Pharmacogn. Mag.
A multifaceted peer reviewed journal in the field of Pharmacognosy and Natural Products
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ORIGINAL ARTICLE

Phytochemical and Biological Evaluations of *Arum hygrophilum* Boiss. (Araceae)

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Submitted: 28-03-2016 Revised: 29-04-2016 Published: 18-04-2017

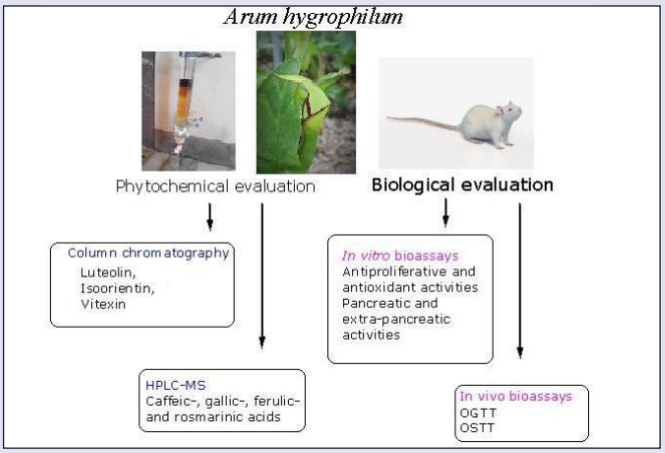
ABSTRACT

Background: *Arum hygrophilum* is a traditional medicinal plant indigenous to Jordan. The present study explores its phytochemistry, antioxidative, antidiabesity, and antiproliferative potentialities. **Materials and Methods:** Column chromatography and HPLC-MS analysis were used for its phytochemical evaluation. Using leaf crude water and ethanol extracts, the antioxidative capacities, their modulation of pancreatic β-cell proliferation, and insulin secretion as well as glucose diffusion and enzymatic bioassays were evaluated. **Results:** Three flavonoids (luteolin, isoorientin, and vitexin) and β-sitosterol have been isolated and their structures determined. HPLC-MS analysis of the ethanol extract further revealed the presence of caffeic, ferulic, gallic, and rosmarinic acids and quercetine-3-O-rhamnoside. The ethanol extract exhibited DPPH and ABTS radical scavenging and antioxidative capacities. *A. hygrophilum* (1), vitexin (2), and rosmarinic acid (3) inhibited pancreatic lipase (PL) dose dependently with PL-IC_{50} (μg/mL) values in an ascending order: (3); 51.28 ± 7.55 < (2); 260.9 ± 21.1 < (1); 1720 ± 10. Comparable to GLP-1-enhanced β-cell proliferation in 2-day treatment wells, a dose-dependent augmentation of BrdU incorporation was obtained with the *A. hygrophilum* aqueous extract (AE) (0.5 and 1 mg/mL, with respective 1.33- and 1.41-folds, $P < 0.001$). *A. hygrophilum* AE was identified as an inhibitor of α-amylase/α-glucosidase with IC_{50} value of 30.5 ± 2.1 mg/mL but lacked antiproliferative effects in colorectal cancer cell lines (HT29, HCT116, and SW620) and insulinotropic effects in β-cell line MIN6. **Conclusion:** *A. hygrophilum* extracts inhibited gastrointestinal enzymes involved in carbohydrate and lipid digestion and absorption.

Key words: *A. hygrophilum Boiss*, Araceae, HPLC-MS, pancreatic lipase, α-amylase/α-glucosidase

SUMMARY

- Phytochemical evaluation of *Arum hygrophilum* recovered flavonoids (luteolin, isoorientin and vitexin) and β-sitosterol
- HPLC-MS analysis of its antioxidative ethanol extract further revealed the presence of caffeic-, ferulic-, gallic- and rosmarinic acids and quercetine-3-O-rhamnoside
- *A. hygrophilum* inhibited α-amylase/α-glucosidase and pancreatic lipase dose-dependently
- *A. hygrophilum* augmented β-cell proliferation dose dependently, but it lacked antiproliferative effects in colorectal cancer cell lines (HT29, HCT116, and SW620) and insulinotropic effects in β-cell line MIN6



Abbreviations used: ABTS: 2,2′-Azino-Bis-3-Ethylbenzothiazoline-6-Sulfonic Acid, AE: Aqueous Extract, ANOVA: Analysis Of Variance, AUC: Area Under Curve, BrdU: 5-Bromo-2'-Deoxyuridine, DPPH: 2,2-Diphenyl-1-Pycriylhydrazyl, ELISA: Enzyme Linked Immunosorbent Assay, GLP1: Glucagon Like Peptide 1, GSIS: Glucose Stimulated Insulin Secretion, HPLC-MS: High Performance Liquid Chromatography –Mass Spectrometry, IC_{50}: 50% Inhibitory Concentration, KRH: Krebs/Ringer/Hepes, MTT: 3-(4,5-Dimethylthiazol-2-Yl)-2,5-Diphenyltetrazolium Bromide, OGTT: Oral Glucose Tolerance Test, ORAC: Oxygen Radical Antioxidant Capacity, OSTT: Oral Starch Tolerance Test, PL: Pancreatic Lipase, SEM: Standard Error Of The Mean, SRB: Sulforhodamine B, TEAC: Trolox Equivalent Antioxidant Capacity, TLC: Thin Layer Chromatography

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DOI: 10.4103/0973-1296.204551

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INTRODUCTION

Plants have been long used for the ethnomedical integrative/complementary treatment of cancer and obesity-diabetes in various systems of medicine.[1-3] Type 2 diabetes and obesity, referred to as diabesity, comprise global health threats with rising prevalence.[4] Diverse studies were conducted to explore medicinal plants as potential therapeutic agents for dual management of diabetes and hyperlipidemia via digestive enzymes' inhibition, namely pancreatic α-amylase, intestinal α-glucosidase, and pancreatic lipase.[5,6] In the Jordanian traditional medicine, the edible *Arum* species, referred with the common Arabic name "Louf," are recommended as a natural anticancer agent against colon cancer.[7] Previously, different flavonoids, such as quercetin, apigenin, vitexin, and isoorientin were isolated from *A. palaestinum* in our laboratories and their antimicrobial activities were established.[8] In a recent comparative study in *in vitro* and *in vivo* experiments, pancreatic lipase (PL) and dual α-amylase/α-glucosidase inhibitory potentials were

Cite this article as: Afifi FU, Kasabri V, Litescu S, Abaza IF, Tawaha K. Phytochemical and biological evaluations of Arum *hygrophilum* boiss. (Araceae). Phcog Mag 2017;13:275-80.

demonstrated for the extracts of *A. dioscorides* and *A. palaestinum* as well as for some of their isolated compounds.[9] Earlier, Karahan *et al.*[10] reported free radical scavenging and ferric-reducing activities of ethanol, methanol, acetone, and water extracts of *A. dioscoridis* leaves. The study concluded that total phenolic and flavonoid contents were greatly influenced by the extraction medium. Also, a literature survey indicated that *A. hygrophillum* is the least evaluated *Arum* species, lacking on both phytochemical and biological evaluations. This study investigates the indigenous *A. hygrophilum* phytochemically and biologically. The *A. hygrophilum* crude aqueous extract (AE) modulation of the extrapancreatic digestive enzymes was examined *in vitro*. Additionally, acute *in vivo* effects were investigated. Antiproliferative potential of this species against colorectal cancer cell lines as well as possible pancreatic effects in β-cell line was evaluated.

MATERIALS AND METHODS

Plant material

Aerial parts of the flowering *A. hygrophilum* were collected in February/March 2013 in Zai, Salt, Jordan. Taxonomic identity of the collected plants was established in comparison with herbarium specimens of the School of Science, The University of Jordan. The identification was confirmed by Prof. K. Tawaha. A vaucher specimen (FMJ-ARA2) was kept in the Department of Pharmaceutical Sciences, School of Pharmacy, The University of Jordan.

Extraction and chromatographic separation

The air-dried flowers and leaves were coarsly powdered and extracted by soaking in EtOH for 3 weeks at RT. After solvent evaporation until dryness, the syrupy residue was extracted successively with $CHCl_3$, EtOAc, and BuOH. Based on similar TLC profile, fractions of EtOAc and BuOH were combined and chromatographed on Silica gel columns (Silicagel 60, Merck) successively. Chloroform/methanol gradients were used for the extraction of flavonoids and plant acids. The isolated compounds were purified by repeated crystallization in MeOH. For the biological experiments, 10%% (w/v) AEs were prepared as reported earlier.[11]

HPLC analysis

Crude EtOH extract was evaluated by HPLC. The experiments were based on the previously developed method published by Cristea *et al.*,[12] adjusted to the current samples' specificity for the measurement of the plant acids and flavonoids.[12] The HPLC measurements were performed using a complete HPLC SHIMADZU system, using a Nucleosil 100-3.5 C18 column,. The system was coupled to a MS detector, LCMS-2010 detector, equipped with an ESI interface. The mobile phase consisted of formic acid in water (pH = 3.0) as solvent A and formic acid in acetonitrile (pH = 3.0) as solvent B. The polyphenolic compounds separation was performed using binary gradient elution: 0 min 5% solvent B; 0.01-20 min 5-30% solvent B; 20-40 min 30% solvent B; 40.01-50 min 30-50% solvent B; 50.01-52 min 50-5% solvent B. The flow rate was 0-5 min 0.1 mL/min; 5.01-15 min 0.2 mL/min; 15.01-35 min 0.1 mL/min; 35.01-50 min 0.2 mL/min; 50-52 min 0.1 mL/min. The analyses were performed at RT for the period of 70 min and the injection volume was 20 µL. Initially full-scan acquisition mode was used in *m*/*z* range 50-800. Stock solutions of the reference substances (1 mg/mL EtOH) were kept at 4°C between the experiments.

Antioxidant efficacy and free radical scavenging properties assessment

The radical scavenging activities of the ethanol extract of *A. hygrophilum* were evaluated using 2,2-diphenyl-1-pycriylhydrazyl (DPPH) and 2,2'-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) radical-scavenging activity assays and expressed as Trolox Equivalent. Antioxidant Capacity (TEAC). The antioxidant property was determined using Oxygen Radical Antioxidant Capacity (ORAC).[13,14]

Insulin secretion static incubation experiments

Glucose-stimulated insulin secretion (GSIS) from MIN6 cells was determined using a static incubation protocol as described earlier.[15]

Cell viability and proliferation assays

Cell viability was assessed by a MTT kit on 96-well plates using kit's manufacturer protocol. Proliferation of MIN6 cells was evaluated with a colorimetric ELISA-based BrdU incorporation kit. Assays were performed in accordance with manufacturer protocol instructions.[15]

Spectrophotometric quantification of PL activity and assaying PL inhibition of test extracts and compounds

The aqueous extract was evaporated until dryness under vacuum at 40°C using a rotary evaporator and dissolved in Tris-HCl buffer. The reference drug orlistat, rosmarinic acid, vitexin, and the AE were prepared in six different concentration ranges and *in vitro* enzymatic PL activity was assayed as described earlier in triplicates.[16,17]

In vitro enzymatic starch digestion assay

In vitro enzymatic starch digestion was assayed with acarbose as the reference drug.[18] The extent of polysaccharide breakdown into glucose was evaluated for the AE in seven concentrations (1, 5, 10, 12.5, 25, 50, and 100 mg/mL). The effects of acarbose at 1000 µg/mL concentration were performed in triplicates.

Glucose movement *in vitro* assay

In vitro glucose movement was assayed as described earlier with guar gum (50 mg/mL) as a positive control.[15] *A. hygrophilum* AEs 10, 25, and 50 mg/mL in 0.22 M glucose in triplicates were dialyzed against 0.15 M NaCl overnight at 37°C with gentle shaking. A parallel plant-free (negative) control was included.[19]

In vivo confirmatory studies: Oral starch tolerance test (OSTT) and oral glucose tolerance test (OGTT)

OSTT and OGTT were conducted as described earlier in the Experimental Animal Laboratory of the School of Medicine, The University of Jordan using rats (*Rattus rattus*) of both sexes weighing 220-260 g, applying The University of Jordan ethical guidelines for animal protection.[20] Experimental approval was obtained from the Scientific Research Council at the Deanship of Academic Research. Aqueous extracts were administered under mild anesthesia in doses 125, 250, and 500 mg/kg body weight ($n = 6$ for each group).

In vitro antiproliferative assay

The cytotoxicity measurements with colorectal cell lines HT29, HCT116, and SW620 were determined using sulforhodamine B (SRB) colorimetric assay for cytotoxicity screening and mechanism of reduction of cell viability as described previously.[21] Doxorubicin IC_{50} values were calculated within treatment concentration range 0.1-50 µg/mL.

Statistical analysis

The values are presented as mean ± standard error of mean (SEM) of three to six independent experiments. Statistical differences between

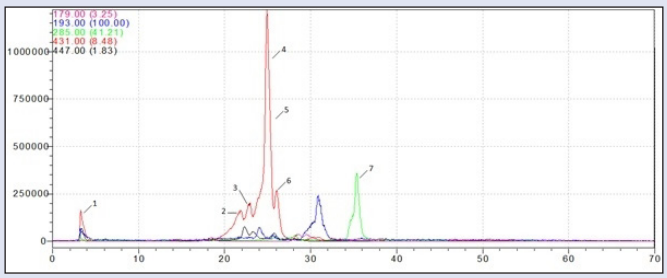

Figure 1: HPLC-MS chromatogram of *Arum hygrophilum*. (1) gallic acid; (2) caffeic acid; (3) isoorientin; (4) vitexin; (5) ferulic acid; (6) quercetin-3-O-rhamnoside; (7) luteolin

Table 1: Modulatory effects of *A. hygrophilum* AE on the viability of pancreatic β-cells MIN6 in 48 h after seeding. Each result indicates the mean ± SEM of four independent experiments

Treatment	MIN6 viability (% control)
Control incubations (plant free)	99.5 ± 10.4
A. hygrophilum AE (0.01 mg/mL)	179.2 ± 7.3***
A. hygrophilum AE (0.05 mg/mL)	154.0 ± 15.2***
A. hygrophilum AE (0.1 mg/mL)	134.2 ± 16.7***
A. hygrophilum AE (0.5 mg/mL)	166.7 ± 4.2***
A. hygrophilum AE (1 mg/mL)	123.6 ± 17.1*
A. hygrophilum AE (5 mg/mL)	142.1 ± 4.8***
A. hygrophilum AE (10 mg/mL)	131.0 ± 16.3***

*P<0.05 and ***P<0.001 compared to control (plant-free) incubations.

control and different treatment groups and AUCs (incremental area under 24-h glucose curve) were determined using Graph Pad Prism one-way analysis of variance (ANOVA) followed by Dunnett's posttest whenever appropriate (version 3.02 for windows; Graph Pad Software, San Diego, CA, USA). AUCs, also, were calculated by Graph Pad Prism. Values were considered significantly different if *P* was less than 0.05 and highly significantly different if *P* was less than 0.01 and less than 0.001.

RESULTS AND DISCUSSION

Arum is a genus of about 26 species of flowering plants in the family Araceae, native to different parts of the globe with the highest species diversity in the Mediterranean region. In Jordan, it is represented by three species: *A. palaestinum*, *A. dioscoridis*, and *A. hygrophilum*.[22] In our previous investigations with the former two species, several flavonoids were isolated, and subsequently flavonoids, coumarins, and plant acids were identified using LC-MS.[8,9] The isolated isoorientin showed myolytic activity on smooth muscle containing preparations from the rat and guinea pig.[23] Also, the antiproliferative activities of both species were evaluated using different cancer cell lines.[9,24]

In the present study, using conventional column chromatography, from the butanol and ethanol extracts of *A. hygrophilum*, three flavonoids (luteolin, isoorientin, and vitexin) and rosmarinic acid were isolated. Chloroform extract yielded β-sitosterol. The structures of the isolated compounds were determined using their physical properties and the different spectroscopic spectra (UV, IR, ^{1}H-NMR, ^{13}C-NMR). The obtained results were in agreement with the values reported for them.[25-28] For all isolated compounds, melting points and mixed melting points with the reference substances were determined and confirmed.

HPLC-MS analysis of the ethanol extract revealed in addition to the isolated flavonoids the presence of quercetin-3-O-rhamnoside (3.33 mg/mL) and several plant acids, caffeic(3.33 mg/mL), ferulic (0.58 mg/mL), and gallic acid (3.58 mg/mL) [Figure 1]. The *in vitro* antioxidant efficacy and free radical scavenging properties assessment of *A. hygrophilum* ethanol extract, expressed as trolox equivalent in micromols per milligram, exhibited good correlation between the values obtained for TEAC (115.29 ± 5.75), DPPH (4.44 ± 0.22), and ORAC (3.61 ± 0.18) and identified polyphenolic compounds of the extract.

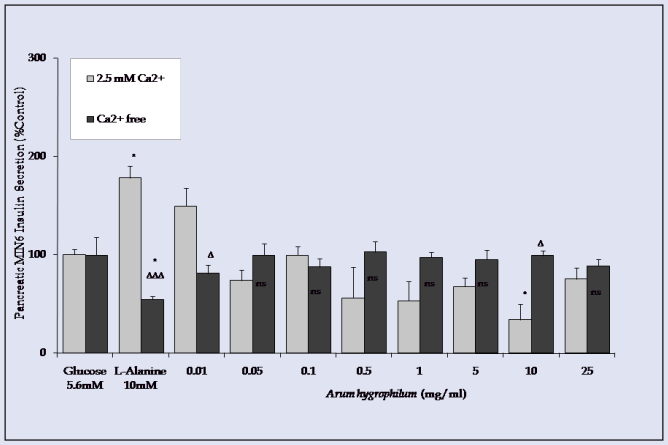

Figure 2: Modulatory effects of *A. hygrophilum* AE (0.01–25 mg/mL) on function of MIN6 pancreatic β-cells. Such augmentation of GSIS following acute 1-h treatments was evaluated by rat insulin ELISA. *A. hygrophilum* treatment wells were co-incubated in corresponding 5.6 mM glucose. Each bar indicates the mean ± SEM of four determinations. *$P < 0.05$ compared to respective 5.6 mM glucose (negative) control wells; $^{\Delta}P < 0.05$ compared to respective treatment conditions in the presence of 2.5 mM Ca^{2+}.

Glucose-dependent modulation of glucose stimulated insulin secretion (GSIS) in pancreatic β-cell by *A. hygrophilum* AEs

L-Alanine (10 mM) was used as a positive control, which enhanced substantially ($P < 0.05$) GSIS in MIN6 by 178.5 ± 17.9% ($n = 4$) following 1-h incubations, compared to untreated (glucose only) controls [Figure 2].[29] With obvious unlikeness to L-alanine, *A. hygrophilum* AE doses lacked any marked augmentation of MIN6 GSIS in acute treatment wells compared with controls [Figure 2]. Surprisingly, *A. hygrophilum* AE (10 mg/mL) seemed to antagonize pancreatic GSIS significantly ($P < 0.05$) [Figure 2]. Cell viability was unaffected, negating against plant inflected cytotoxicity. Changes in β-cell cytosolic Ca^{2+} concentrations, whether by an influx of extracellular Ca^{2+} or by release of Ca^{2+} from intracellular stores, are thought to be a primary trigger for the initiation of insulin exocytosis machinery. Figure 2 illustrates that the marked insulinotropic trend of l-alanine was highly significantly (54.9 ± 8.6%, $P < 0.001$) abolished in Ca^{2+} depleted KRH, as compared to corresponding Ca^{2+} free glucose-only (negative control) wells. Apparently, Ca^{2+} depleted *A. hygrophilum* treatments (0.01 mg/mL) had substantial reduction ($P < 0.05$) in pancreatic secretory function, compared with respective Ca^{2+} buffered conditions [Figure 2].

Pancreatic β-cell viability/expansion modulation by *A. hygrophilum* AEs

Compared to control untreated cells, the MTT method revealed that *A. hygrophilum* AEs (0.01-10 mg/mL) treatment in 48h post seeding preserved β-cell integrity. *A. hygrophilum* induced highly significantly pancreatic monolayers expansion by 1.23-1.76 folds (P<0.05-0.001 vs. basal plant-free control, Table 1). The higher concentrations,

Table 2: Effect of ascending concentrations of *A. hygrophilum* (AE) (mg/mL) on percentage reduction of enzymatic starch digestion *in vitro*. Results expressed as percentage decrease in control values are mean ± SEM (n = 3 independent replicates). *P < 0.05 and ***P < 0.001 compared to control (drug-free or plant-free) incubations

Plant AE (mg/mL)	0.1	0.5	1	1.25	2.5	5	10
A. hygrophilum	0.3 ± 1.9	4.5 ± 1.2	7.0 ± 0.9***	6.9 ± 1.1***	7.5 ± 1.0***	17.4 ± 2.1***	22.2 ± 0.4***

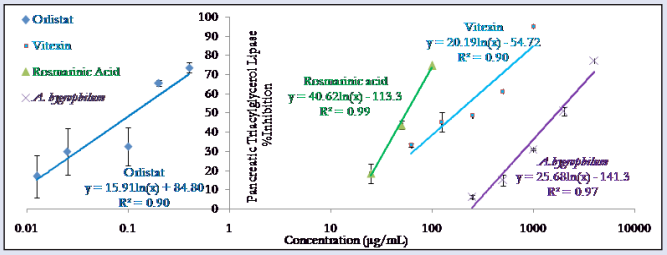

Figure 3: *In vitro* inhibitory effects of *A. hygrophilum* (AE) (IC_{50} = 1.7 ± 0.01 mg/mL), vitexin (IC_{50} = 51.3 ± 7.6 µg/mL [0.6 ± 0.1 mM]), rosmarinic acid (IC_{50} = 260.9 ± 21.1 µg/mL [0.1 ± 0.02 mM]), and orlistat (IC_{50} = 114.0 ± 4.0 ng/mL [0.2 ± 0.0 µM]) on pancreatic triacylglycerol lipase activity. Results are mean ± SEM (n = 3 independent replicates)

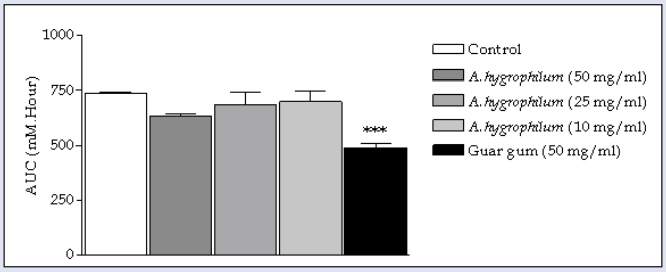

Figure 4: *In vitro* effects of *A. hygrophilum* AE concentrations (mg/mL) on the incremental AUC of 24-h glucose movement. ***P < 0.001 compared to control (drug-free or plant-free) incubations

however, proved ineffective and noncytotoxic (the same table). A colorimetric immunoassay of BrdU incorporation into MIN6 β-cell genome was recruited to ascertain proliferative principles of chronic plants treatments. The gut hormone glucagon-like peptide-1 (GLP-1) agonists have been shown to stimulate the growth and differentiation of pancreatic cells, as well as to exert cytoprotective and antiapoptotic effects on β-cells.[30] GLP-1 (500 nM) highly significantly promoted a maximal extent of BrdU incorporation by 1.33-1.5-fold (P < 0.001, n = 4) in comparison to basal BrdU incorporation (spontaneous control). Similar to 48-h MTT findings, *A. hygrophilum* AE 0.5 and 1 mg/mL induced a highly substantial concentration related increase in pancreatic BrdU incorporation (with respective 1.33 and 1.41 folds, P < 0.001 vs. basal controls). With its safety profile, pancreatic proliferative capacities could be ascribed to *A. hygrophilum* AEs. This suggests the plant's potential utility in diabetes regenerative therapeutics.[15] The present study has revealed that water-soluble bioactive principles in *A. hygrophilum* AEs lacked any glucose-evoked insulin-releasing effect in pancreatic β-cells, unlike many other herbal remedies reputed for substantial insulin secretagogue activity.[15,29]

In vitro inhibitory effects of *A. hygrophilum* AEs, vitexin, and rosmarinic acid on PL activity

PL inhibition is one of the most widely studied mechanisms to determine the potential efficacy of natural products and ethnomedicinal botanicals as obesity-modulating agents, since they are generally considered to be less toxic with less side effects than totally synthetic compounds.[16] In this current study, the pancreatic triacylglycerol antilipase activity profiles of the crude AE of *A. hygrophilum*, vitexin, and rosmarinic acid were determined [Figure 3]. Orlistat's PL-IC_{50} value of 114.0 ± 4.0 ng/mL, equivalent to 0.2 ± 0.0 µM, is comparable to reported PL-IC_{50} values.[16] Comparable to orlistat performance, a marked concentration-dependent PL inhibition trend was obtained per tested extracts as well as their isolated components [Figure 3]. PL-IC_{50} values obtained for triple separate determinations are also illustrated [Figure 3]. The importance of polyphenolic substances as potential inhibitors of PL were discussed recently by Buchholz and Melzig.[31] Several flavonoids, as the largest class of polyphenolic substances, have been evaluated and the studies indicated that the flavonoids having hydroxyl or methoxy groups at C3' and C4' in ring B as well as C-glycosidic flavones favor the PL inhibition.[31-33] Additionally, the efficacy of phenolic acids, namely caffeic-, rosmarinic-, and ferulic acids, as PL inhibitors are well described.[34,35] In effect, the results of the present study indicate that the PL inhibitory efficacy of *A. hygrophilum* may be attributable to their multiple phenolic components acting additively or synergistically.[36]

In vitro extrapancreatic inhibitory effects of *A. hygrophilum* AE on α-amylase/α-glucosidase

In acarbose (0.1 mg/mL) incubations as the reference drug, glucose liberation from starch was inhibited by 97.6% highly substantially (P < 0.001, vs. drug-free control incubations, n = 3). With an IC_{50} value of 30.5 ± 2.1 mg/mL, the significant dose-related (P < 0.001) percentage decreases in enzymatic starch hydrolysis by *A. hygrophilum* dosage gradient (1-10 mg/mL) are summarized in Table 2. The overall dual α-amylase and α-glucosidase inhibitory propensities of *A. hygrophilum* could be the result of the combination of its several constituents performing in concert. Additionally, similar digestive enzymes modulatory outcomes were obtainable for both *A. dioscoridis* and *A. palaestinum*.[9,18,20]

Extrapancreatic modulation of glucose movement *in vitro* by *A. hygrophilum* AEs

Using the diffusion model as described; mean AUCs (area under 24-h glucose curve) for the viscous water-soluble gel-forming guar gum (50 mg/mL) were decreased highly significantly by 30.8 ± 2.5% (P < 0.001, n = 3) [Figure 4] compared to overnight negative control. The efficacy of guar gum as a classical positive control has been elsewhere detailed.[37] Incomparable to guar gum, *A. hygrophilum* AEs (10, 25, and 50 mg/mL) lacked any marked glucose diffusional hindrances into external solution across dialysis membrane (with respective 8.1 ± 4.1%, 8.8 ± 5.1% AUC % reductions in *A. hygrophilum* 10, 25, and 50 mg/mL overnight incubations, P > 0.05) [Figure 4].

Confirmatory *in vivo* studies: OSTT and OGTT

The administration of acarbose 3 mg/kg body weight reduced highly significantly the starch-induced postprandial hyperglycemia at 45, 90, and 135 min after corn starch load at 0 min, thus evoking highly substantial reduction (P < 0.001 vs. untreated animals, n = 6) of the overall glycemic excursion AUC compared to controls. Compared

to control normal rats, administration of *A. hygrophilum* AEs in starch loaded fasting normoglycemic rats did not minimize in overall glycemic excursions, neither did they reduce the acute starch-induced postprandial hyperglycemia at any determination time point. In the OGTT oral administration of *A. hygrophilum* AEs did not evoke any marked improvement of glucose tolerance AUCs in comparison to control determinations respective AUCs contrary to metformin and glipizide therapeutic propensities metformin (300 mg/kg body weight) or glipizide (0.6 mg/kg body weight). Equally *A. dioscoridis* and *A. palaestinum* lacked *in vivo* efficacies in acute carbohydrate tolerance tests performed in overnight fasting normoglycemic animals.[9]

Antiproliferative activity in colorectal cancer cell lines

Like *A. dioscoridis* and *A. palaestinum;*[9] *A. hygrophilum* (25 µg/mL) aqueous extract lacked on antiproliferative efficacies in any of the colorectal carcinomas panel incubations; despite its notable activity at the concentration 200 µg/mL. Doxorubicin respective IC_{50} (µg/mL) values for the tested cell lines were 0.09 ± 0.01 (HT29); 0.11 ± 0.02 (HCT116), and 0.7 ± 0.01 (SW620).

CONCLUSIONS

Flavonoids and phenolic acids are considered valuable in the maintenance of glucose homoeostasis by different mechanisms. In the present study, *A. hygrophilum* AEs exhibited pancreatic MIN6 proliferative propensities. *A. hygrophilum* AEs inhibited crucial gastrointestinal enzymes involved in carbohydrate and lipid digestion and absorption. Our findings with the colon cancer cell lines indicate that traditionally claimed anticancer properties of *A. hygrophilum* have to be pharmacologically evidenced. Still, further studies with other cancer cell lines are warranted.

Acknowledgement

This work was supported by a grant (UJ 1134) of the Deanship of Academic Research, The University of Jordan and the Scientific Research Fund, The Ministry of Higher Education (MPH/1/05/2014).

Financial support and sponsorship

Nil

Conflicts of interest

There are no conflicts of interest

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