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UPLC-MS Profile and Anti-Proliferative Activity of the Berries of an Aggressive Wild-Growing Weed: *Solanum elaeagnifolium* Cav. (Solanaceae)

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ABSTRACT

Although, *Solanum elaeagnifolium* Cav. is described as a problematic weed difficult to eradicate, it is used in the traditional medicine of different countries for the treatment of different ailments, such as tooth ache and constipation. Its occurrence in Jordan is widespread. In the present study, the aim was to identify the phytochemical composition of the berries and to screen for different biological activities. Phytochemically, the hydro-alcoholic extracts of the berries of *S. elaeagnifolium* were analyzed using Ultra Performance Liquid Chromatography-Mass Spectrometry (UPLC-MS). Pharmacologically, using Sulforhodamine B (SRB) assay, the anti-proliferative effect of the extracts against four colorectal cancer cell lines (HT29, HCT116, SW620 and CACO2) was tested. The aqueous extracts of the ripe and unripe berries were further evaluated for their *in vitro* anti-lipidemic and anti-obesity activities. UPLC-MS analysis resulted in the identification of seven bioactive compounds in both extracts. Three of them, namely; apigenin, hyperoside and luteolin were identified in these berries for the first time. Also,chlorogenic acid, kaempferol, solasodine and solamargine were identified. The extracts exhibited anti-proliferative activity but lacked anti-obesity activity *in vitro*. This is the first report on the phytochemical and pharmacological evaluation of the ripe and unripe berries of *S. elaeagnifolium* Cav., growing wild in Jordan.

Keywords: Colorectal cancer, Jordan, Obesity, *Solanum elaeagnifolium* Cav., Solanaceae, UPLC-MS.

Introduction

Colorectal cancer or colon cancer is the second most commonly occurring cancer in Jordan and one of the most prevalent cancers worldwide and chemotherapy is the main therapy for the disease.[1] It is the third most common cancer in men and the second among women (10% and 9.2% of the total cancer prevalence, respectively, in more than 180 different countries worldwide 2012).[2] Many medicinal plants have been screened and approved to prevent and/or treat many cancers including colorectal cancer; in a bid to find an alternative therapy due to the side effects and resistance that have limited the use of conventional chemotherapy.[3] In Jordan, several plants including *Inula graveolens* (L.) Des, *Ephedra aphylla*, *Varthemia iphionides*[4-6]have been tested for their anti-cancer effects and many others still need to be screened.

Obesity is a growing unsolved challenge in both developing and developed countries, and a major risk factor for numerous serious diseases including cancer, cardiovascular diseases, and diabetes mellitus. Previous studies correlated obesity as well as the increase in body mass index to colorectal cancer prevalence and mortality; both identified as a risk for colorectal cancer. Also, insulin resistance as a result of obesity can contribute to colorectal cancer progression.[3, 7] One of the significant management strategies of obesity is to inhibit digestion and absorption of dietary nutrients (carbohydrates and fats) using drugs like acarbose and orlistat. Therefore, natural inhibitors of amylase enzymes and human pancreatic lipase are preferred since they are better tolerated than the synthetic inhibitors.[8]It was reported that the anti-obesity effect of plant is related to the presence of various secondary metabolites. Like in other countries, several plants of the Jordanian flora have been screened for their anti-obesity and anti-proliferative activities, including colorectal cancer.[9,10] In Jordan, plant-based traditional medicine is still widely practiced, especially by the inhabitants of the rural areas.[11]

The genus *Solanum is* the largest among the genera of the family Solanaceae containing more than 1700 species spread worldwide. In Jordan, the genus *Solanum* is represented by eight wild-growing species namely, *S. cornutum* Lam., *S. dulcamara* L., *S. esculentum* L., *S. incanum* L., *S. luteum* Miller., *S. nigrum* L., *S. sinaicum* Boiss. And *S. elaeagnifolium* Cav.[12]

S. elaeagnifolium (silver-leaf nightshade) is a woody perennial or chamaephyte, erect, spreadingly branched and multi-stemmed plant that grows up to 1m. It has over 2m deep extensive roots system, with olive-green leaves on the upper face, whitish on the lower face which gives the plant the name "silver shade". Weak straight reddish-yellow colored spikes usually occur on various parts, especially on older stems. The flowers are bright blue to purple, but occasionally white with yellow anthers. Berry globes, about 1 cm diameter, yellow, smooth, glabrous and is looking like a little tomato. Flowering occurs during the period extending from May to August and fructification from the end of spring till autumn.[13]*S. elaeagnifolium* has been described as a problematic weed in many countries, because of the

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aggressive vegetative growth from deep rootstocks; which explains the difficulty in controlling the plant mechanically, chemically and biologically. Additionally, the economic, social, and environmental negative impacts of this plant are linked to crops losses, as well as loosing rental and resale values of the infected land. On the other hand, the European Plant Protection Organization EPPO (2011) reported that the ripe berries are toxic to livestock and lethal to sheep and the toxicity symptoms include: respiratory complications, trembling, diarrhea, bloating, nasal discharge and excessive salivation.[13] Nevertheless, *S. elaeagnifolium* is used in the traditional medicine of different countries: Indians chewed the roots of the plant to remove the tooth pain and to treat snakebite. Mexican folk healers utilized the plant to treat sneezing and the berries to treat constipation.[14]

Similarly, earlier studies described different biological activities of *S. elaeagnifolium*; such as anti-microbial, antioxidant, hepatoprotective, analgesic, anti-inflammatory activities and its anti-proliferative activity was screened against several cell lines, such as breast cancer (MCF7), liver cancer (HPG2), melanoma, cervical carcinoma (HeLa) and colon cancer (LIM-1863).[15, 16] Phytochemically, the occurrence of some flavonoids and alkaloids has been reported.[17] To the best of our knowledge, *S. elaeagnifolium* grown wild in Jordan has not been evaluated, neither biologically nor phytochemically. Hence, the objective of the present study was to determine the main secondary metabolites of the ripe and unripe berries using UPLC-MS analysis and to evaluate the antiproliferative activity, as well as the α-amylase and Pancreatic Triacylglycerol Lipase (PL) inhibitory potentials of *S. elaeagnifolium.* The reported occurrence of genetic and morphological variations of this plant strengthens the phytochemical and biological evaluation of *S. elaeagnifolium,* grown in Jordan.[18-20]

Materials and Methods

Instruments, chemicals and biochemicals

All reagents and chemicals were obtained from Sigma (Dorset, UK) (unless stated otherwise). Glucose GOD-PAP kit was purchased from BioLabo Reagents, France. In the UV determinations; a UV-VIS spectrophotometer (Spectro Scan 80D (UK)) was used. Additionally, Sonicator (Bandelin Sonorex, Bandelin Electronics, Germany), RPMI 1640, (PAA Laboratories GmbH, Austria and rotary evaporator (Laborota 4000-efficient, Heidolph, Germany) were used. HRMS (High Resolution Mass Spectrometric) data were acquired using a Thermo QExactive Plus mass spectrometer equipped with a heated electrospray ionization source operating on both positive and negative ionization modes (Thermo Fisher Scientific).

Plant collection

The ripe and unripe berries were collected from Deir Alla, located in the northern region of the Jordan Valley, in August 2019. The plant was identified by Prof. Dawood Al-Eisawi at the Department of Biological Sciences, School of Science, The University of Jordan, Amman, Jordan. The green unripe and yellow-orange ripe berries were separated and purified from extraneous material and were used for extraction. A voucher specimen has been deposited in the Department of Pharmaceutical Sciences, School of Pharmacy, The University of Jordan (FMJ-SOLA-SE1f).

Preparation of the extracts of S. elaeagnifolium unripe and ripe berries

Aqueous extracts of *S. elaeagnifolium* unripe and ripe berries were prepared as described earlier.[10] Briefly, each 10 g of the fresh unripe or ripe berries -coarsely sliced were refluxed with 100 mL distilled water for 15 min until boiling. The overnight kept extracts were filtered twice through filter paper and the volumes of the filtered solutions were increased to 100 mL with distilled water to obtain 10% (equivalent to 100 mg/mL) crude aqueous solutions. Sonication of the stock crude extracts, or testing concentrations was performed when needed. For the Pancreatic Triacylglycerol Lipase (PL) experiments, water was removed by lyophilization at -60ºC. The solid residue collected was stored in dry conditions until analysis. For the preparation of the hydro-alcoholic extracts of *S. elaeagnifolium* unripe and ripe berries were prepared using each 10 g of the fresh and coarsely sliced berries with 70% ethanol for 30 min and kept overnight. Then, after filtration, the solvents were evaporated, and crude extracts were obtained. For the cytotoxicity assay, each 100 mg of the hydro-alcoholic extract was dissolved in 10 mL dimethyl sulphoxide (DMSO) for the preparation of the stock solutions.[9, 10]

Phytochemical Analysis of S. elaeagnifolium berries extracts

Ultra-performance-liquid-chromatography high-resolution mass spectrometric (UPLC-HR-ESI-MS) evaluation of hydro-alcoholic extracts of S. elaeagnifolium unripe and ripe berries

Phytochemical screening of the hydro-alcoholic extracts of *S. elaeagnifolium* unripe and ripe berries was carried out using UPLC-HR-ESI-MS as described earlier.[21]The QExactive Plus was adjusted to collect data from 150 to 2000 m/z at a resolution of 70 000. The parameters in the tune method were spray voltage 4000 V; capillary temperature 320°C; sheath gas 50 arb; aux gas 25 arb; spare gas 2 arb; probe heater temp. 30°C. Nitrogen was utilized for the sheath and auxiliary gases. Thermo Scientific Xcalibur 2.3 software was used for instrument control and data analysis. UPLC was carried out on a Waters Acquity system. The column used was a BEH C18 (2.1 × 50 mm, 1.7 µm) (Waters Corp., Milford, MA, USA) that was equilibrated at 40 °C. The sample temperature was set at 10°C. A mobile phase consisting of acetonitrile: water mixture CH_3CN-H_2O (acidified with 0.1% formic acid) was used, starting with 15:85 and increased linearly to 100% CH_3CN within 8 min, holding for 1.5 min, and then returning to the starting conditions within 0.5 min. An Acquity UPLC photodiode array detector was used to acquire PDA spectra, which were collected from 190 to 500 nm with 4 nm resolution. The total run time was 10 min, injection volume was 3 µL.[21]

Pharmacological activities of S. elaeagnifolium berries extracts

In vitro antiproliferative assay for S. elaeagnifolium extracts of unripe and ripe berries

In vitro antiproliferative assay of the hydro-alcoholic extracts of the unripe and ripe berries of *S. elaeagnifolium* was performed using Sulforhodamine B (SRB) colorimetric assay as described earlier.[10] The used obesity-related colorectal cell lines (SW620, SW480, HT29 and HCT116) were gifts from Dr. R. F. Thorne (University of Newcastle, Australia) and Prof. Y. Bustanji, School of Pharmacy, The University of Jordan (CACO2). The latter cell line was cultured in RPMI 1640 medium containing 10% FBS, HEPES Buffer (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) (10 mM), L-glutamine (2 mM), gentamicin (50 µg/mL), penicillin (100 U/mL), and streptomycin sulfate (100 mg/mL), while the used cell lines (SW620, SW480, HT29 and HCT116) were cultured in high glucose DMEM containing 10% FCS (Fetal Calf Serum) (Bio Whittaker, Verviers, Belgium). The cytotoxicity screening and mechanism of reduction of cell viability were performed according to. [9,10] The validation of selective cytotoxicity was tested using human periodontal fibroblasts (PDL). Cisplatin [Sigma-Aldrich, purity: ≥ 99%] in concentrations (0.1-200 µg/mL) was used as robust positive control.All the assays were in three independent experiments and the calculated cytotoxic properties were calculated and reported as the IC_{50} mean values ± Standard deviation (n = 3).

In vitro pancreatic lipase inhibition for aqueous extracts of S. elaeagnifolium unripe and ripe berries and the reference drug Orlistat

In vitro, enzymatic PL activity was assayed using the spectrophotometric quantification method. Orlistat was used as a reference drug. Briefly, four primary stock solutions (concentration range 0.1–100 mg/mL) were prepared by dissolving the aqueous extracts of the unripe and ripe berries in Tris-HCl buffer (2.5 mM (Promega, USA), pH 7.4 with 2.5 mM NaCl). The reference drug, orlistat [Sigma-Aldrich, purity: ≥98%], (in DMSO; 1 mg/mL), was prepared in six different stock solutions in the concentration range of 0.625-20 µg/ mL. Then, 20 µL aliquot of the stock solution was used in the reaction mixture to give a final concentration range of 0.0125-0.4 µg/mL. For the determination of the concentration required for PL

50% inhibition (IC_{50}) the tested extracts and orlistat were measured in comparison to control readings.[10]

In vitro enzymatic starch digestion assay

In these *in vitro* experiments, the efficacy of the aqueous extracts of the unripe and ripe berries was evaluated in seven concentrations ranges (1, 5, 10, 12.5, 25, 50 and 100 mg/mL) in comparison to the reference drug acarbose [Sigma-Aldrich, purity: ≥95%]. Control (distilled water only) samples were free of acarbose and berries extracts as described earlier.[10]

Statistical analysis

The cell survival percentage was calculated as [Mean (Absorbance $_{Test}$ − Absorbance $_{Blank}$) / Mean (Absorbance $_{Control}$ – Absorbance $_{Blank}$)] × 100%. All the values are presented as mean ± SD (Standard Deviation) of three to four independent experiments and were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's multiple-comparison posttest whenever appropriate, with $\alpha = 0.05$. Values were considered significantly different if $P < 0.05$. Statistical analysis was performed with GraphPad Prism 8.4.2 (GraphPad Software, Inc., San Diego, CA, USA).

Results and Discussion

Phytochemical analysis of the extracts of S. elaeagnifolium berries

Ultraperformance liquid chromatography-high resolution mass spectrometric (UPLC- HRESIMS) analysis of the hydro-alcoholic extracts of the unripe and ripe berries

Four flavonoids (apigenin, hyperoside, luteolin and kaempferol), two alkaloids (solasodine and solamargine) and one phenolic acid (chlorogenic acid) were detected in both berries' extracts. The (+)-ESI-SIC and (+)-HR-ESI-MS of the identified compounds in the hydro-alcoholic extracts in comparison to the standards are given in Figures 1, 2 and 3 for both, unripe and ripe berries.

In the present study, the aim was to identify the phytochemical composition of the berries and to screen the different biological activities of them with the hope to benefit from this widespread aggressive weed as a curative agent. In the current study, the flavonoids apigenin, hyperoside and luteolin are identified for the first time in these berries. Earlier, the flavonoids kaempferol 3*β*-D-(6"-*O*-cis-cinnamoyl glucoside) and kaempferol 3-glucoside and the alkaloids solamargine and solasodine were isolated from *S. elaeagnifolium*, grown in Spain.[22] These steroidal alkaloids have been commercially extracted from *S. elaeagnifolium* berries, grown in India.[23]The occurrence of steroidal alkaloids with their diverse biological activities such as antifungal, antiviral and cytotoxic activities, is common in the genus *Solanum*. Solanine and solamargine have cytotoxic activity against colorectal (HT29) cell line.[16] In the present study, the identification of solamargine in the unripe and ripe berries extracts may explain the observed cytotoxicity of these extracts on the colorectal cancer cell line (HT29). It is well-known that secondary metabolites in plants such as phenolic compounds including phenolic acids and flavonoids provide many pharmacological benefits to the plants such as anti-proliferative [26] or anti- α-amylase and anti-α-glucosidase activities.[25]

Pharmacological activities of the extracts of S. elaeagnifolium berries

Anti-proliferative activity of the hydro-alcoholic extracts of the unripe and ripe berries against obesity-related colorectal cancer cell lines

According to the National Cancer Institute guidelines, crude extracts that have IC_{50} less than 100µg/mL in the initial assay are considered active while crude extracts with IC_{50} value less than 30µg/mL are considered cytotoxic.[9,10] The obtained results of the anti-proliferative activities of the tested extracts and the control drug cisplatin, against the four colorectal carcinoma cell lines and Human periodontal fibroblasts (PDL), are given in Table 1 and the dose-response curves for both extracts were generated (Figure 4). Both hydro-alcoholic extracts showed cytotoxic activities over 72h incubations against HT29, HCT116, SW620 and CACO2 cell lines and the extracts lacked selective cytotoxicity in PDL fibroblasts ($P<0.05$) with a significant difference between both extracts against SW620 cell line at $P< 0.05$.Nevertheless, it should be taken into consideration that extracts are mixtures of numerous compounds while the control is a pure compound, making direct comparison difficult. This is the first report of the cytotoxicity of *S. elaeagnifolium* berries against the cell lines investigated. There was a significant difference in the cytotoxicity of each extract on the tested colorectal cell lines ($P<0.05$), besides, both extracts elicited significant difference ($P<0.001$) against one cell line (SW620), therefore further investigation is required to explain these findings. Similarly, the antiproliferative effects of luteolin on human colorectal carcinoma (CRC) derived cell line, HCT15 and CO115 have been confirmed.[26]

In vitro inhibitory effects of the aqueous extracts of the unripe and ripe berries of S. elaeagnifolium on PL and enzymatic starch digestion

The results of the pancreatic triacylglycerol lipase modulatory profiles of the aqueous extracts of both berries and their IC_{50}'s (µg/mL)are given in Table 2. Orlistat's PL-IC_{50} of 114.0 ± 4.0 ng/mL equivalent to 0.2 ± 0.0 µM agrees with the reported PL-IC_{50} values earlier. In comparison to Orlistat's performance, the aqueous extracts of both berries exhibited a concentration-dependent PL inhibition. On the other hand, the reference drug acarbose inhibited glucose liberation from starch and exhibited an IC_{50} value of 0.2± 0.02 µg/mL (Table 2). The significant dose-related reductions of aldohexose release from culinary polymeric cornstarch achieved by the extracts of both tested berries with their IC_{50} values (mg/mL) are given in Table 2.In the present *in vitro* experiments, the aqueous extracts of the unripe and ripe berries of *S. elaeagnifolium* did not exhibit α-amylase or PL-enzyme inhibitory effects. Conversely, Houda *et al*. (2014) reported anti-glycation activity for the aqueous ripe and unripe berries extracts of *S. elaeagnifolium.*[27] These contradicting findings may be influenced by several factors, such as genetic variation reported for this species environmental factors, collection time of the plant material as well as the methods of preparation. Such contradicting observations were also, reported for other *Solanum* species,[18-20] for their anti-obesity activities. *S. linnaeanum* and *S. tuberosum* evoked reduction of lipid metabolism.[28,29] *S. melongena* exhibited moderate anti-amylase activity without anti-lipase activity and *S. betaceum* and *S. diphyllum* L. elicited anti-amylase and anti-glucosidase activities.[30,31] Still, further investigations of the antidiabetics activity of this weed are required.

Conclusion

In conclusion, the extracts of the unripe and ripe berries of *S. elaeagnifolium* exhibited cytotoxic effects against the tested four colorectal cancer cell lines while no anti-obesity or anti-lipidemic effects could be detected *in vitro* and further investigations are needed. The UPLC/MS analysis revealed the presence of bioactive constituents; flavonoids (apigenin, hyperoside and luteolin) for the first time in this species, phenolic acids and two steroidal alkaloids that potentially exert the antiproliferative activity. Further studies with the identified pure compounds as well as the screening of the biological activities of other parts of this quick-spreading species are recommended.

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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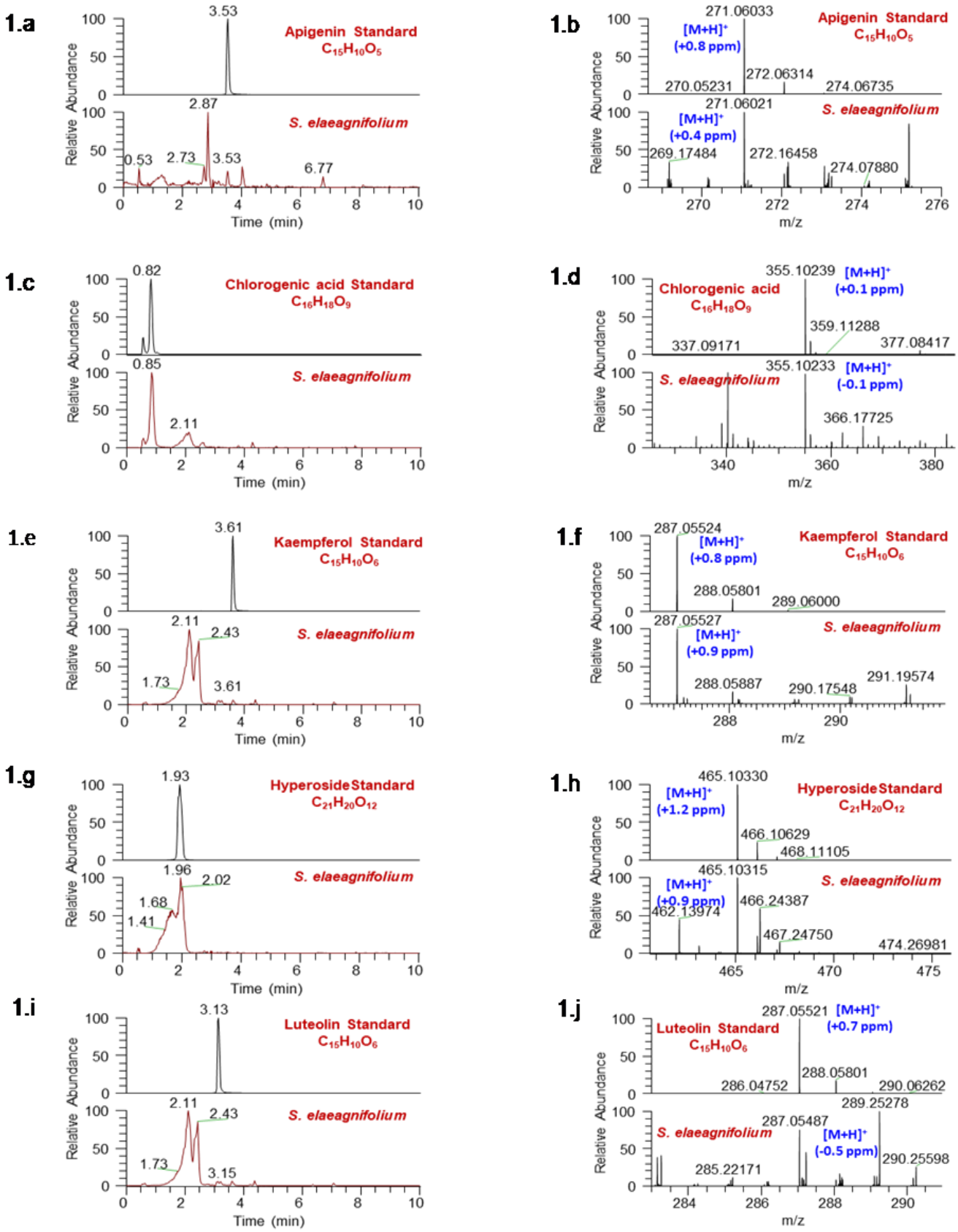

Figure 1: Overlay of chromatographic peaks of the (+)-ESI SIC of different flavonoids standards and the crude hydro-alcoholic extract (unripe berries) of *S. elaeagnifolium*; Figures 1.a, 1.c, 1.e,1.g and 1.i. (+)-HRESIMS of the flavonoids; Figures 1.b, 1.d, 1.f, 1.h, and 1.j.

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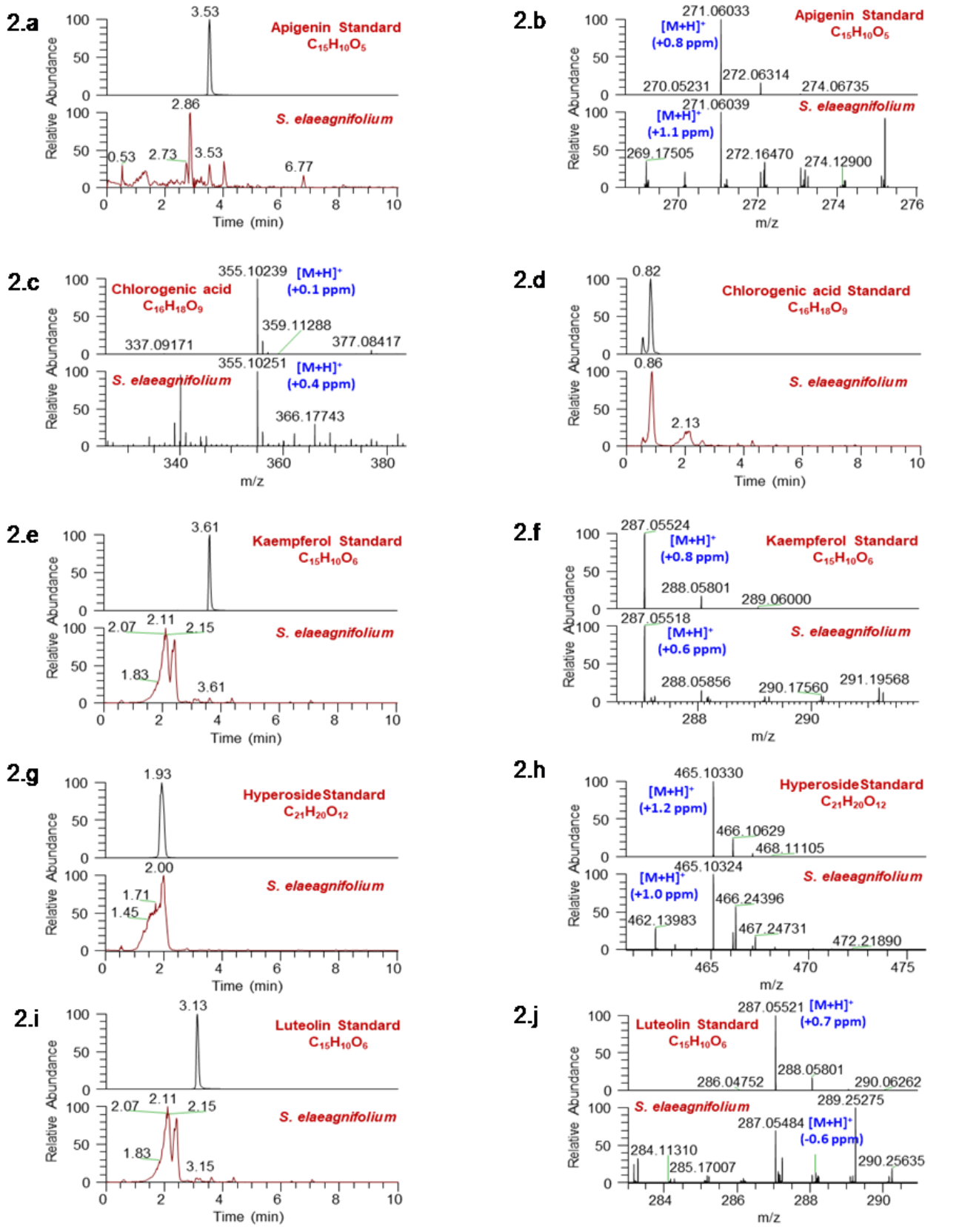

Figure 2: Overlay of chromatographic peaks of the (+)-ESI SIC of different flavonoids standards and the crude hydro-alcoholic extract (ripe berries) of *S. elaeagnifolium*; Figures 2.a, 2.c, 2.e, 2.g and 2.i. (+)-HRESIMS of the flavonoids; Figures 2.b, 2.d, 2.f, 2.h, and 2.j.

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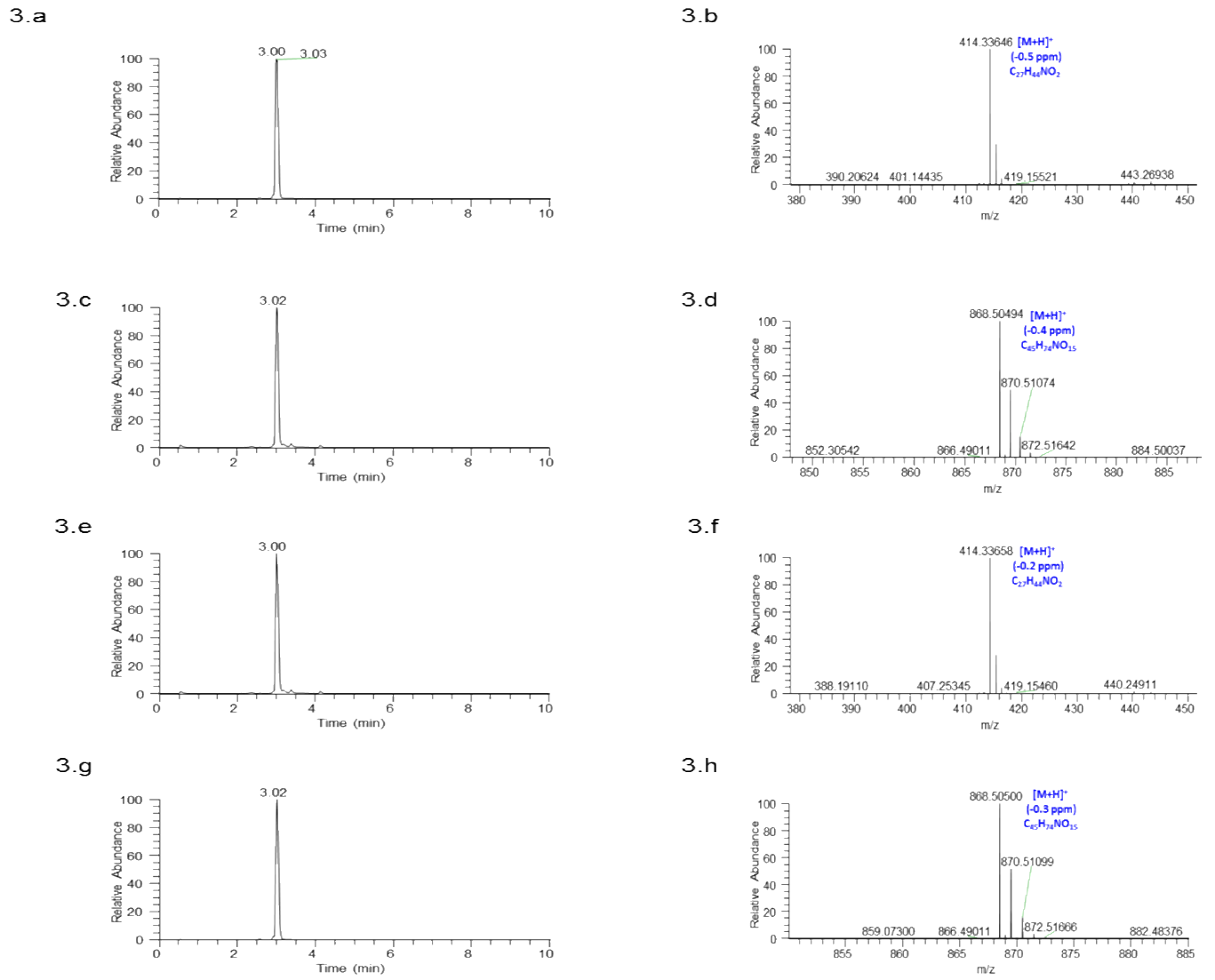

Figure 3: (+)-ESI SIC of crude hydro-alcoholic ripe berries extract of *S. elaeagnifolium* (*m/z*: 869; solamargine), (*m/z*: 414; solasodine); Figures 3.a and 3.c, respectivelly.(+)-HRESIMS of solamargine and solasodine; Figures 3.b and 3.d, respectively. (+)-ESI SIC of crude ethanolic extract (unripe berries) of *S. elaeagnifolium*, (*m/z*: 869; solamargine), (*m/z*: 414; solasodine); Figures 3.e and 3.g, respectivelly. (+)-HRESIMS of solamargine and solasodine; Figures 3.f and 3.h, repectively

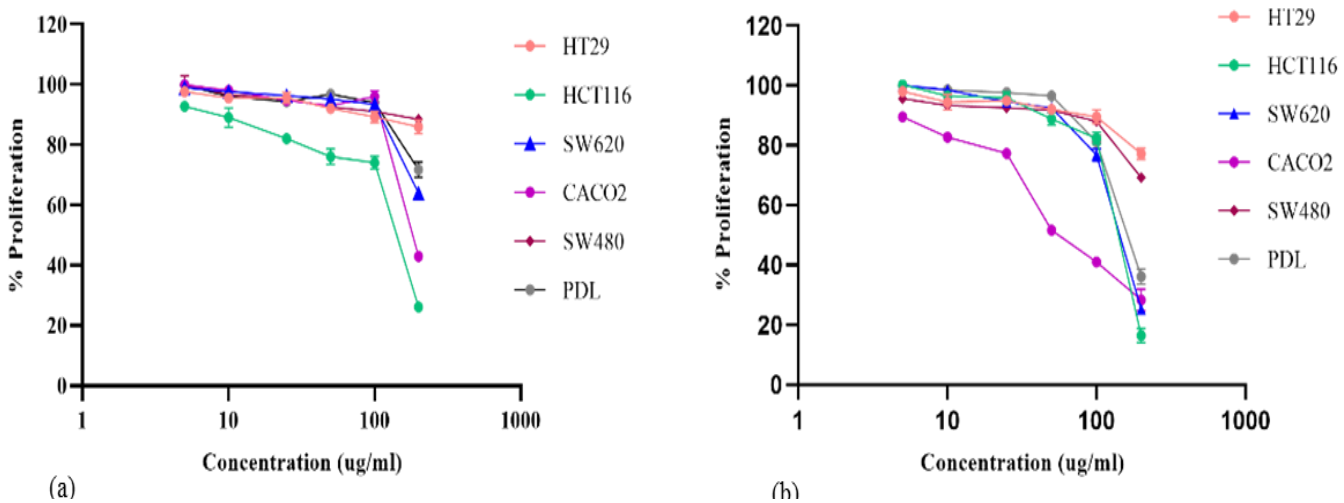

Figure 4: Dose-response curves representing the effect of *S. elaeagnifolium* extracts (a) unripe berries (b) ripe berries on the proliferation of colorectal cancer cell lines and PDL.
Results represent the mean percent cell proliferation vs the concentration of the extracts in µg/mL.

Table 1: Determination of IC_{50}values (µg/mL) for the crude hydro-alcoholic extracts of the unripe and ripe berries of *S. elaeagnifolium* against colorectal cancer cell lines

***S. elaeagnifolium* hydro-alcoholic Extracts**	**Cytotoxicity (as % of Control) IC_{50} value (µg/mL)***					
	HT29	**HCT116**	**SW620**	**SW480**	**CACO2**	**Fibroblasts**
Unripe Berries	14.8 ± 1.300	16.3 ± 1. 00	18.0 ± 2.40[a]	16.5 ± 2.300	11.3 ± 0.800	18.8 ± 1.300
Ripe Berries	17.3 ± 0.700	18.2 ± 1.100	11.1 ± 0.70[b]	19.9 ± 1.700	13.8 ± 0.100	20.4 ± 1.800
Cisplatin	2.4 ± 0.130	0.04 ± 0.006	2.2 ± 0.100	2.06 ± 0.320	3.52 ± 0.400	2.4 ± 0.130

*Results are mean ± SD (n = 3-4 independent experiments). 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. Means with different letters are significantly different at $P< 0.001$ in each column.

Table 2: Determination of PL and enzymatic starch digestion for the aqueous extracts of the unripe and ripe berries of *S. elaeagnifolium*

***S. elaeagnifolium* Aqueous Extracts**	**Pancreatic Triacylglycerol Lipase IC_{50} (µg/mL)***	**Enzymatic Starch Digestion IC_{50} (mg/mL)***	**Sugar (mM) Interferences at 100 mg/mL**
Unripe Berries	949.00 ± 60.810	3.91 ± 0.590	2.280
Ripe Berries	164.70 ± 23.050	7.10 ± 1.070	1.960
Reference	Orlistat	Acarbose	-
Drugs	0.114 ± 0.01 µg /mL	0.2± 0.02 µg /mL	

*Results are mean ± SD (n = 3-4 independent experiments).

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References

1. Taha H, Jaghbeer MA, Shteiwi M, Alkhaldi S, Berggren V. Knowledge and Perceptions about Colorectal Cancer in Jordan. Asian Pac J Cancer Prev. 2016; 16(18):8479-8486.
2. Ferlay J, Soerjomataram I, Dikshit R, Eser S, Mathers C, Rebelo M, Parkin DM, Forman D, and Bray F. Cancer incidence and mortality worldwide: Sources, methods and major patterns in GLOBOCAN 2012. Int J Cancer. 2015; 136(5):E359-E386.
3. Benarba B and Pandiella A. Colorectal cancer and medicinal plants: Principle findings from recent studies. Biomed Pharmacother. 2018; 107:408-423.
4. Abu-Rish EY, Kasabri V, Hudaib MM, Mashalla SH, Alalawi LH, Tawaha K, Mohammad MK, Mohamed YS, Bustanji Y. Evaluation of antiproliferative activity of some traditional anticancer herbal remedies from Jordan. Trop J Pharm Res. 2016; 15(3):469-474.
5. Al-Tawarah NM, Qaralleh H, Khlaifat AM, Nofal MN, Alqaraleh M, Khleifat KM, Al-Limoun MO, and Al Shhab MA. Anticancer and Antibacterial Properties of *Varthemia iphionides* Essential Oil/Silver Nanoparticles. Biomed Pharmacol J. 2020; 13(3):1175-1184.
6. Kasabri V, Afifi FU, Abu-Dahab R, Mashallah S. Mitigating Efficacy of *Inula graveolens* (L.) Desf.(Asteraceae) In Breast Adenocarcinoma MCF7 and T47D Proliferation: *In Vitro* Mechanistic Studies of A Selected Ethnomedicinal Plant from Jordan. Rom Biotechnol Lett. 2017; 22(6):13044.
7. Jochem C and Leitzmann M. Obesity and Colorectal Cancer. Recent Results Cancer Res. 2016; 208:17-41.
8. Seetaloo AD, Aumeeruddy MZ, Rengasamy Kannan RR, Mahomoodally MF. Potential of traditionally consumed medicinal herbs, spices, and food plants to inhibit key digestive enzymes geared towards diabetes mellitus management — A systematic review. S Afr J Bot. 2019; 120:3-24.
9. Afifi FU, Kasabri V, Litescu S, Abaza IF, Tawaha K. Phytochemical and biological evaluations of *Arum hygrophilum* Boiss. (Araceae). Pharmacogn Mag. 2017; 13(50):275-280.
10. Al-Hamaideh KD, El-Elimat T, Afifi FU, Kasabri V. Phytochemical Screening and Pharmacological Activities of *Echium judaeum* Lacaita Extracts Growing Wild in Jordan. J Pharm Sci. 2017; 10(3):153-164.
11. Haddad MA, Dmour H, Al-Khazaleh JFM, Obeidat M, Al-Abbadi A, Al-Shadaideh AN, Al-Mazra'awi MS, Shatnawi MA, Iommi C. Herbs and Medicinal Plants in Jordan. J AOAC Int. 2020; 103(4):925-929.
12. Al-Eisawi D. Flora of Jordan Checklist, Revised; The University of Jordan Press; 2013. 168 p.
13. Brunel S. Pest risk analysis for *Solanum elaeagnifolium* and international management measures proposed. EPPO Bull. 2011; 41(2):232-242.
14. Camazine S and Bye RA. A study of the medical ethnobotany of the Zuni Indians of New Mexico. J Ethnopharmacol. 1980; 2(4):365-388.
15. Badawy A, Zayed R, Ahmed S, Hassanean H. Phytochemical and pharmacological studies of *Solanum elaeagnifolium* growing in Egypt. J Nat Prod India. 2013; 6:156-167.
16. Lee K-R, Kozukue N, Han J-S, Park J-H, Chang E-Y, Baek E-J, Chang J-S, Friedman M. Glycoalkaloids and Metabolites Inhibit the Growth of Human Colon (HT29) and Liver (HepG2) Cancer Cells. J Agric Food Chem. 2004; 52(10):2832-2839.

17. Kaunda JS and Zhang Y-J. The genus *Solanum*: an ethnopharmacological, phytochemical and biological properties review. Nat prod bioprospect. 2019; 9(2):77-137.
18. Zhu XC, Wu HW, Stanton R, Burrows GE, Lemerle D, Raman H. Morphological variation of *Solanum elaeagnifolium* in south-eastern Australia. Weed Res. 2013; 53(5):344-354.
19. Zhu XC, Wu HW, Raman H, Lemerle D, Stanton R, Burrows GE. Genetic variation and structure of *Solanum elaeagnifoliumin* Australia analysed by amplified fragment length polymorphism markers. Weed Res. 2013; 53(5):337-343.
20. Qasem JR, Al Abdallat AM, Hasan SM. Genetic diversity of *Solanum elaeagnifolium*, an invasive problematic weed in Jordan. Weed Res. 2019; 59(3):222-234.
21. El-Elimat T, Figueroa M, Ehrmann BM, Cech NB, Pearce CJ, Oberlies NH. 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(9):1709-1716.
22. Chiale CA, Cabrera JL, Juliani HR. Kaempferol 3-(6″-cis-cinnamoylglucoside) from *Solanum elaeagnifolium*. Phytochemistry. 1991; 30(3):1042-1043.
23. Kulkarni M and Pendse G. Glycoalkaloid contents in leaf, stem and root of *Solanum elaeagnifolium*. Planta Med. 1974; 25(03):249-252.
24. Tariq A, Sadia S, Pan K, Ullah I, Mussarat S, Sun F, Abiodun OO, Batbaatar A, Li Z, Song D, Xiong Q, Ullah R, Khan S, Basnet BB, Kumar B, Islam R, Adnan M. A systematic review on ethnomedicines of anti-cancer plants. Phytother Res. 2017; 31(2):202-264.
25. Sergent T, Vanderstraeten J, Winand J, Beguin P, Schneider Y-J. Phenolic compounds and plant extracts as potential natural anti-obesity substances. Food Chem. 2012; 135(1):68-73.
26. Xavier CP, Lima CF, Preto A, Seruca R, Fernandes-Ferreira M, Pereira-Wilson C. Luteolin, quercetin and ursolic acid are potent inhibitors of proliferation and inducers of apoptosis in both KRAS and BRAF mutated human colorectal cancer cells. Cancer Lett. 2009; 281(2):162-170.
27. Houda M, Derbre S, Jedy A, Tlili N, Legault J, Richomme P, Limam F, Saidani-Tounsi M. Combined anti-ages and antioxidant activities of different solvent extracts of *Solanum elaeagnifolium* Cav (Solanaceae) fruits during ripening and related to their phytochemical compositions. EXCLI J. 2014; 13:1029-1042.
28. Ku SK, Sung SH, Choung JJ, Choi J-S, Shin YK, Kim JW. Anti-obesity and anti-diabetic effects of a standardized potato extract in ob/ob mice. Exp Ther Med. 2016; 12(1):354-364.
29. Mahomoodally FM and Ramcharun S. *In vitro* kinetics of inhibition of lipase, antioxidant activity, glucose entrapment and polyphenolic content of *Solanum linnaeanum*. J Biol Act Prod Nat. 2015; 5(6):383-396.
30. Hossain SJ, El-Sayed MA, Mohamed A-HH, Sheded MG, Aoshima H. Phenolic content, anti-oxidative, anti-alpha-amylase and anti-alpha-glucosidase activities of *Solanum diphyllum*. Bangladesh J Bio. 2009; 38(2):139-143.
31. Orqueda ME, Rivas M, Zampini IC, Alberto MR, Torres S, Cuello S, Sayago J, Thomas-Valdes S, Jimenez-Aspee F, Schmeda-Hirschmann G, Isla MI. Chemical and functional characterization of seed, pulp and skin powder from chilto (*Solanum betaceum*), an Argentine native fruit. Phenolic fractions affect key enzymes involved in metabolic syndrome and oxidative stress. Food Chem. 2017; 216:70-79.