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

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**ABSTRACT**

**Background:** *Arum hygrophilum* is a traditional medicinal plant indigenous to Jordan. The present study explores its phytochemistry, antioxidative, antidiabetic, and antiproliferative potentials. **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 quercetin-3-O-rhamnose. 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 PlIC50 (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 IC50 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 quercetin-3-O-rhamnose
- 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

**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.⁴⁻¹⁰ 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.¹³⁻¹⁴ In the Jordanian traditional medicine, the edible *A. palaestinum* species, referred with the common Arabic name “Louf,” are recommended as a natural anticancer agent against colon cancer.¹⁵ Previously, different flavonoids, such as quercetin, apigenin, vitexin, and isoorientin were isolated from *A. palaestinum* in our laboratories and their antimicrobial activities were established.¹⁶ In a recent comparative study in *in vitro* and *in vivo* experiments, pancreatic lipase (PL) and dual α-amylase/α-glucosidase inhibitory potentials were...
demonstrated for the extracts of *A. dioscorides* and *A. palaestinum* as well as for some of their isolated compounds.\[^9\]\[^10\] 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. hygrophilum* 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 voucher 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 coarsely 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.\[^11\]\[^12\]

**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 50-50% solvent B; 50.01-52 min 50-50% 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-picrylhydrazyl (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
Modulatory effects of *Arum 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; \( P < 0.05 \) compared to respective 5.6 mM glucose (positive) control wells.

**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 myotropic 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.\(^{[22,24]}\)

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.\(^{[22,29]}\) For all isolated compounds, melting points and mixed melting points with the reference substances were determined and confirmed.

HPLC-MS analysis of the ethanolic extract revealed in addition to the isolated flavonoids the presence of quercetin-3-O-rhamnose (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.

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 infused 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 insulinoergic 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,

| 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.
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*** |

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. **[36]** 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. **[18]** 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. **[35,39]**

**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 ethnomedical 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-IC₅₀ value of 114.0 ± 4.0 ng/mL, equivalent to 0.2 ± 0.0 μM, is comparable to reported PL-IC₅₀ 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-IC₅₀ 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. **[21]** 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 flavonones favor the PL inhibition. **[30-33]** 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 IC₅₀ 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. palaeus*. **[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 glyceremic 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 IC50 (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 homeostasis 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.

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Conflicts of interest

There are no conflicts of interest

REFERENCES

1. Afifi-Yazar FU, Kasabri V, Abu-Dahab R. Medicinal plants from Jordan in the treatment of diabetes: traditional uses vs. in vitro and in vivo evaluations-Part 2. Planta Med 2011;77:1210-20.
2. Afifi FU, Wazafy M, Jabr M, Treish E. The use of herbal preparations as complementary and alternative medicine (CAM) in a sample of patients with cancer in Jordan. Complement Therap Clin Pract 2010;16:208-12.
3. Wang CZ, Calway T, Yuan CS. Herbal medicines as adjuvants for cancer therapeutics. Am J Chin Med 2012;40:657-69.
4. Tschop MH, DiMarchi RD. Outstanding scientific achievement award lecture 2011: defeating diabetes: the case for personalized combinatorial therapies. Diabetes 2012;61:1309-14.
5. Rani N, Sharma SK, Vasudeva N. Assessment of antiobesity potential of Azharrhynchos aspera Linn.seed. Evid Based Complement Alternat Med 2012;2012:715912.
6. Sun X, Zhang K, Ji X, Wang Y, Jeffrey Z, Tong Y, et al. Screening of pancreatic lipase and alpha-glucosidase inhibitors from Chinese dietary herbs. Zhongguo Zhong Yao Za Zhi 2012;37:1319-23.
7. Abu-Imaleh B, Afifi F. Treatment with medicinal plants in Jordan. Dirasat 2000;27:53-74.
8. Afifi FU, Sherervington A, Darwish R. Phytochemical and biological evaluation of Arum palaestinum Part 1: Flavone-C-glycosides. Acta Technolog Legis Medicament 1997;VIII:105-11.
9. Afifi F, Kasabri V, Litescu SC, Abaza IF. In vitro and in vivo comparison of the biological activities of two traditionally and widely used Arum species from Jordan: Arum diasoridis Sibth and Sm and Arum palaestinum Boiss. Nat Prod Res 2015;1-10.10.1080/14786419.2015.1072713.
10. Karahan F, Kulak M, Uru E, Gozucuk HG, Boyumey T, Serengil Y, et al. Total phenolic content, ferric reducing and DPPH scavenging activity of Arum diasoridis. Nat Prod Res 2014;29:1678-83.
11. Hamdan II, Afifi FU. 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;93:117-21.
12. Cristea V, Deliu C, Oltean B, Keul A, Brummer A, Abu C, et al. Soilless cultures for pharmaceutical use and biodiversity conservation. Acta Horticult 2009;843:157-64.
13. Hammam HM, Matar SA, Litescu SC, Abuhamedah S, Al Jaber HL, Afifi FU. Biological activities of the hydro-alcoholic and aqueous extracts of Achillea fragrantissima (Forssk.) grown in Jordan. Nat Sci 2014;6:23-30.
14. Litescu SC, Eremia S, Rudu GL. Methods for the determination of antioxidant capacity in food and raw materials. Adv Exp Med Biol 2010;698:241-9.
15. Kasabri V, Abu-Dahab R, Afifi FU, Naffa N. Modulation of pancreatic MIN6 insulin secretion and proliferation and extrapancreatic glucose absorption with Achilles santolina. Eryngium creticum and Pistacia atlantica extracts: In vitro evaluation. J Exp Integ Med 2012;2:245-54.
16. Habtemariam S. The antiobesity potential of sigoimoid A. Pharm Biol 2012;50:1519-22.
17. Bustanji Y, Issa A, Mohammad M, Hudak M, Tawaha K, AlKharih H, et al. Inhibition of hormone sensitive lipase and pancreatic lipase by Rosmarinus officinalis extract and selected phenolic constituents. J Med Plant Res 2010;4:2235-42.
18. Foddia M, Kasabri V, Pretetro GL, Azara E, Sias A, Afifi FU, et al. In vitro inhibitory effects of Limonium contortifolium (Mabile) Erben and Limonium virgatum (Wildt) Fourr extracts from Sardinia on α-amylase, α-glucosidase and pancreatic lipase. Nat Prod Commun 2014;9:181-4.
19. Gallagher AM, Flatt PR, Duggy G, Abdel-Wahab YHA. The effects of traditional antidiabetic plants on in vitro glucose diffusion. Nutr Res 2003;23:413-24.
20. Kasabri V, Afifi FU, Hamdan II. In vitro and in vivo acute antihyperglycemic effects of five selected indigenous plants from Jordan used in traditional medicine. J Ethnopharmacol 2011;133:888-96.
21. Abu-Dahab R, Kasabri V, Afifi FU Evaluation of volatile oil composition and antiproliferative activity of Laurus nobilis L. (Lauraceae) on breast cancer cell line models. Rec Nat Prod 2014;8:136-47.
22. Al-Esawi DM List of Jordan vascular plants. Mtt Bot München 1982;18:173-82.
23. Afifi FU, Khalil E, Abdalla S Effect of isorientin isolated from Arum palaestinum on uterine smooth muscle of rats and guinea pigs. J Ethnopharmacol 1999:65:173-7.
24. Abu-Dahab R, Afifi FU Aniproliferative activity of selected medicinal plants of Jordan against a breast adenocarcinoma cell line. Sci Pharm 2007;75:121-36.
25. Aggrawal PK Carbon-13 NMR of flavonoids. In Studies in Organic Chemistry, Vol.39. Amsterdam: Elsevier; 1989:320-21, 329-31.
26. Nawwar MAM, El-Mousallamy AMD, Barakat HH, Buddrus J, Linscheid M Flavonoid lactates from leaves of Marrubium vulgare. Phytochemistry 1989;28:32016.
27. Harborn J.B The flavonoids: Advances in Research Since 1986. London: Chapman and Hall; 1994. 448-50.
28. Zhang Y, Zhao J, Liu C, Wu X, Zhang Y. Isolation and purification of four flavone C-glycosides from antioxidant of bamboo leaves by macroporous resin column chromatography and preparative high-performance liquid chromatography. Food Chem 2008;107:1326-36.
29. Kasabri V, Flatt PR, AbdelWahab Y In vitro modulation of pancreatic insulin secretion and extrapancreatic insulin action, and peptide glycation by Curcuma longa aqueous extracts. J Exp Integ Med 2014;4:187-93.
30. List JF, Habener JF. Glucagon-like peptide 1 agonists and the development of and growth of pancreatic β-cells. Am J Physiol Endocrinol Metab 2004;286:E875-81.
31. Bucholtz T, Melzig MF. Polyphenolic compounds as pancreatic lipase inhibitors. Planta Med 2015;81:771-83.
32. Lee EM, Lee SS, Chung BY, Cho JY, Lee IC, Ahn SR, et al. Pancreatic lipase inhibition by C-glycosidic flavones isolated from Eremonchloa ophiuroides. Molecules 2010;15:8251-9.
33. Shimura S, Itoh Y, Yamashita A, Kitano A, Hatano T, Yoshida T, et al. Inhibitory effects of flavonoids on lipase. Nippon Shokuhin Kogyo Gakkashi 1994;41:947-50.
34. Dalar A, Türker M, Zabaras D, Konczak I. Phenolic composition, antioxidant and enzyme inhibitory activities of Eryngium Bornmuelleri leaf. Plant Foods Hum Nutr 2014;69:30-6.

35. YHS Wu, Chiu CH, Yang DJ, Lin YL, Tseng JK, Chen YC. Inhibitory effects of litchi (Litchi chinensis Sonn.) flower-water extracts on lipase activity and diet-induced obesity. J Funct Foods 2013;5:923-9.

36. Chuang CM, Wang HE, Peng CC, Chen KC, Peng RY. Hypolipidemic effects of different angiocarp parts of Alpinia zerumbet. Pharm Biol 2011;49:1257-64.

37. Butt MS, Ahmad A, Sharif MK. Influence of pectin and guar gum composite flour on plasma biochemical profile of streptozocin-induced diabetic male albino rats. Int J Food Prop 2007;10:345-61.