

Antidiabetic, dermatoprotective, antioxidant and chemical functionalities in Zizyphus lotus leaves and fruits.

Marmouzi, Ilias; Kharbach, Mourad; El Jemli, Meryem; Bouyahya, Abdelhakim; Cherrah, Yahia; Bouklouze, Abdelaziz; Vander Heyden, Yvan; Faouzi, My El Abbas

Published in:
Industrial Crops and Products

DOI:
[10.1016/j.indcrop.2019.02.007](https://doi.org/10.1016/j.indcrop.2019.02.007)

Publication date:
2019

License:
CC BY

Document Version:
Accepted author manuscript

[Link to publication](#)

Citation for published version (APA):

Marmouzi, I., Kharbach, M., El Jemli, M., Bouyahya, A., Cherrah, Y., Bouklouze, A., Vander Heyden, Y., & Faouzi, M. E. A. (2019). Antidiabetic, dermatoprotective, antioxidant and chemical functionalities in Zizyphus lotus leaves and fruits. *Industrial Crops and Products*, 132, 134-139. <https://doi.org/10.1016/j.indcrop.2019.02.007>

Copyright

No part of this publication may be reproduced or transmitted in any form, without the prior written permission of the author(s) or other rights holders to whom publication rights have been transferred, unless permitted by a license attached to the publication (a Creative Commons license or other), or unless exceptions to copyright law apply.

Take down policy

If you believe that this document infringes your copyright or other rights, please contact openaccess@vub.be, with details of the nature of the infringement. We will investigate the claim and if justified, we will take the appropriate steps.

**Antidiabetic, dermatoprotective, antioxidant and chemical
functionalities in *Zizyphus lotus* leaves and fruits**

Ilias Marmouzi^{1,*}, Mourad Kharbach^{2,3}, Meryem El Jemli⁴, Abdelhakim Bouyahya⁵, Yahia Cherrah^{1,2,4}, Abdelaziz Bouklouze², Yvan Vander Heyden³, My El Abbes Faouzi¹

¹ Faculté de Médecine et de Pharmacie, Laboratoire de Pharmacologie et Toxicologie, équipe de Pharmacocinétique, Mohammed University V in Rabat, Rabat, Morocco.

²Pharmaceutical and Toxicological Analysis Research Team, Laboratory of Pharmacology and Toxicology, Faculty of Medicine and Pharmacy, University Mohammed V- Rabat-Morocco

³Department of Analytical Chemistry, Applied Chemometrics and Molecular Modelling, Vrije Universiteit Brussel (VUB), Laarbeeklaan 103, B-1090 Brussels, Belgium

⁴Pharmacodynamy Research Team, ERP, Laboratory of Pharmacology and Toxicology, Faculty of Medicine and Pharmacy, Mohammed V University, BP 6203, Rabat-Instituts, Rabat, Morocco

⁵Laboratory of Human Pathologies Biology, Department of Biology, Faculty of Sciences, and Genomic Center of Human Pathologies, Mohammed V University, Rabat, Morocco

*** Corresponding author:** Ilias Marmouzi; **E-mail:** ilias.marmouzi@gmail.com

Highlights

- Chemical analysis revealed 20 phenolic compounds in *Zizyphus lotus* leaves and fruits.
- *Zizyphus lotus* exhibited antidiabetic activity by means of digestive enzyme inhibition.
- *Zizyphus lotus* demonstrated dermatoprotective effect through tyrosinase inhibition.
- *Zizyphus lotus* leaves presented potent antioxidant properties.

ABSTRACT

Medicinal plants provide important sources of new chemical substances with potential therapeutic effects. They have been used in traditional medicine for the treatment of several diseases. The present study investigated the antidiabetic (anti-amylase and anti-glucosidase) and dermatoprotective (anti-tyrosinase) activities of *Zizyphus lotus* (Rhamnaceae) leaves and fruits. This plant is known for several medicinal applications; the phenolic profile of aqueous extracts of *Z. lotus* was also developed using HPLC-DAD-QTOF-MS analysis. The chemical analysis resulted in the identification and quantification of twenty phenolic compounds in the fruits and/or leaves. *Zizyphus lotus* was highly dosed in gallic acid with 2715 mg/kg in leaves and 15,640 mg/kg in fruits. *Z. lotus* leaves and fruits, demonstrated inhibitory effects against α -amylase (IC₅₀: 20.40–31.91 μ g/mL), and α -glucosidase (IC₅₀: 8.66–27.95 μ g/mL). In tyrosinase inhibition, the fruits exhibited a higher effect (70.23 μ g/mL) than the leaves. Furthermore, the extracts did show interesting antioxidant activity; thus *Z. lotus* constitutes a promising traditional medicine and this study contributed to identify some functional compounds responsible for its bioactivity.

Keywords: *Zizyphus lotus*; leaves, fruits; tyrosinase; glucosidase; phenolic compounds.

1. Introduction

Functional foods and natural products have attracted widespread attention because of their medicinal value. *Zizyphus lotus* (*Z. lotus*), is a widespread traditional medicine, known also as Jujube. Botanically, it belongs to the angiosperm Rhamnaceae family that contains about 135–170 species (Maraghni et al., 2010). *Zizyphus lotus* is a Mediterranean medicinal plant distributed in several Asian countries, such as China, Iran, South Korea; in Africa; and in European countries, such as Cyprus, Spain, Greece (Richardson et al., 2004; Adeli and Samavati, 2015). In Morocco, the plant is known as "Sedra" and spread in many habitats in arid and semi-arid regions (Rsaissi et al., 2013). *Zizyphus lotus* is being used in nutrition, health, and cosmetics in several forms, for example, occurring in honey, tea, jam, juice, oil, loaf, and cake. This plant offers a delicious red fruit that is consumed fresh, dried or processed in gastronomic traditions in different cultures (Abdoul-Azize, 2016). Moreover, several parts are used in traditional medicine for the treatment of diseases, such as insomnia, anxiety, urinary problems, diabetes, skin infections, fever, diarrhea, insomnia, sedative, bronchitis and urolithiasis (Lahlou et al., 2002; Adzu et al., 2003; Yoon et al., 2010; Khouchlaa et al., 2016) Extracts from *Z. lotus* did show pharmacological properties, such as antibacterial (Rsaissi et al., 2013; Ghazghazi et al., 2014), antioxidant (Ghazghazi et al., 2014; Ghalem et al., 2014; Bakhtaoui et al., 2014; Hammi et al., 2015; Adeli and Samavati, 2015), anti-inflammatory and analgesic (Borgi et al., 2007, 2008; El Hachimi et al., 2016), litholytic (Khouchlaa et al., 2017a, b), antidiarrheal (Adzu et al., 2003), antifungal (Lahlou et al., 2002; Ghazghazi et al., 2014) and mollusucidal (Lahlou et al., 2002). Phenolic compounds of *Z. lotus* extracts prevent oxidative stress and inflammation by reducing reactive oxygen species (ROS) (Abdoul-Azize et al., 2013; 2016). Indeed, *Z. lotus* scavenges free radicals preventing lipid

peroxidation and cell damages (Boulanouar et al., 2013; Ghazghazi et al., 2014; Bakhtaoui et al., 2014; Adeli et Samavati, 2015). In vivo, *Z. Lotus* increases the rate of haemolysis and the expression of glutathione reductase enzymes and decreases catalase and glutathione peroxidase activities (Abdoul-Azize, 2016). In fact the root and leaf extracts from *Z. Lotus* demonstrated a hypoglycemic effect in vivo (Abdoul-Azize, 2016). *Zizyphus lotus* fruits contain bioactive compounds, such as glutamic acid, sterols, vitamins, tocopherols, fibers, amino acids, triacylglycerol, fatty acids, carbohydrates, tannins, phenolic acids and flavonoids (Borgi et al., 2008; Ghazghazi et al., 2014; Maciuk et al., 2004). On the other hand, the leaves are particularly rich in phenolic acids, flavonoids, tannins and saponins notably jujuboside B, jujubogenin glycosides, and jujubasaponine IV (Maciuk et al., 2004). Few papers discussed the functional compounds and pharmacological properties of *Z. lotus* leaves and fruit. Thus, the aim of this study was to compare the functional chemistry in leaves and fruit and to evaluate their antioxidant, antidiabetic and dermatoprotective activities.

2. Materials and methods

2.1. Plant material and extraction

Zizyphus lotus fresh leaves and fruits were collected from different trees (5 samples) in the region of Sidi Sliman (Morocco) in Mars 2015, identified and deposited at the herbarium of the laboratory. *Zizyphus lotus* fruits and leaves were dried at room temperature and separately ground into powder. The plant extract was prepared by infusing 200 g of the powdered dried material in 500 mL boiled distilled water for 30 min, to mimic the locals' ethnopharmacy. The extract was filtered, lyophilized and its constituents analyzed using HPLC-DAD-QTOF-MS analysis.

2.2. Phytochemical and HPLC-DAD-QTOF-MS analysis

The HPLC–DAD-QTOF-MS system consisted of a binary pump (G1312 A; Agilent 1100) and an autosampler (G1330B) coupled to a mass spectrometer equipped with an electrospray ionizer source (MS; ESI-; Micromass Quattro Micro; Waters, Milford, MA, USA). Reversed phase HPLC separation was carried out using a Zorbax C18 column (100 mm x 2.1 mm x 1.7 μ m). The mass spectrometer was operated in negative ion mode with the following parameters: capillary voltage, 3.0 kV; cone voltage, 20 V; and extractor, 2 V. Source temperature was 100 °C, desolvation temperature 350 °C, cone gas flow was 30 L/h, and desolvation gas flow 350 L/h. The mobile phase components were 0.1% aqueous formic acid (A) and acetonitrile with 0.1% formic acid (B). The mobile phase gradient was: 0 min, 90% A; at 18 min, 30% A; 18–23 min, 30% A; at 25 min, 90% A; 25–30 min, 90% A. The injection volume was 10 μ L and the column temperature 35 °C. The flow rate of the mobile phase was 0.5 mL/min. The phenolic acids, flavanols and flavonols were identified on the basis of their retention times, MS spectra and molecular-ion properties/masses.

2.3. Antidiabetic activities

2.3.1. α -amylase Inhibitory Assay

The α -amylase inhibitory potential was investigated by reacting different concentrations of the extracts with α -amylase enzyme and starch solution (Marmouzi et al., 2016). A mixture of 250 μ L sample and 250 μ L 0.02 M sodium phosphate buffer (pH = 6.9) containing α -amylase (240 U/mL) was incubated at 37 °C for 20 min. Then, 250 μ L 1% starch solution in 0.02 M sodium phosphate buffer (pH = 6.9) was added to the reaction mixture, which was incubated at 37 °C for 15 min. Thereafter, 1.0 mL dinitrosalicylic acid (DNS) was added, and the mixture incubated in a boiling water bath for 10 min. Then, the

reaction mixture was diluted by adding 2.0 mL distilled water, and the absorbance measured at 540 nm. Acarbose was used as positive control.

2.3.2. α -glucosidase Inhibitory Assay

The α -glucosidase inhibitory activity of the extracts was determined using the substrate p-nitrophenyl β -D-glucopyranoside (pNPG). Briefly, a mixture of 150 μ L sample and 100 μ L 0.1 M sodium phosphate buffer (pH = 6.7) containing α -glucosidase (0.1 U/mL) was incubated at 37 °C for 10 min. After preincubation, 200 μ L 1 mM pNPG solution in 0.1 M sodium phosphate buffer (pH = 6.7) was added. The reaction mixture was incubated at 37 °C for 30 min. After incubation, 1.0 mL 0.1 M Na₂CO₃ was added and the absorbance determined at 405 nm. The α -glucosidase inhibitory activity was expressed as percentage inhibition, and the 50% inhibitory concentration (IC₅₀) determined. Acarbose was used as positive control.

2.4. Dermatoprotective effect

The tyrosinase inhibitory activity was used to evaluate the dermatoprotective effect and determined according to the method described by Batubara et al. (2010). Briefly, the sample (25 μ L) was added to the tyrosinase solution (100 μ L, 333 unit/mL in phosphate buffer 50 mM, pH 6.5) and kept at 37 °C for 10 min. Then, 300 μ L substrate (L-DOPA, 5 mM) was added. After 30 min incubation at 37 °C, the absorbance was determined at 510 nm. The percent inhibition of the tyrosinase activity was determined at sample concentrations of 40, 60, 120 and 160 μ g/mL, and IC₅₀. Quercetin was used as a positive control.

2.5. Antioxidant activity

2.5.1. 2,2-diphenyl-1-picrylhydrazyl radical scavenging activity assay

The radical scavenging activity of the extracts was measured using the stable radical DPPH (2,2-diphenyl-1-picrylhydrazyl) (Blois, 1958). A solution of DPPH (0.2 mM) was prepared and 0.5 mL was mixed with 2.5 mL extract (1.33–1.66 µg/mL). The reaction mixture was vortexed thoroughly and left in the dark at room temperature for 30 min. The absorbance of the mixture was measured at 517 nm and the results expressed as trolox equivalents relative to a calibration curve.

2.5.2. 2,2-diphenyl-1-picrylhydrazyl radical scavenging activity assay

The ability of our extracts to scavenge the 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) radical was determined according to a described method by Re el al., (Re et al., 1999). A solution of ABTS radical cation (ABTS^{•+}) was prepared by the reaction between 10 mL of 2.0 mM ABTS in H₂O and 100 µL 70 mM potassium persulphate at room temperature in the dark for 24 h. The ABTS^{•+} solution was then diluted with methanol to obtain an absorbance of 0.70 ± 0.02 at 734 nm. Samples were prepared in triplicate by diluting 200 µL extract in 2 mL of the diluted ABTS^{•+} solution and allowed to react for 1 min. The absorbance was recorded at 734 nm. The antioxidant activities of samples were expressed as trolox equivalents.

2.5.3. Ferric reducing/antioxidant power (FRAP) assay

2.5. The reducing power was assayed as earlier described (Marmouzi et al., 2017; Oyaizu, 1986). In brief, the extract (1.0 mL) was mixed with 2.5 mL phosphate buffer (0.2 M, pH 6.6) and 2.5 mL 1% potassium ferricyanide. The mixture was then incubated at 50 °C for 20 min. Then, 2.5 mL trichloroacetic acid (10%) was added to the mixture, which was centrifuged at 3000 rpm for 10 min. Finally, 2.5 mL supernatant was mixed with 2.5 mL distilled water and 0.5 mL FeCl₃ solution (0.1%, w/v). The absorbance was measured at

700 nm. The results were expressed as ascorbic acid equivalent per gram of extract dry weight (mg AAE/g edw).

2.6. Statistical and chemometric analysis

The significance of differences between multiple averages was determined by one-way analysis of variance (ANOVA), followed by a Tukey's post hoc test at 5% significance level. Principal component analysis (PCA), an exploratory technique, was performed with the PLS_Toolbox 8.1 software, (Eigenvector).

3. Results

3.1. Chemical composition

The chemical composition was determined using HPLC-DAD-QTOF-MS analysis. Table 1 summarizes the results obtained. Twenty phenolic compounds were identified and quantified. The t-test analysis revealed significant differences ($p < 0.05$) between leaves and fruits for all measured phenolic compounds (Table 1). Gallic acid was the major component of fruits and leaves: $15,640 \pm 310$ mg/kg and 2715 ± 112 mg/kg, respectively. Similarly, ferulic and vanillic acids, rutin, catechin and epicatechin were much more abundant in fruits than leaves. On the other hand, pyrogallol, naringin, chlorogenic, caffeic, syringic, p-coumaric, sinapic, salicylic, rosmarinic acids were more elevated in leaves than fruits extract's (Table 1).

3.2. Antidiabetic activity

The antidiabetic activity was evaluated by two in vitro anti-enzymatic methods: the α -amylase and α -glycosidase inhibition assays. The α -amylase catalyzes the cleavage of α -(1-4) glycosidic binding to dextrin, maltose or maltotriose. Generally, incubation of the α -amylase with its substrate leads to the generation of maltose. In our study, the addition of *Z. lotus* leaves and fruits extracts significantly inhibited the generation of sugars in a dose-

dependent manner, and was compared to acarbose (α -amylase and α -glucosidase inhibitor). Table 2 summarizes the inhibitory effect (IC_{50}) of *Z. lotus* extracts and acarbose on α -amylase activity. The extracts from leaves and fruits of *Z. lotus* showed higher inhibiting activities against α -amylase than acarbose, with the respective IC_{50} values : $20.40 \pm 1.30 \mu\text{g/mL}$ and $31.91 \pm 1.53 \mu\text{g/mL}$. The acarbose effect was characterized by an IC_{50} of $396.42 \pm 3.54 \mu\text{g/mL}$. On the other hand, α -glucosidase catalyzes the hydrolysis of 1-4 linked α -glucose and generates glucose molecules. *Zizyphus lotus* demonstrated a potent α -glucosidase inhibiting activity when compared to the acarbose (Table 2). Indeed, the IC_{50} inhibition values of *Z. lotus* leaves and fruits were $8.66 \pm 0.62 \mu\text{g/mL}$ and $27.95 \pm 2.45 \mu\text{g/mL}$ respectively, while the acarbose IC_{50} value was $199.53 \pm 2.45 \mu\text{g/mL}$.

3.3. Dermatoprotective effect

During skin aging, the derm loses its tensile strength, simultaneously roughness and dryness arise along with abnormalities such as hypo- or hyper-pigmentation. Tyrosinase inhibitors are strategic agents in hyperpigmentation therapy. The targeted enzyme catalyzes the first two steps in mammalian melanogenesis. It is a glycosylated, and copper-containing oxidase, which catalyze the oxidations of monophenols and o-diphenols into reactive o-quinones. To evaluate the dermatoprotective effect of our extracts, the *in vitro* inhibition of tyrosinase was evaluated as a functional assay. Fig. 1 summarizes the percentage of the enzyme inhibition *versus* the concentration of *Z. lotus* extract. The inhibitory activity increased with increasing concentration, *i.e.* from $40 \mu\text{g/mL}$ to $160 \mu\text{g/mL}$. To compare the obtained results, the IC_{50} values of the enzyme inhibition were calculated. As summarized in Table 2, *Z. lotus* fruits extract showed higher inhibition than the extract from leaves; the IC_{50} were $70.23 \pm 5.94 \mu\text{g/mL}$ and $129.11 \pm 9.40 \mu\text{g/mL}$, respectively. Surprisingly, both

extracts demonstrated higher efficacy compared to the reference compound used in our assays (Quercetin). Quercetin exhibited an IC₅₀ of 246.90 ± 1.90 µg/mL.

3.4. Antioxidant activity

The antioxidant activity was assessed using three in vitro antioxidant methods: the DPPH, ABTS, and FRAP assays. The results are expressed in trolox equivalents for the DPPH and ABTS assays, and by ascorbic acid equivalent for FRAP (Table 3). As shown in Table 3, the *Z. lotus* leaves extracts showed the highest antioxidant capacities, compared to the fruits in the three tests. Significant differences ($p < 0.05$) were registered in favor of the leaves in all assays: DPPH, ABTS and FRAP.

3.5. Chemometric exploratory data analysis of *Z. Lotus* extracts

Comparative studies of phytochemical composition are usually carried out applying the univariate procedures (component to component, or sample to sample). However, principal component analysis (PCA) as a multivariate technique can allow further interpretation of the results. Principal component analysis is an unsupervised technique to explore relationships between different samples (extracts) characterized by given profiles, occasionally as metabolic fingerprints (Cuadros-Rodríguez et al., 2016). Principal component analysis is a useful technique in data exploratory analysis to visualize the variability in the data set, to reveal the important variables, as well as to identify outliers, to observe clusters in the data set, or to detect biomarkers. Principal component analysis defines latent variables that capture the largest remaining variation in the data (Bro and Smilde, 2014).

Principal component analysis is a visualization technique whose main goal here is to visualize the relationships existing between the variables (phenolic acids) and their variability in the pharmacological properties, determined in the two matrices (leaves and fruits extracts). Principal component analysis was applied on a data set composed of 10 samples (5 from leaves and 5 from fruits) and 20 phenolic-acid variables from the leave and fruit extracts. The PCA results are displayed in a graphical manner as “scores” and “loadings” plots. The scores and loading as the first two principal components (PC1 vs PC2) are shown in Fig. 2 and explain about 98% of the total variability based on the auto-scaled data. The score plot (Fig. 2a) shows a clear discrimination between the two clusters, cluster 1 included *Z. Lotus* leaves samples and cluster 2 fruits samples. The results show that samples from fruits or leaves were easily identified and discriminated. The loading plot indicated the phenolic variables characterizing the discrimination between both groups of samples (Fig. 2b). In addition, PCA was performed in order to identify phenolic biomarkers, which contribute to sample group separations between the leave and fruit extracts of *Z. Lotus*. It can be concluded from the PCA results that the phenolic molecules presented different distributions in the leaves and fruits extracts.

4. Discussion

The biosynthesis of secondary metabolites in a plant varies between organs and tissues. This synthesis regulation is mediated, essentially, by several epigenetic factors, such as DNA methylation and histone modifications (Vriet et al., 2015; Avramova, 2015). Previous studies revealed the rich bioactive compounds content in *Z. lotus* extracts (Ghazghazi et al., 2014; Borgi et al., 2008; Maciuk et al., 2004), and their pharmacological properties, such as antidiabetic and hypoglycemic effects (Maciuk et al., 2004; Bakhtaoui et al., 2014). The fluctuations in *Z. lotus* extracts across reported experimental results could be

attributed to several factors that affect the synthesis and secretion of phenolic compounds, such as phenological stage, climatic conditions, methods of extraction, used parts and the geographical location (Rodrigues et al., 2013; Karray-Bouraoui et al., 2009; Alipor and Saharkhiz, 2016; Abdoul-Azize, 2016). In our study, the differences in antioxidant activity registered between fruits and leaves is apparently linked to the variability in the contents of functional compounds. On the other hand, the variability between antioxidant assays can be explained by the mechanisms the compounds will react, with the used radicals. The antioxidant effects of *Z. lotus* extracts have also been investigated in previous studies (Bakhtaoui et al., 2014; Adeli and Samavati, 2015; Khouchlaa et al., 2017a). Our study is in accordance with the results obtained in previous works with some variability that may due to the difference in chemical composition (Espinosa et al., 2015). The fluctuation in compounds contents may be responsible of the variability between fruits and leaves in the obtained results. In a earlier report (Abderrahim et al., 2017), the antioxidant activity expressed by ascorbic acid equivalent was significantly higher in *Z. lotus* stem extract (480.20 ± 40.64 mg/g extract) compared with *P. harmala* seed extract (240.32 ± 50.56 mg/g extract). *Z. lotus* leaves exhibited the best activity in DPPH and ABTS assays compared with fruit extract conducted by Ghazghazi et al., 2014. Furthermore, the antioxidant capacity of *Z. lotus* pulp extracts, expressed as ascorbic acid equivalents (Ghalem et al., 2018), showed that polyphenol extract had the highest antioxidant capacity (0.704 ± 0.011 mg/mg extract) compared with the tannins, flavonoids-containing butanol phase, and the ethyl acetate phase containing flavonoids.

Regarding the antidiabetic activity, evaluated by two in vitro assays, IC₅₀ values of *Z. lotus* leaves and fruit extracts, against α -amylase and α -glucosidase, demonstrated a higher effect of the extracts compared than the standard drug acarbose. This latter is a potent inhibitor of α -glucosidase and α -amylase. However, several side effects are related to the use of acarbose (Nakhaee and Sanjari, 2013). For instance it provokes diarrhea by excessive inhibition of the amylase enzyme in the gastrointestinal tract (Kast, 2002). The excessive inhibition of pancreatic amylase can lead to abnormal bacterial fermentation of carbohydrate foods in the colon, which may lead to adverse digestive disorders (Kast, 2002; Apostolidis et al., 2007). Other studies have reported hepatotoxicity and hepatic injury (Andrade et al., 1998; Fujimoto et al., 1998) and elevation of liver enzyme levels (Gentile et al., 1998) resulting from acarbose intake. Medicinal plants may have high potency and less adverse effects than existing drugs (Shimada et al., 1992; Mahomoodally, 2013). For this reason, research is continuously conducted to find alternatives source from medicinal plants as treatment for type 2 diabetes. In fact, nutritional plants are of interest, because they are used daily and their pharmacological properties could be considered as nutraceuticals (Cencic and Chingwaru, 2010). *Zizyphus lotus* leave and fruit extracts are rich in phytochemical constituents (e.g, phenols, flavonoids, and vitamin C). *Zizyphus lotus* compounds could be helpful in the management of diabetes and could decrease the risk of other diabetes related chronic disorders. Previous studies have revealed the antidiabetic effects of *Z. lotus* roots and leaves extract on animal models and this effect was attributed to vitamin A (Abdel-Zaher et al., 2005). Indeed, Jeyakumar et al. (2011) have reported that vitamin A improve the sensitivity toward insulin via the activation of insulin receptor. The actual study revealed for the first time a possible mechanism of the antidiabetic activity attributed to *Z. lotus* leaves and fruits extracts.

The tyrosinase inhibitory assay is the commonly in vitro method to reveal to the dermatoprotective activity of plant extracts and their derivatives. Tyrosinase (monophenol monooxygenase, E:C:1.14. 18.1), known as polyphenol oxidase (PPO), is a copper containing enzyme. The enzyme is involved in the first two steps of melanin biosynthesis (Karioti et al., 2007). Melanin formation by mean of tyrosinase activity is the main cause of enzymatic browning in human skin. Therefore, tyrosinase inhibitors have become increasingly an important dermatoprotective target. The difference in inhibitory effects between fruit and leaves extracts of *Z. lotus* is certainly due the difference in chemical functional compounds. *Zizyphus lotus* fruits extract is rich in phenolic compounds such as catechin, gallic acid and rutin. These compounds are known inhibitors of tyrosinase (Karioti et al., 2007). At the best of our knowledge, no previous study reported dermatoprotective effect of *Z. lotus*. Our findings revealed that the antioxidant activity correlates positively with the strong anti-tyrosinase effect. Such observations regarding the antioxidant and anti-tyrosinase activities of plants extracts (Masuda et al., 2005) has been reported in several studies.

5. Conclusion

This is the first attempt to provide a multivariate comparative analysis of the chemical composition and pharmacological (antioxidant, antidiabetic and dermatoprotective) properties of *Z. lotus* leaves and fruits from different trees. The study revealed a moderate antioxidant activity, especially attributed to the leaves extracts. Traditional medicine uses *Z. lotus* for diabetes. In fact, the extracts exhibited a potent in vitro antidiabetic activity compared with the acarbose. Moreover, regarding their potent tyrosinase inhibitory effect, the samples are endowed with a potentially high dermatoprotective activity.

Results from this study discriminated a functional activity of fruits (dermatoprotective) and leaves (antioxidant and antidiabetic).

Conflicts of interest

The authors declare no conflict of interest.

References

- Abdel-Zaher, A. O., Salim, S. Y., Assaf, M. H., Abdel- Hady, R. H., 2005. Antidiabetic activity and toxicity of *Zizyphus spinachristi* leaves. J. Ethnopharmacol, 101, 129-138.
- Abdoul-Azize, S., 2016. Potential Benefits of Jujube (*Zizyphus Lotus* L.) Bioactive Compounds for Nutrition and Health. J. Nutr & Metab, 2016, dx.doi.org/10.1155/2016/2867470.
- Abdoul-Azize, S., Bendahmane, M., Hichami, A., 2013. Effects of *Zizyphus lotus* L. (Desf.) polyphenols on Jurkat cell signaling and proliferation. International Immunopharmacology, 15, 364-371.
- Adeli, M., Samavati, V., 2015. Studies on the steady shear flow behavior and chemical properties of water-soluble polysaccharide from *Zizyphus lotus* fruit. Inter J Biolo Macromol, 72, 580-587.
- Adzu, B., Amos, S., Amizan, MB., Gamaniel, K., 2003. Evaluation of the antidiarrheal effects of *Zizyphus spinachristi* stem bark in rats. Acta Trop, 87, 245-250.
- Alipor, M., Saharkhiz, MJ. 2016. Phytotoxic activity and variation in essential oil content and composition of Rosemary (*Rosmarinus officinalis* L.) during different phenological growth stages. Biocat Agric Biotechnol, 7, 271-278.
- Andrade, RJ., Lucena, M., Vega, JL., et al. 1998. Acarbose-associated hepatotoxicity. Diabetes Care, 21, 2029-2030.
- Apostolidis, E., Kwon, YI., Shetty, K., 2007. Inhibitory potential of herb, fruit, and fungalenriched cheese against key enzymes linked to type 2 diabetes and hypertension. Innov Food Sci Emerg Technol, 8, 46-54.
- Asnaashari, M., Farhoosh, R., Sharif, A., 2014. Antioxidant activity of gallic acid and methyl gallate in triacylglycerols of Kilka fish oil and its oil-in-water emulsion. Food Chem. 15;159:439-44.
- Avramova, Z., 2015. Transcriptional 'memory' of a stress: Transient chromatin and memory (epigenetic) marks at stress-response genes. Plant J, 83, 149-159.
- Bakhtaoui, F.Z, Lakmichi, H., Megraud, F., Chait, A., Gadhi, C.E.A., 2014. Gastro-protective, anti-Helicobacter pylori and, antioxidant properties of Moroccan *Zizyphus lotus* L. J Appl Pharma Sci, 4, 81-87,
- Batubara, I., Darusman, L.K., Mitsunaga, T., Rahminiwati, M., Djauhari, E. 2010. Potency of Indonesian medicinal plants as tyrosinase inhibitor and antioxidant agent. J Biol Sci, 10(2), 138-144.

- Blois, M.S., 1958. Antioxidant determinations by the use of a stable free radical. *Nature*, 181(4617), 1199.
- Borgi, W., Ghedira, K., Chouchane, N., 2007. Antiinflammatory and analgesic activities of *Zizyphus lotus* root barks. *Fitoterapia*, 78, 16-19.
- Borgi, W., Recio, M.C., Rios, J. L., Chouchane, N. 2008. Antiinflammatory and analgesic activities of flavonoid and saponins fractions from *Zizyphus lotus* (L.) Lam., *South Afric J Bot*, 74, 320-324,
- Boulanouar, B., Abdelaziz, G., Aazza, S., Gago, C., Miguel, M.G., 2013. Antioxidant activities of eight Algerian plant extracts and two essential oils," *Indust Crop Prod*, 46, 85-96.
- Bro, R., Smilde, AK., 2014. Principal component analysis. *Analytical Methods*, 6, 2812-2831.
- Cencic, A., Chingwaru, W., 2010. The Role of Functional Foods, Nutraceuticals, and Food Supplements in Intestinal Health. *Nutrients*, 2, 611-625.
- Cuadros-Rodríguez, L., Ruiz-Samblás, C., Valverde-som, L., Pérez-Castano E., Ganzalez-Casado A., 2016. Chromatographic fingerprinting: An innovative approach for food'identification'and food authentication–A tutorial. *Analytica Chimica Acta*, 909, 9-23.
- El Hachimi, F., Alfaiz, C., Bendriss, A., Cherrah, Y., Alaoui, K., 2016. Anti-inflammatory activity of the seed oil of *Zizyphus lotus* (L.) Desf. *Phytothérapie*, DOI 10.1007/s10298-016-1056-1.
- Espinosa, RR., Inchingolo, R., Alencar, SM., Rodriguez-Estrada, MT., Castro, IA., 2015. Antioxidant activity of phenolic compounds added to a functional emulsion containing omega-3 fatty acids and plant sterol esters. *Food Chem*. 182: 95-104.
- Fujimoto, Y., Ohhira, M., Miyokawa, N., Kitamori, S., Kohgo, Y., 1998. Acarbose induced hepatic injury. *Lancet*, 351, 340.
- Gentile, S., Turco, S., Guarino, G., Sasso, FC., Torella, R., 1998. Aminotransferase activity and acarbose treatment in patients with type 2 diabetes. *Diabetes Care*. 22, 1217-1218.
- Ghalem, M. Merghache, S., Belarbi, M., 2014. Study on the antioxidantactivities of root extracts of *Zizyphus lotus* from the western region of Algeria. *Pharmacognosy Journal*, 6, 32-42,
- Ghazghazi, H. Aouadhi, C. Riahi, L. Maaroufi, A. Hasnaoui, B., 2014. Fatty acids composition of Tunisian *Ziziphus lotus* L. (Desf.) fruits and variation in biological activities between leaf and fruit extracts. *Nat Prod Res*, 28, 1106-1110,
- Hammi, K.M., Jdey, A., Abdelly, C., Majdoub, H., Ksouri, R., 2015. Optimization of ultrasound-assisted extraction of antioxidant compounds from Tunisian *Zizyphus lotus* fruits using response surface methodology. *Food Chemistry*, 184, 80-89.
- Jeyakumar, S.M., Vijaya Kumar, P., Giridharan, N.V., Vajreswari, A., 2011. Vitamin A improves insulin sensitivity by increasing insulin receptor phosphorylation through protein tyrosine phosphatase 1B regulation at early age in obese rats of WNIN/Ob strain. *Diabetes, Obesity and Metabolism*, 13, 955-958.
- Karioti, A., Protopappa, A., Megoulas, N., Skaltsa, H., 2007. Identification of tyrosinase inhibitors from *Marrubium velutinum* and *Marrubium cylleneum*. *Bioorg Med Chem*, 15, 2708-2714.
- Karray-Bouraoui, N., Rabhi, M, Neffati, M., Baldan, B, Ranieri, A., Marzouk, B., Lachaal, M., Smaoui, A., 2009. Salt effect on yield and composition of shoot essential

oil and trichome morphology and density on leaves of *Mentha pulegium*. *Indus Crop Prod*, 30, 338-343.

- Kast, RE., 2002. Acarbose related diarrhea: increased butyrate upregulates prostaglandin E. *Inflamm Res*, 51, 117-118.
- Khouchlaa, A., Bouyahya, A., Ait Lahcen, S., Youssef, B., Nadia, D., Tijane, M., 2017a. Phytochemical screening, evaluation of antioxidant activity and litholytic effect of *Zizyphus lotus* l. extracts. *World J Pharma Res*, 6, 1354-1367.
- Khouchlaa, A., El-Mehyaoui, A., Bouyahya, A., Tijane, M., 2017b. Determination of phenol content and evaluation of *in vitro* litholytic effects on urolithiasis of Moroccan *Zizyphus lotus* L. extract. *Phytothérapie*, DOI 10.1007/s10298-017-1106-3.
- Khouchlaa, A., Tijane, M., Chebat, A., Hseini, S., Kahouadji, A., 2016. Enquête ethnopharmacologique des plantes utilisées dans le traitement de la lithiase urinaire au Maroc. *Phytothérapie*, DOI 10.1007/s10298-016-1073-4.
- Lahlou, M., El Mahi, M., Hamamouchi, J., 2002. Evaluation of antifungal and mollusicidal activities of Moroccan *Zizyphus lotus* (L.) Desf]. *Ann Pharm Fr*, 60, 410-414.
- Maciuk, A. Lavaud, C. Thépenier, P. Jacquier, M.-J. Ghédira, K., 2004. Zèches-Hanrot, M., Four new dammarane saponins from *Zizyphus lotus*. *J Nat Prod*, 67, 1639-1643.
- Mahomoodally, MF., 2013. Traditional medicines in Africa: an appraisal of ten potent African medicinal plants. *Evid Based Complement Alternat Med*, 2013, 14.
- Maraghni, M., Gorai, M., Neffati, M., 2010. Seed germination at different temperatures and water stress levels, and seedling emergence from different depths of *Zizyphus lotus*. *South Afri J Bot*, 76, 453-459.
- Marmouzi, I., Saidi, N., Meddah, B., Bouksaim, M., Gharby, S., El Karbane, M., Serragui, S., Cherrah, Y., Faouzi, M.E.A. 2016. Nutritional characteristics, biochemical composition and antioxidant activities of Moroccan Oat varieties. *Food Measure*, 10(1), 156-165.
- Marmouzi, I., El Karbane, M., El Hamdani, M., Kharbach, M., Naceiri Mrabti, H., Alami, R., Dahraoui, S., El Jemli, M., Ouzzif, Z., Cherrah, Y., Derraji, S., Faouzi, M.E.A. 2017. Phytochemical and pharmacological variability in Golden Thistle functional parts: comparative study of roots, stems, leaves and flowers. *Nat Prod Res*, 1-6.
- Masuda, T., Yamashita, D., Takeda, Y., Yonemori, S., 2005. Screening for tyrosinase inhibitors among extracts of seashore plants and identification of potent inhibitors from *Garcinia subelliptica*. *Biosci Biotechnol Biochem*, 69, 197-201.
- Nakhaee, A., Sanjari, M., 2013. Evaluation of effect of acarbose consumption on weight losing in non-diabetic overweight or obese patients in Kerman. *J Res Med Sci*, 18, 391-394.
- Oyaizu, M., 1986. Antioxidative activities of browning reaction prepared from glucosamine. *Jpn. J. Nutr.*, 44, pp.307-315.
- Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M. and Rice-Evans, C., 1999. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free radical biology and medicine*, 26(9-10),1231-1237.
- Richardson, J.E., Chatrou, L.W., Mols, J.B., Erkens, R.H.J., Pirie, M.D., 2004. Historical biogeography of two cosmopolitan families of flowering plants: annonaceae and rhamnaceae, *Philosophical Transactions of the Royal Society B: Biol Sci*, 359, 1495-1508.
- Rodrigues, L., Pova, O, Teixeira, G, Figueiredo, AC, Maldao, M., Monteiro, A., 2013. Trichomes micromorphology and essential oil variation at different

developmental stages of cultivated and wild growing *Mentha pulegium* L. populations from Portugal. *Indust Crop Prod*, 43, 692-700.

- Rsaissi, N., Bencharki, B., Hillali, L., Bouhache, M., 2013. Antimicrobial activity of fruits extracts of the wild jujube "*Zizyphus Lotus* (L.) Desf. *Intern J Scient Engineering Res*, 4, 1521-1528.
- Shimada, K., Fujikawa, K., Yahara, K., Nakamura, T. 1992. Antioxidative properties of xanthan on the autoxidation of soybean oil in cyclodextrin emulsion. *J. Agri. Food Chem*, 40, 945-948.
- Vriet, C., Hennig, L., Laloi, C., 2015. Stress-induced chromatin changes in plants: of memories, metabolites and crop improvement. *Cell. Mol. Life Sci*, 72, 1261-1273.
- Yoon, JI., Al-Reza, SM., Kang, SC., 2010. Hair growth promoting effect of *Zizyphus jujube* essential oil. *Food Chem Toxicol*, 48, 1350-1354.

Table 1. HPLC-DAD-QTOF-MS analysis of phenolic compounds fruits and leaves content (mean \pm SD) in mg/kg; n, number of samples (with two replicates for each measure).

| Compound | Rt (min) | MW (in Da) | Fruits (n = 5) | Leaves (n = 5) |
|---------------------------------------|---------------------|-----------------------|---------------------------------|-----------------------------------|
| 1 Gallic acid | 0.79 | 169.0151 | 15,640 ^a \pm 310 | 2715.45 ^b \pm 112.23 |
| 2 Pyrogallol | 1.00 | 125.0255 | 29.89 ^a \pm 1.32 | 124.20 ^b \pm 5.34 |
| 3 Chlorogenic acid | 1.42 | 353.0885 | 105.60 ^a \pm 3.78 | 398.00 ^b \pm 11.32 |
| 4 Catechin | 1.48 | 289.6523 | 22.81 ^b \pm 2.12 | 0.75 ^a \pm 0.09 |
| 5 Rutin | 1.49 | 609.1461 | 58.87 ^b \pm 5.37 | 1.95 ^a \pm 0.11 |
| 6 <i>p</i>-Hydroxybenzoic acid | 1.59 | 137.0233 | 128.10 ^a \pm 11.21 | 109.58 ^a \pm 8.86 |
| 7 Catechol | 1.78 | 109.0308 | 3.31 \pm 0.51 | nd |
| 8 Caffeic acid | 1.82 | 179.0357 | 56.26 ^a \pm 7.43 | 247.90 ^b \pm 18.43 |
| 9 Vanillic acid | 1.89 | 167.0935 | 254.10 ^b \pm 21.34 | 152.29 ^a \pm 16.80 |
| 10 Epicatechin | 1.95 | 289.0726 | 6.33 ^b \pm 0.65 | 3.33 ^a \pm 0.31 |
| 11 Syringic acid | 2.00 | 197.7523 | 7.70 ^a \pm 0.24 | 41.01 ^b \pm 8.92 |
| 12 <i>p</i>-Coumaric acid | 2.60 | 163.0410 | 40.82 ^a \pm 4.02 | 148.01 ^b \pm 17.65 |
| 13 3-hydroxycinnamic acid | 3.00 | 163.0396 | 37.49 ^a \pm 2.43 | 91.43 ^b \pm 3.11 |
| 14 Ferulic acid | 3.07 | 193.0517 | 29.79 ^b \pm 1.39 | 15.64 ^a \pm 1.62 |
| 15 Sinapic acid | 3.09 | 223.0620 | 1.81 ^a \pm 0.08 | 43.31 ^b \pm 5.60 |
| 16 Naringin | 3.58 | 579.1714 | 5.21 ^a \pm 0.27 | 105.59 ^b \pm 1.97 |
| 17 Salicylic acid | 4.21 | 137.0254 | 1.96 ^a \pm 0.07 | 30.12 ^b \pm 1.98 |
| 18 Rosmarinic acid | 4.31 | 359.2321 | 33.60 ^a \pm 1.23 | 64.13 ^b \pm 2.76 |
| 19 Resveratrol | 4.35 | 227.0721 | 0.43 ^a \pm 0.09 | 0.88 ^b \pm 0.15 |
| 20 Quercetin | 5.56 | 301.0359 | nd | 27.72 \pm 1.66 |

(a to b) in the same row differs significantly at $p < 0.05$, with b expressing the highest value.

Table 2. In vitro antidiabetic and dermatoprotective activities of *Z. Lotus* fruits and leaves

| | α-amylase | α-glucosidase | tyrosinase |
|------------------|------------------------------------|--|--------------------------------|
| Fruits | 31.91 ^b \pm 1.53 | 27.95 ^b \pm 2.45 | 70.23 ^a \pm 5.94 |
| Leaves | 20.40 ^a \pm 1.30 | 8.66 ^a \pm 0.62 | 129.11 ^b \pm 9.40 |
| Acarbose | 396.42 ^c \pm 3.54 | 199.53 ^c \pm 2.45 | – |
| Quercetin | – | – | 246.90 ^c \pm 1.90 |

Data are reported as mean (n = 5) \pm SD (standard deviation). Values in the same column not sharing a common letter (*a* to *c*) differs significantly ($p < 0.05$). *a* indicates the smallest and *c* the highest value.

Table 3. Antioxidant activities of Z. Lotus fruits and leaves

| | Fruits | Leaves |
|-------------|----------------------------|-----------------------------|
| DPPH | 74.87 ^a ± 16.74 | 241.75 ^b ± 17.37 |
| FRAP | 55.30 ^a ± 2.30 | 160.10 ^b ± 2.30 |
| ABTS | 46.31 ^a ± 11.02 | 301.34 ^b ± 8.26 |

Data are reported as mean (n = 5) ± SD. DPPH (2,2-diphenyl-1-picrylhydrazyl) and ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)) are expressed in mg Trolox (TE)/g edw, FRAP (Ferric reducing/antioxidant power) in Ascorbic acid equivalent (AAE)/g edw (extract dry weight). Values in the same row not sharing a common letter (a to b) differ significantly at p < 0.05, with a expressing the smallest value.

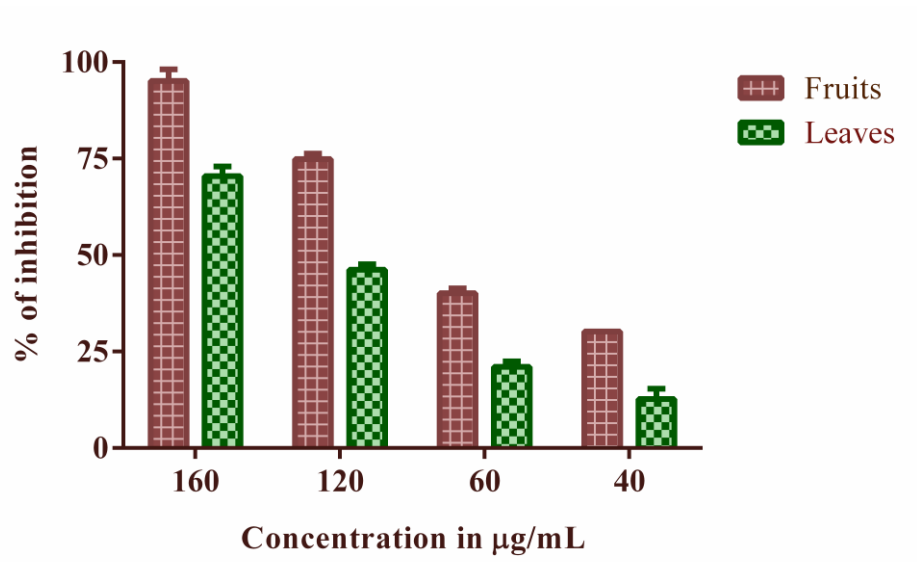


Fig.1. Anti-tyrosinase activity of *Z. Lotus* fruits and leaves.

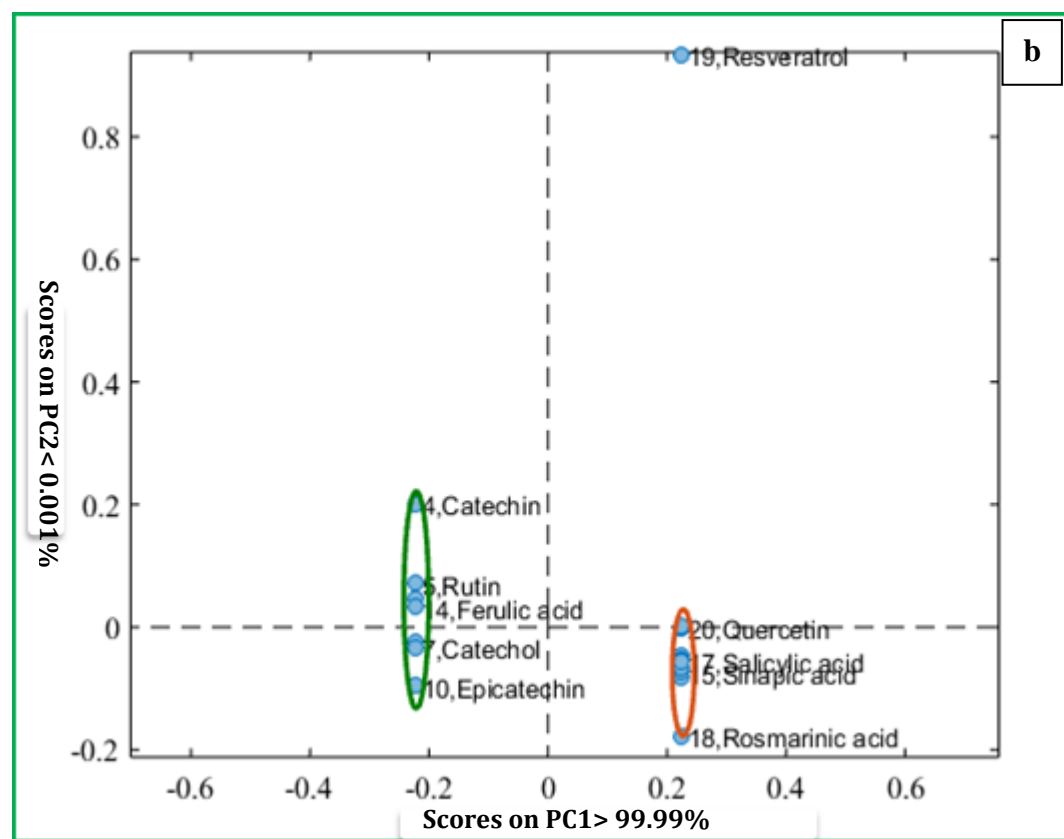
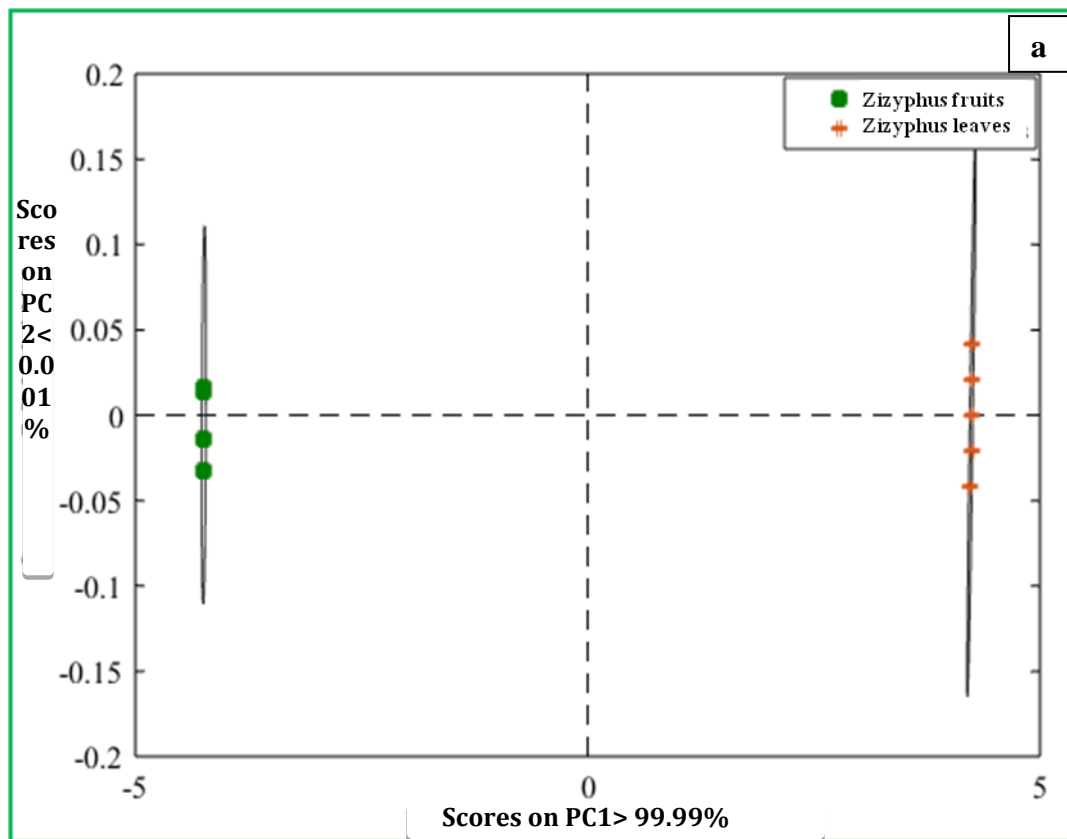


Fig. 2. Principal component analysis (PCA): (a) PC1-PC2 scores plot, and (b) PC1-PC2 loadings plot for Zizyphus lotus fruits and leaves extracts, (measured variables: phenolic acid concentration).