Metabolomics and chemometrics approaches to unravel the metabolic diversity and in-vitro antidiabetic potential of two Ziziphus species

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Introduction

Ziziphus, commonly known as sider or ber, is renowned in traditional medicine for its diverse pharmacological properties. *Z. lotus* has a long history of use as antidiabetic and analgesic plant while, *Z. spina-christi* was used in folk medicine as antibacterial, antidiabetic, and antinociceptive **[1]**. Phytochemical studies on Z. lotus demonstrated the presence of flavonoids and saponins alkaloids and tannins, while metabolic profiling of *Z. spina-christi* showed predominance of glycosides, resins, saponin glycosides **[2]**. Extensive research into their nutrients and polyphenolic compounds is required as they have exhibited insulin-like effects in glucose metabolism and served as potent inhibitors of key enzymes like α-amylase and α-glucosidase that are commonly upregulated in type 2 diabetes. In spite of the various hypoglycemic activity testing studies carried out on some *Ziziphus* species, scarce information are available concerning the hypoglycemic activity of *Z. spina-christi*. This study hypothesis include that the metabolic variation in different organs of *Z. spina-christi* and *Z. lotus* will reveal diverse bioactive compounds with potential anti-diabetic properties.

Materials and Methods

Plant samples collection and preparation:

Z. lotus and Z. spina-christi leaves, seeds and mature fruits were collected from Sohag governorate, Egypt in 2022. The authentication of the sample involved a thorough examination conducted by Professor Sania Ahmad from the Faculty of Science, Alexandria University. Each sample was dried then ground and separately extracted by maceration in 250 mL of 70% ethanol and ultra-sonication for 40 mins at 50 ◦C. Finally, samples were filtered then concentrated under reduced pressure.

Chemical profiling of different Ziziphus extracts using UPLC-MS/MS:

an UPLC XEVO TQD triple quadruple instrument Waters Corporation, Milford, MA01757 U.S.A was used with BEH C18 column (50 mm x 2.1 mm). A flow rate of 0.2 ml/ min and mobile phase of two phases ; Phase A (ultrapure water + 0.1% formic acid) and Phase B (methanol + 0.1% formic acid) were operated. Negative ionization mode was selected using a triple quadrupole (TQD) mass spectrometer coupled to electrospray ionization (ESI) source.

Evaluation of α-amylase, α-glycosidase inhibitory and glucose uptake:

Evaluation of α-amylase, α-glycosidase inhibitory and glucose uptake effects of different Ziziphus extracts was done using previously described methods **[3,4].**

Statistical analyses:

Statistical analyses *was performed* using Metaboanalyst 4.0 was utilized to create hierarchical cluster analysis (HCA) heat maps. Moreover, OPLS-DA and OPLS were constructed using SIMCA-P version 14.0 software (Umetrics, Sweden) to unravel the clustering of samples in relation to their chemical profile and to antidiabetic activity, respectively. Furthermore, coefficient plots were constructed to pick out the biomarkers positively correlated to antidiabetic activity.

Results

1. Annotation of compounds in different Ziziphus extracts:

Base peak chromatograms of the extracts of seeds and ripe fruits in addition to leaves' extracts of Z. lotus and Z. spina-christi showed the presence of total of 50 compounds representing different chemical classes such as, amino acids, alcohol glycosides, phenolic acids, flavonoids, cyclopeptide alkaloids, aporphine alkaloids, isoquinoline alkaloids, triterpenes, and fatty acids.

2. Comparative chemical profiling of the different organs of Z. lotus and Z. spina-christi using UPLC-MS-multivariate data analyses

To establish an accurate depiction of the relationships among samples, an orthogonal projection to latent structures-discriminant analysis (OPLS-DA) model was developed. In the score scatter plot **(Fig. 1)**, the leaves samples of both species clustered on the negative side of LV1, while the fruits and seeds samples of both species clustered on the positive side of LV1. By analyzing the coefficients plots, the flavonoidal glycosides quercetrin, rutin and diferuloylspinosin, the chalcone di-C-glycosylphloretin, the triterpenoidal compounds jujubasaponin I, jujubogenin, betulinic acid and colubrinic acid were the main metabolites which were positively correlated to the discrimination of the leaves samples. Meanwhile, arginine, lysine, ferulic acid and caffeic acid and the alkaloids integerrenine and apetaline A were the metabolites positively associated with the discrimination of mature fruits . Finally, xylopyrine H, amphibine, B, Daechuine S7, nummularine T, mauritine A and sativanine A and the fatty acids linoleic acid, oleic acid and margaroleic acid were positively correlated to the discrimination of seeds samples

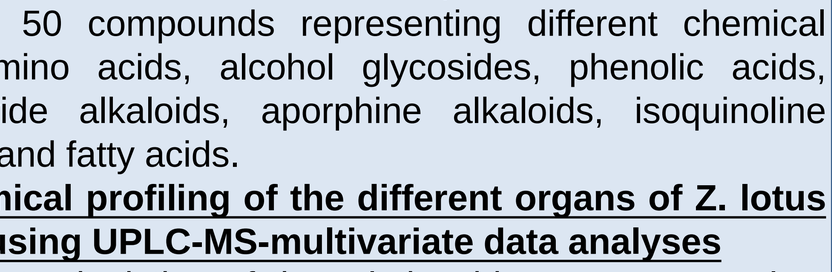

Fig 1: OPLS-DA score scatter plot of leaves, seeds and fruits of the studied Ziziphus samples

3. In-vitro α-amylase and α-glycosidase inhibitory effect of extracts and their effect on glucose reuptake:

As depicted in **Fig. 3,** the extracts from Z. spina-christi leaves and fruits displayed the lowest IC50 values (1.296 mg/mL). Regarding inhibition of α-amylase enzyme, fruits of Z. lotus exhibited significant inhibition scoring an IC50 value of 4.98 mg/mL. Moreover, all extracts except Z. lotus seeds (LS), provoked higher glucose utilization in hepatic cells than the positive control, berberine.

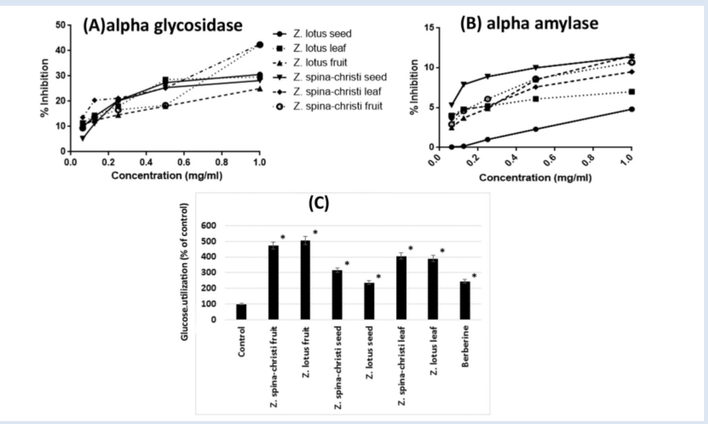

Fig 3: The dose-response curves of the tested samples against alpha glycosidase (A) and alpha amylase (B). The Effect of Ziziphus samples on glucose uptake using HepG2 cells (C)

4. Multivariate statistical analysis for prediction of the metabolites with putative in-vitro anti-diabetic activity in the tested Ziziphus samples:

The OPLS model biplot **(Fig. 4A)** showed that clear discrimination was revealed between mature fruits and leaves, with mature fruits clustering along the negative side of LV1 and showing spatial correlation with α-glucosidase inhibitory and glucose uptake activities.The results indicated that while fruits and leaves impose their in-vitro antidiabetic activity through α-glucosidase inhibition and glucose uptake activities, the seeds act mainly through inhibition of the enzyme α-amylase. The coefficients plot **(Fig.4B-D)** depicted that caffeic acid, ferulic acid and hemsine A were positively correlated to the inhibition of α-amylase enzyme while caffeic acid, ferulic acid and betulinic acid were revealed to be positively correlated to α-glucosidase inhibitory activity. On the other hand, zizyotin, O-desmethylspinorhamnoside, apetaline, quercetrin, jujubogenin, hemsine A, and zizyphursolic acid positively contributed to glucose uptake activity.

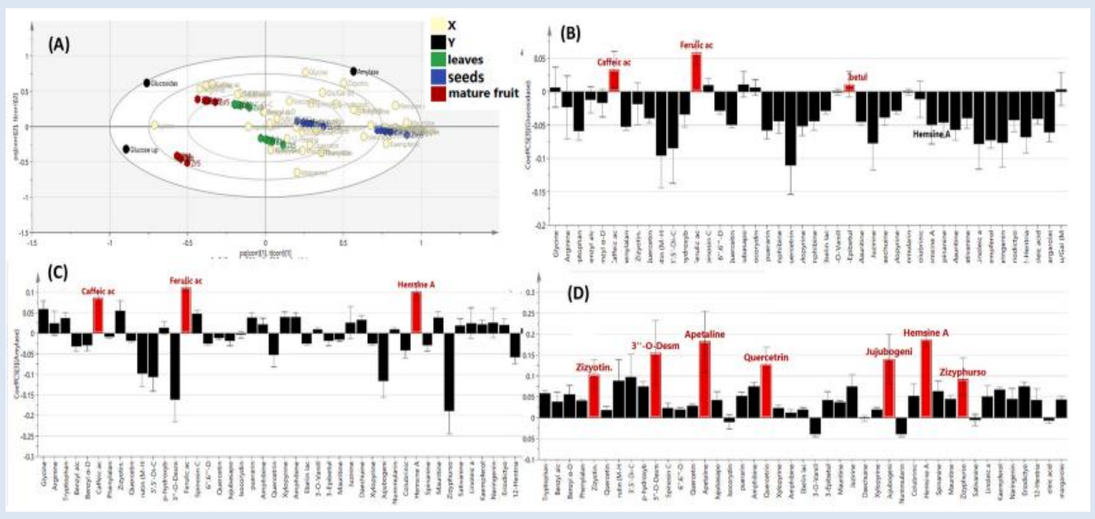

Fig. 4. OPLS biplot of the tested samples in relation to the alpha amylase, alpha glycosidase inhibitory and Glucose Uptake activities (A). Correlation analysis of the identified metabolites to the alpha amylase (B) alpha glucosidase (C) inhibition and Glucose Uptake (D) activities.

Conclusions

The analysis demonstrated variations in the chemical composition of different plant parts, with amino acids predominantly found in fruits, cyclopeptide alkaloids in seeds, and flavonoidal glycosides in leaves. The in-vitro assays evaluating the inhibitory effects of the Ziziphus extracts on α-amylase and α-glycosidase enzymes demonstrated dose-dependent suppression, with Z. spina-christi extracts exhibiting the lowest IC50 values. The glucose uptake assays in HepG2 cells revealed increased utilization in cells treated with Z. lotus extracts, indicating their potential in promoting glucose uptake. Multivariate statistical analysis provided clustering patterns based on the chemical profiles of the different plant parts, highlighting the significance of each metabolite in distinguishing sample classes. Compounds such as caffeic acid, ferulic acid, betulinic acid, jujubogenin, and quercetrin were identified as potential bioactive compounds associated with anti-diabetic activities. Nonetheless, these findings are just a starting point where extensive in-vivo experiments are required in the future search to afford deep understanding to their molecular and pharmacological mechanisms of action as anti-diabetic drugs. Further investigations into the isolation, identification, and characterization of these bioactive compounds are warranted for their future development as natural anti-diabetic agents.

References

[1] Alsayari, A., Wahab, S., 2021. Genus Ziziphus for the treatment of chronic inflammatory diseases. Saudi J. Biol. Sci. 28, 6897–6914. https://doi.org/10.1016/j. sjbs.2021.07.076.

[2] El Maaiden, E., El Kharrassi, Y., Moustaid, K., Essamadi, A.K., Nasser, B., 2019. Comparative study of phytochemical profile between Ziziphus spina christi and Ziziphus lotus from Morocco. J. Food Meas. Charact. 13, 121–130. https://doi.org/ 10.1007/s11694-018-9925-y.

[3] Odeyemi, Samuel Wale, and Anthony Jiede Afolayan. 2018. "Identification of Antidiabetic Compounds from Polyphenolic-Rich Fractions of Bulbine Abyssinica A. Rich Leaves." *Pharmacognosy research* 10(1): 72–80.

[4] Sun, Wenli, Hesam Mohamad Shahrajabian, and Qi Cheng. 2021. "Fenugreek Cultivation with Emphasis on Historical Aspects and Its Uses in Traditional Medicine and Modern Pharmaceutical Science." *Mini-Reviews in Medicinal Chemistry* 21(6): 724–30. http://www.eurekaselect.com/article/111909.