the control group was treated with distilled water only. The mice in both, the test and control groups had free access to food and water. Animals were continuously observed after extract administration for 2, 4, 6, and 12 h. to detect any possible changes in the behavioral responses. Finally, they were monitored for any mortality after 24 hours to estimate median lethal dose (LD_{50}). The animals were observed further during the following 14 days.

Spectrophotometric quantification of PL inhibition *in vitro* for *E. judaeum* aqueous extract and the reference drug Orlistat

E. judaeum aqueous extract was initially dissolved in Tris-HCl buffer (2.5 mM (Promega, USA), pH 7.4 with 2.5 mM NaCl) to give initial stock solution with a concentration range 0.1–100 mg/mL. Subsequently, 20 µL aliquot of the stock solution was used in the reaction mixture to give a final concentration range of 2-2000 µg/mL. The extract was prepared according to the traditional indications of use, thus DMSO or any other organic solvent; even to the minimum concentration was avoided.[8] Finally, orlistat, the reference drug (in DMSO; 1 mg/mL), was prepared in six different stock solutions with a concentration range of 0.625-20 µg/ mL.[34] Thereafter, 20 µL aliquot of each stock solution was used in the reaction mixture to give a final concentration range of 0.0125-0.4 µg/ mL. *In vitro* enzymatic PL activity was assayed according to Al-Hallaq et al. (2013).[5] Subsequent measurements were undertaken for the tested extract and orlistat in comparison to control evaluations, to calculate the concentration required for PL 50% inhibition (IC_{50}).

***In vitro* enzymatic starch digestion assay**

In vitro enzymatic starch digestion was assayed with acarbose, as the reference drug.[8] The extent of polysaccharide breakdown into glucose was evaluated in a concentration range of 1, 5, 10, 12.5, 25, 50 and 100 mg/mL of *E. judaeum* aqueous extract. The effects of acarbose at 1000 µg/mL concentration were evaluated as well. Control (tap water only) samples contained neither acarbose nor plant extract.

***In vitro* anti-proliferative assay**

Obesity related colorectal cell lines HT29, HCT116, SW620 and SW480 were generously provided by Dr. R. F. Thorne (University of Newcastle, Australia) and were cultured in high glucose DMEM containing 10% FCS (Bio Whittaker, Verviers, Belgium). CACO2 cell line was a gift of Prof. Y. Bustanji, School of Pharmacy, The University of Jordan. CACO2 cell line was cultured in RPMI 1640 containing 10% FBS, HEPES Buffer (10 mM), L-glutamine (2 mM), gentamicin (50 µg/mL), penicillin (100 U/mL), and streptomycin sulfate (100 mg/mL). The cytotoxicity measurements were determined using Sulforhodamine B (SRB) colorimetric assay for cytotoxicity screening and mechanism of reduction of cell viability as described previously.[8] Human periodontal fibroblasts (PDL) are a primary cell culture for verification of selective cytotoxicity with the least antiproliferative IC_{50} value obtained. As a robust and classical antineoplastic reference agent, cisplatin (0.1-200 µg/mL), was recruited for comparison purposes.[8] All of the assays were performed in triplicate and the calculated antiproliferative activities were reported as the mean values ± SD (n=3).

RESULTS AND DISCUSSION

Ultraperformance liquid chromatography-high resolution mass spectrometric (UPLC- HRESIMS) method analysis of the hydro-alcoholic extract

The phytochemical screening using HRESIMS and different reference compounds revealed the identification of six compounds; two alkaloids (echiumine, echimidine) and four polyphenols (esculin, rosmarinic acid, luteolin and kaempferol) (Table 1, Figures 1-6). Previous studies reported the occurrence of kaempferol, rosmarinic acid, echiumine and echimidine in other *Echium* species.[35-38] This is the first report to detect these secondary metabolities in *E. judaeum*.

Table 1. UPLC- HRESIMS screening results of *E. judaeum* hydroalcoholic extract

Identified compound	Chemical class
Luteolin	Flavonoid
Kaempferol	Flavonoid
Esculin	Coumarin
Rosmarinic acid	Polyphenol
Echiumine	Alkaloid
Echimidine	Alkaloid

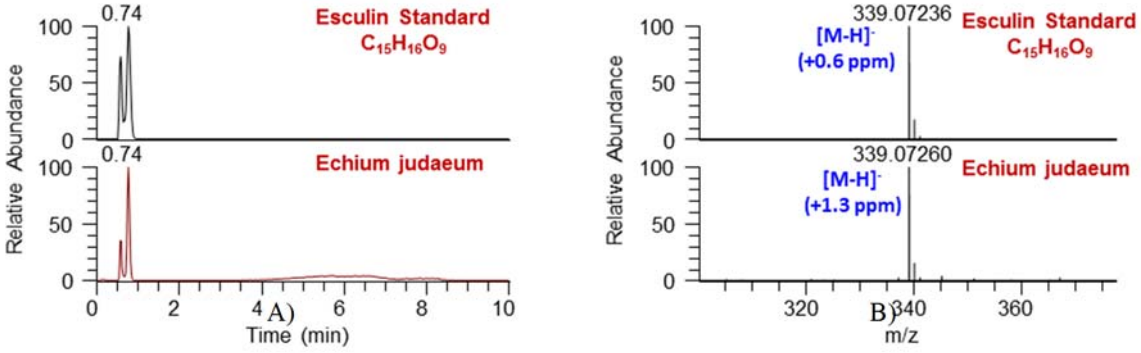

Figure 1: (A) Overlay of chomatographic peaks of the (-)-ESI SIC of esculin stanadrd and crude *Echium judaeum* extract. (B) (-)-HRESIMS of Esculin

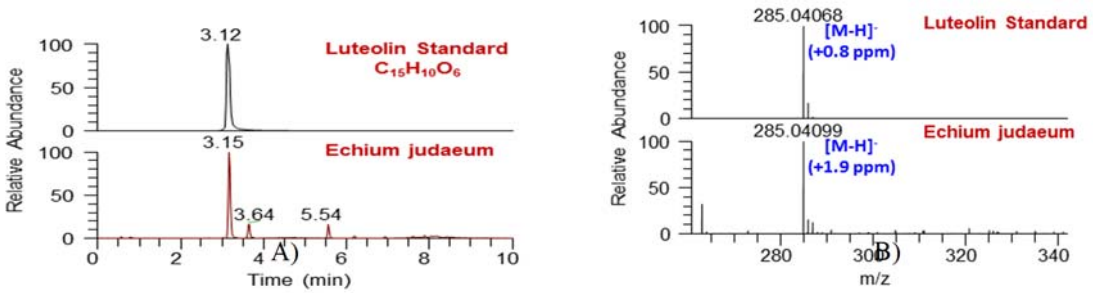

Figure 2: (A) Overlay of chomatographic peaks of the (-)-ESI SIC of luteolin stanadrd and crude *Echium judaeum* extract. (B) (-)-HRESIMS of Luteolin

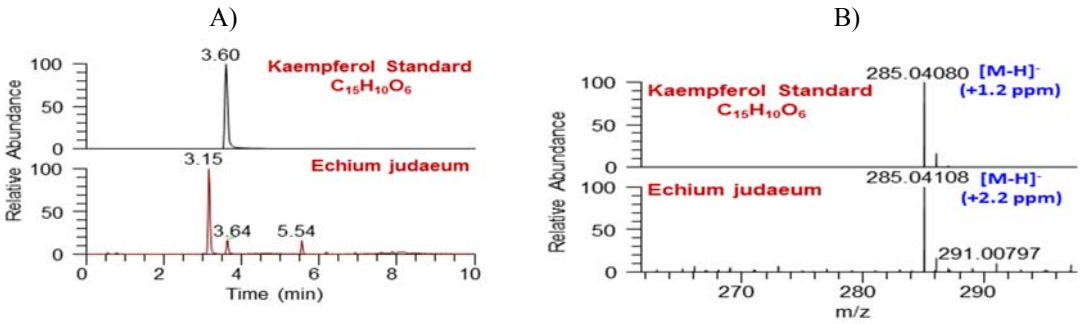

Figure 3: (A) Overlay of chomatographic peaks of the (-)-ESI SIC of kaempferol stanadrd and crude *Echium judaeum* extract. (B) (-)-HRESIMS of Kaempferol

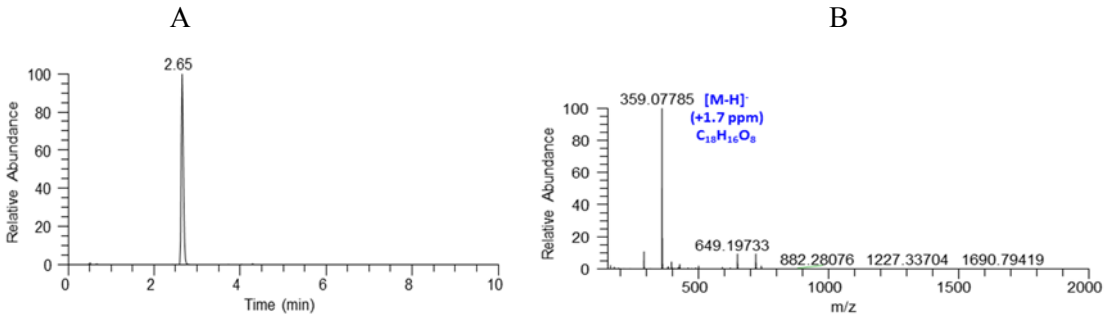

Figure 4: (A) (-)-ESI SIC of crude *Echium judaeum* extract (*m/z*: 359; rosmarinic acid). (B) (-)-HRESIMS of Rosmarinic acid

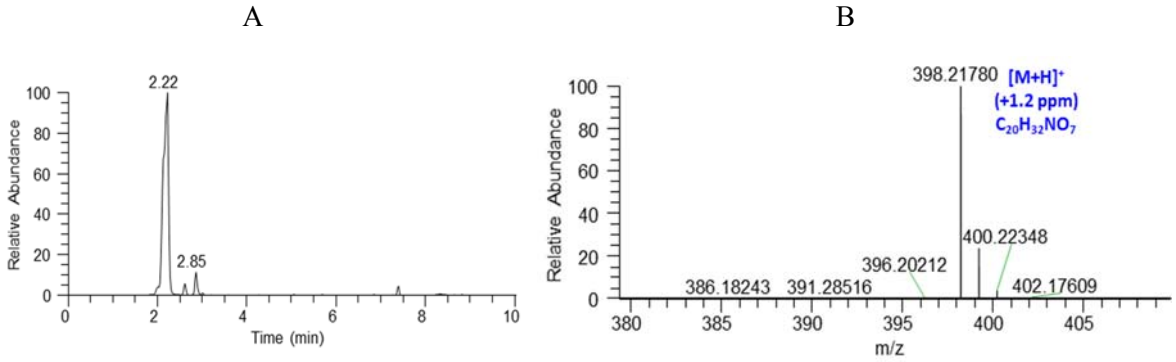

Figure 5: (A) (+)-ESI SIC of crude *Echium judaeum* extract (*m/z*: 398; echimidine). (B) (+)-HRESIMS of Echimidine

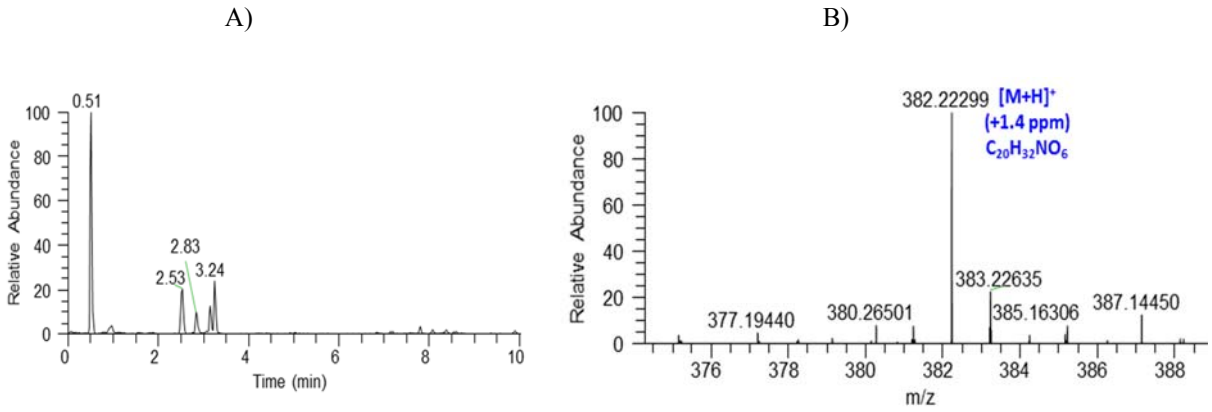

Figure 6: (A) (+)-ESI SIC of crude *Echium judaeum* extract (*m/z*: 382; echiumine). (B) (+)-HRESIMS of Echiumine

Determination of Total Phenolic Content and Estimation of Total Flavonoids

Total phenolic content and total flavonoids were determined for the hydroalcoholic extract of *E. judaeum* and their values were 23.92 ± 0.28 mg GAE/g plant extract and 12.44 ± 0.16 mg RE/g plant extract respectively, as shown in Table 2. To the best of our knoweldge this first time to estimate the total flavonoids of *E. judaeum.*

Table 2. Total phenolic and total flavonoids contents of *E. judaeum* hydroalcoholic extract*

Total phenolic content (mg GAE/g plant extract)[a]	23.92 ± 0.28
Total flavonoid content (mg RE/g plant extract)[b]	12.44 ± 0.16

* All data are shown as mean ±SD. All measurements were performed in triplicate.

[a] Data are expressed as mg of gallic acid equivalents (GAE) per g plant extract.

[b] Data are expressed as mg of rutin equivalents (RE) per g plant extract.

Phenolic compounds have the high activity to counteract free radicals.[39] Many natural polyphenolic compounds have been reported to inhibit both lipase and α-amylase enzymes *in vitro*.[5, 8, 39, 40]

Consequently, phenolics quantification in food and medicinal plant extracts is a common practice. The Folin-Ciocalteu assay is the most frequently used procedure to estimate total phenolic content of food and plant extracts.[39] Therefore, in the present study, total phenolic and total flavonoid contents of *E. judaeum* extract were determined. The total phenolic content was determined for both the aqueous and methanol extracts of *E. judaeum* in a previous study and were estimated as 11.7 and 11.5 mg GAE /g dry extract weight respectively[41], values that differ from the results of the present study. These variations could be explained by the different extraction solvents used in both studies in addition to seasonal and regional factors in the locations from where the plant samples were collected.

***In vitro* inhibitory effects of *E. judaeum* aqueous extract on PL and enzymatic starch digestion**

The pancreatic triacylglycerol lipase (PL) modulatory profiles of the aqueous extract of *E. judaeum* are shown in Table 3. For orlistat's PL-IC_{50} was 114.0 ± 4.0 ng/mL (equivalent to 0.2 ± 0.0 μM), which is comparable to the reported PL-IC_{50} values elsewhere.[34] Similar to orlistat

performance, a marked concentration dependent PL inhibition trend was obtained for the tested extract. With acarbose the reference drug, glucose liberation from starch was inhibited with an IC_{50} value of 0.2± 0.02 µg /mL (Table 3). The *E. judaeum* aqueous extract, on the other hand showed a dose-related reduction in glucose release from culinary polymeric cornstarch with IC_{50} of 20.9 ± 3.14 (mg/mL) as listed in Table 3. Although recently Buchholz and Melzig (2015) have reported PL-inhibitory activity for *E. vulgare*, no pancreatic lipase or α-amylase enzymes inhibitory effects were detected for *E. judaeum* extracts.[42]

Table 3. *In vitro* PL and enzymatic starch digestion IC_{50} values for *E. judaeum* aqueous extract, compared to reference drugs*

Tested material	Pancreatic Triacylglycerol Lipase IC_{50} (µg/mL)*	Enzymatic Starch Digestion IC_{50} (mg/mL)*	Sugar (mM) interferences at 100 mg/mL
E. judaeum Aqueous Extract	551.85±69.51	20.9±3.14	2.51
Reference Drugs	Orlistat 0.114 ± 0.01 µg /mL	Acarbose 0.2± 0.02 µg /mL	

*Results are mean ± SD (n = 3 independent replicates).

Determination of LD_{50}

Before the determination of various biological activities of natural product, oral acute toxicity in mice should be estimated, as they are sensitive to plant noxious substances.[43] The application of the extracts in increasing amounts assists the evaluation of the toxicity level.[44] After 24 h of oral administration, the *E. judaeum* extract did not create lethality or any sign of acute intoxication in the mice in dose concentration up to 3000 mg/kg. The animals were healthy after two weeks observation-without noticing any clinical abnormalities in comparison with the control group, which in turn is an indication of the safety of the plant under investigation. Therefore, aqueous extract of *E. judaeum* was safe up to a dose of 3000 mg/kg body weight.

***E. judaeum* anti-proliferative activity in obesity related colorectal cancer cell lines**

NCI nominates a medicinal herb for its therapeutic or prophylactic effects if its crude extract shows an antiproliferative IC_{50} value of less than 30 µg/mL.[45] The anti-proliferative efficacies of *E. judaeum* hydro-alcoholic extract compared to the reference agent (cisplatin) in all tested colorectal carcinomas are illustrated in Table 4. *E. judaeum* hydro-alcoholic extract cytotoxicity against HT29, HCT116, SW620 and CACO2 proved substantially potent over 72h incubations; nevertheless, it lacked selective cytotoxicity in PDL fibroblasts wells.

Colon cancer is the second most commonly occurring cancer in Jordan which is in certain cases correlated to obesity.[46-49] In the present study *E. judaeum* extract did not exhibit any anti-proliferative activity towards the tested colon cancer cell lines. However, it is recommended to screen other colon cancer cell lines. Moreover Moallerm et al. (2007) reported that the two *Echium* species, namely *E. vulgare* and *E. amoneum* growing in Iran are used as mood stimulant and they showed that the extracts of both species exhibit antidepressant activities.[37] Therefore it is suggested to evaluate the antidepressant activity of *E. judaeum* in future studies.

Table 4. IC_{50} values (µg/mL) of *in vitro* anti-proliferative activity of crude *E. judaeum* extract on colorectal cancer cell lines

Tested material	Cytotoxicity (as of % control) IC_{50} value (µg/mL)*					
E. judaeum hydro-alcoholic extract	HT29	HCT116	SW620	CACO2	SW480	Fibroblasts
	NI	NI	NI	179.4±45.1	NI	114.7±0.4
Cisplatin	2.4±0.13	0.04±0.006	2.2±0.1	3.52±0.4	2.06±0.32	2.4±0.13

* Results are mean ± SD (n = 3-4 independent replicates). IC_{50} values (concentration at which 50% inhibition of cell proliferation took place in comparison to non-induced basal 72 h incubations) were calculated within 0.1-200 µg/mL range.

CONCLUSION

This is the first report of phytochemical screening, LD_{50} estimation and determination of pancreatic lipase and starch digestion inhibitory potential as well as the anti-proliferative evaluation of *E. judaeum* species growing wild in Jordan. Since *E. judaeum* was found to contain polyphenolic compounds that have a wide range of approved biological benefits, screening of the crude aqueous and hydro-ethanol extracts for other *in vitro* biological activities (antimicrobial, antioxidant, antidepressant and anti-proliferative effects using different cancer cell lines) is recommended, especially that *E. judaeum* was approved to be safe in this study.

Acknowledgments

This work was supported by a grant of the Deanship of Academic Research, Al-Balqa Applied University. The high-resolution mass spectrometric data were acquired in the Triad Mass Spectrometry Laboratory at the University of North Carolina at Greensboro.

REFERENCES

(1) Das S. K., Chakrabarti R. Antiobesity therapy: emerging drugs and targets. *Curr. Med. Chem.* 2006;13:1429-1460.

(2) Bustanji Y., Mohammad M., Hudaib M., Tawaha K., Al-Masri I. M., AlKhatib H. S., Issa A. and Alali F. Q. Screening of some medicinal plants for their pancreatic lipase inhibitory potential. *Jordan J. Pharm. Sci*. 2011; 4.

(3) Buchholz T. and Melzig M. F. Medicinal plants traditionally used for treatment of obesity and diabetes mellitus. Screening for pancreatic lipase and α-amylase inhibition. *Phytother. Res.* 2016; 30:260-266.

(4) Birari R. B. and Bhutani K. K. Pancreatic lipase inhibitors from natural sources: unexplored potential. *Drug Discov. Today*. 2007; 12:879-889.

(5) Al-Hallaq E. K., Kasabri V., Abdalla S. S., Bustanji Y. K. and Afifi F. U. Anti-obesity and antihyperglycemic effects of *Crataegus aronia* extracts: *in vitro* and *in vivo* evaluations. *Int. J. Food Nutr. Sci.* 2013; 4: 97 2-983.

(6) Singh G., Suresh S., Bayineni V. and Kadeppagari R. Lipase inhibitors from plants and their medical applications. *Int. J. Pharm. Pharm. Sci.* 2015; 7:1-5.

(7) Bray G. A. T. L. A. Medicinal strategies in the treatment of obesity. *Nature.* 2000; 404:672-677.

(8) Kasabri V., Afifi F. U., Abu-Dahab R., Mhaidat N., Bustanji Y. K., Abaza I. and Mashallah S. *In vitro* modulation of metabolic syndrome enzymes and proliferation of obesity related-colorectal cancer cell line panel by *Salvia* species from Jordan. *Rev Roum Chim*. 2014; 59:693-705.

(9) Alali F. Q., Tahboub Y. R., Ibrahim E. S., Qandil A. M., Tawaha K., Burgess J. P., Sy A., Nakanishi Y., Kroll D. J. and Oberlies N. H. Pyrrolizidine alkaloids from *Echium glomeratum* (Boraginaceae). *Phytochemistry*.

2008; 69:2341-2346.

(10) Alali F. Q., Tawaha K., El-Elimat T., Qasaymeh R., Li C., Burgess J., Nakanishi Y., Kroll D. J., Wani M. C. and Oberlies N. H. Phytochemical studies and cytotoxicity evaluations of *Colchicum tunicatum* Feinbr and *Colchicum hierosolymitanum* Feinbr (Colchicaceae): two native Jordanian meadow saffrons. *Nat. Prod. Res*. 2006; 20:558-566.

(11) Feinbrun-Dothan N. Flora Palaestina: part 4. Text. Alismataceae to Orchidaceae. Israel Academy of Sciences and Humanities: Jerusalem.1986; 2:*p 462.-illus., maps, chrom. nos., keys.. En Icones, Maps, Chromosome numbers. Geog*.

(12) Al Eisawi D. M. Field guide to wild flowers of Jordan and neighbouring countries. Amman: Commercial Press. Jordan Press Foundation'Al Rai'296p.-col. illus. En, Ara Icones. *Geog*. 1998; 2.

(13) Oran S. A. Selected wild plant species with exotic flowers from Jordan. *Inter. J. Biod. Cons*. 2015; 7:308-320.

(14) Watson L., Dallwitz M. The families of flowering plants: descriptions, illustrations, identification, and information retrieval. Version: 14th December 2000. *biodiversity. uno. edu/delta*. 1992.

(15) Al-Eisawi D. Flora of Jordan Checklist, Revised; The University of Jordan Press: Jordan. 2013.

(16) Feinbrun-Dothan N. Ericaceae To Compositae; The Israel Academy of Sciencee and Humanties: Jerusalem. 1978, p 74-78.

(17) Heyn C. C., Herrnstadt I., editors. Bryophyte flora of Israel and adjacent regions: *Isr. Acad. Sci. Humanit*. 2004.

(18) El-Shazly A., Abdel-All M., Tei A. and Wink M. Pyrrolizidine alkaloids from *Echium rauwolfii* and *Echium horridum* (Boraginaceae). *Z. Naturforsch C*. 1999; 54:295-300.

(19) Culvenor C. C., Edgar J. A. and Smith L. W. Pyrrolizidine alkaloids in honey from *Echium plantagineum* L. *J. Agric. Food Chem.* 1981; 29:958-960.

(20) Mehrabani M., Ghannadi A., Sajjadi E., Ghassemi N. and Shams-Ardakani M. Toxic pyrrolizidine alkaloids of *Echium amoenum* Fisch.and Mey. *Daru, J. Pharm. Sci.*. 2006;14:122-127.

(21) Colegate S. M., Edgar J. A., Knill A. M. and Lee S. T. Solid-phase extraction and HPLC-MS profiling of pyrrolizidine alkaloids and their N-oxides: a case study of *Echium plantagineum*. *Phytochem. Anal*. 2005;16:108-119.

(22) Cheeke P. Toxicity and metabolism of pyrrolizidine alkaloids. *J. Anim. Sci.* 1988; 66:2343-2350.

(23) Stegelmeier B. L. Pyrrolizidine Alkaloid–Containing Toxic Plants (*Senecio, Crotalaria*, *Cynoglossum*, *Amsinckia*, *Heliotropium*, and *Echium* spp.). *Vet. Clin. North Am. Food Anim. Pract.* 2011; 27:419-428.

(24) Boppre M. The ecological context of pyrrolizidine alkaloids in food, feed and forage: an overview. *Food Addit Contam: Part A*. 2011; 28:260-281.

(25) Afifi F. and Abu-Irmaileh B. Herbal medicine in Jordan with special emphasis on less commonly used medicinal herbs. *J. Ethnopharmacol.* 2000; 72:101-110.

(26) Qasem J. R. Prospects of wild medicinal and industrial plants of saline habitats in the Jordan valley. *Pak. J. Bot*. 2015; 47:551-570.

(27) Albaba I. A List of important Honeybee nectariferous and polleniferous plant species in the West Bank Governorates, Palestine. *J. Agr. Sci.Tech*. 2015; 5: 114-121.

(28) Hamdan I. and Afifi F. Studies on the *in vitro* and *in vivo* hypoglycemic activities of some medicinal plants used in treatment of diabetes in Jordanian traditional medicine. *J. Ethnopharmacol.* 2004; 93:117-121.

(29) El-Elimat T., Figueroa M., Ehrmann B. M., Cech N. B., Pearce C. J. and Oberlies N. H. High-resolution MS, MS/MS, and UV database of fungal secondary metabolites as a dereplication protocol for bioactive natural products. *J. Nat. Prod.* 2013; 76:1709-1716.

(30) Hossain M. A., ALsabari K. M., Weli A. M., Al-Riyami Q. Gas chromatography–mass spectrometry analysis and total phenolic. *J. Taibah.Univ. Scie.* 2013; 7:209-215.

(31) Stanković M. S. Total phenolic content, flavonoid concentration and antioxidant activity of *Marrubium peregrinum* L. extracts. *Kragujevac J Sci*. 2011; 33:63-72.

(32) Foltz C. J. and Ullman-Cullere M. Guidelines for assessing the health and condition of mice. *Lab Animal*. 1999; 28.

(33) Guideline P.-B. T. OECD guideline for the testing of chemicals. *The Hershberger*. 2001; 601.

(34) Habtemariam S. The anti-obesity potential of sigmoidin A. *Pharm. Biol.* 2012; 50:1519-1522.

(35) Ferreres F., Pereira D. M., Valentão P. and Andrade P. B. First report of non-coloured flavonoids in *Echium plantagineum* bee pollen: differentiation of isomers by liquid chromatography/ion trap mass spectrometry. *Rapid Commun. Mass Spectrom.* 2010; 24:801-806.

(36) Mehrabani M., Shams-Ardakani M., Ghannadi A., Ghassemi-Dehkordi N. and Sajjadi-Jazi S. Production of rosmarinic acid in *Echium amoenum* Fisch. and CA Mey. cell cultures. *Iran. J. Pharm. Res*. 2005; 2:111-115.

(37) Moallem S. A., Hosseinzadeh H. and Ghoncheh F. Evaluation of antidepressant effects of aerial parts of *Echium vulgare* on mice. *Iran. J. Basic Med. Sci.* 2007;10:189-196.

(38) Boppré M., Colegate S. M. and Edgar J. A. Pyrrolizidine alkaloids of *Echium vulgare* honey found in pure pollen. *J. Agric. Food Chem.* 2005;53:594-600.

(39) Badarinath A., Rao K. M., Chetty C. M. S., Ramkanth S., Rajan T. and Gnanaprakash K. A review on *in-vitro* antioxidant methods: comparisons, correlations and considerations. *Int.J. PharmTech. Res*. 2010; 2:1276-1285.

(40) Tucci S. A., Boyland E. J. and Halford J. The role of lipid and carbohydrate digestive enzyme inhibitors in the management of obesity: a review of current and emerging therapeutic agents. *Diabetes Metab. Syndr. Obes.* 2010; 3:125-143.

(41) Alali F. Q., Tawaha K., El-Elimat T., Syouf M., El-Fayad M., Abulaila K., Nielsen S. J., Wheaton W. D., III J. O. F. and Oberlies N. H. Antioxidant activity and total phenolic content of aqueous and methanolic extracts of Jordanian plants: an ICBG project. *Nat. Prod. Res*. 2007; 21:1121-1131.

(42) Buchholz T. and Melzig M. F. Polyphenolic compounds as pancreatic lipase inhibitors. *Planta Med.* 2015; 81:771-778.

(43) Parra A. L., Yhebra R. S., Sardiñas I. G. and Buela L. I. Comparative study of the assay of *Artemia salina* L. and the estimate of the medium lethal dose (LD_{50} value) in mice, to determine oral acute toxicity of plant extracts. *Phytomedicine*. 2001; 8:395-400.

(44) Ogbonnia S., Mbaka G., Anyika E., Osegbo O. and Igbokwe N. Evaluation of acute toxicity in mice and subchronic toxicity of hydroethanolic extract of *Chromolaena odorata* (L.) King and Robinson (Fam. Asteraceae) in rats. *Agric. Biol. J. North Am.*. 2010;1:859-865.

(45) Afifi F. U. and Abu-Dahab R. Phytochemical screening and biological activities of *Eminium spiculatum* (Blume) Kuntze (family Araceae). *Nat Prod Res*. 2012; 26:878-882.

(46) Ahmad M. M., Dardas L., Dardas L. and Ahmad H. Colorectal cancer in Jordan: Prevention and care. *Global Health Promotion*. 2015; 22:39-47.

(47) Tarawneh M., Nimri O., Arkoob K. and Zaghal M. Cancer incidence in Jordan 2009. *Non-Communicable Diseases Directorate, Jordan Cancer Registry. Ministry of Health*. 2009.

(48) Peeters P. J., Bazelier M. T., Leufkens H. G., de Vries F. and De Bruin M. L. The risk of colorectal cancer in patients with type 2 diabetes: associations with treatment stage and obesity. *Diabetes Care*. 2015; 38:495-502.

(49) James W. WHO recognition of the global obesity epidemic. *Int. J. Obes.* 2008; 32:S120-S126.

التحليل الكيميائي والمسح البيولوجي لمستخلصات نبات حميم الغور *Echium judaeum Lac.* في الأردن

خولة الحمايدة[1]، تمام عليمات[2]، فاطمة عفيفي[3]، فيولت كسابري[1]

[1] كلية الطب، جامعة البلقاء التطبيقية، السلط، عمان، الأردن.

[2] كلية الصيدلة، جامعة العلوم والتكنولوجيا الأردنية، اربد، الأردن.

[3] كلية الصيدلة، الجامعة الأردنية، عمان، الأردن.

ملخص

هذه المرة الأولى التي يدرس فيها نبات حميم الغور في الأردن من حيث التعرف إلى مركباته الكيميائية ودراسة خصائصه العلاجية. خلال الدراسة الحالية تم تحليل المستخلص الإيثانولي المائي للنبتة المدروسة للمرة الأولى باستخدام الفصل الكروماتوغرافي الفائق للسائل والمطيّاف الكتلي الذي نتج عنة التعرف إلى ستة مركبات هي: من مجموعة الفلافونويدات (luteolin و kaempferol) ومن مجموعة الأحماض الفينولية (rosmarinic acid) ومن مجموعة الكومارين (esculin) ومن مجموعة القلويدات (echiumine و echimidine). كذلك تم تحليل المحتوى الفينولي والمحتوى الفلافونويدي لنفس المستخلص. على صعيد النشاط الحيوي لم يظهر المستخلص أي أثر فاعل عند دراسته من حيث النشاط المضاد للأورام ضد خلايا سرطان القولون والمستقيم من نوع (HT29, HCT116, SW620 and CACO2) باستخدام طريقة SRB وعند دراسة مفعول مكافحة السمنة وخفض مستوى الدهون بالجسم لمستخلص النبتة المائي لم يظهر المستخلص أي أثر. وتبين أن المستخلص المائي للنبتة آمن وغير سام عند دراسة الجرعة القاتلة على فئران BALB/C والتي بلغ مقدارها أكبر من 3000 ملغم لكل كيلوغرام والتي تدرس للمرة الأولى. كما وتوصي الدراسة بمزيد من الفحوصات للأثر العلاجي للنبتة حيث إنها آمنة ولمكوناتها الكثير من الخصائص العلاجية الأخرى التي لم تدرس بعد.

الكلمات الدالة: حميم الغور، الحمحمية، هضم النشا، السمنة، الجرعة القاتلة، سرطان القولون والمستقيم، الفصل الكروماتوغرافي الفائق للسائل والمطياف الكتلي.

تاريخ استلام البحث 2017/2/27 وتاريخ قبوله للنشر 2017/6/18.