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Phytochemistry / Fitoquímica

Phytochemical composition of *Eriobotrya japonica* (Rosaceae) leaves extracts from central Veracruz, Mexico, and its effect on α-glucosidase enzyme inhibition

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Abstract
Background: *Eriobotrya japonica* is economically significant as an ornamental tree, and its leaves have medicinal properties.
Question: What are the main chemical components of loquat leaves grown in a population of Central Veracruz Mexico? and does the methanolic extract have potential antidiabetic properties through α-glucosidase inhibition?
Species study: *Eriobotrya japonica* (Thunb.) Lindl. (Rosaceae)
Study site and date: Xalapa, Veracruz, Mexico, 2021-2022.
Methods: Total carbon (C) and nitrogen (N) content, phosphorus (P), sodium (Na), potassium (K), calcium (Ca), and magnesium (Mg) were determined in leaves powder. Leaf methanol extract of *E. japonica* was tested for α-glucosidase inhibition. Also, different groups of secondary metabolites were detected by the phenolics/volatile-targeted metabolomic analysis.
Results: The leaves of *E. japonica* are rich in C and minerals such as Na, K, Ca, and Mg, and contain high levels of flavonoids, and procyanidin B2. The leaf methanol extract (LME) effectively inhibited α-glucosidase activity (86.05 ± 0.73 %) *in vitro*. In addition, a leaf petroleum ether extract (LPE) contains mainly phytol, palmitic acid, linoleic acid, stearic acid, and phytol acetate.
Conclusions: The leaf methanolic extract exhibited antidiabetic potential due to its potent α-glucosidase inhibition, and the presence of diverse phenolic compounds, including flavonoids and their glycoside derivatives, and some fatty acids further supports the traditional use of *E. japonica* as an herbal medicine with antidiabetic properties.
Keywords: antidiabetic activity, flavonoids, fatty acids, mass spectrometry.

Resumen
Antecedentes: *Eriobotrya japonica* es de importancia económica como árbol ornamental, y sus hojas tienen propiedades medicinales.
Pregunta: ¿Cuáles son los principales componentes químicos de las hojas de níspero en una población del centro de Veracruz México? ¿Tiene el extracto metanólico propiedades antidiabéticas potenciales a través de la inhibición de la α-glucosidasa?
Especie de estudio: *Eriobotrya japonica* (Thunb.) Lindl. (Rosaceae)
Sitio y año de estudio: Xalapa, Veracruz, México, 2021-2022.
Materiales y métodos: Se determinó el contenido total de carbono (C) y nitrógeno (N), fósforo (P), sodio (Na), potasio (K), calcio (Ca) y magnesio (Mg) en el polvo foliar. Se analizó el extracto metanólico de hoja (LME) de *E. japonica* para determinar la inhibición de la α-glucosidasa. Además, se detectaron diferentes grupos de metabolitos secundarios mediante el análisis metabolómico dirigido a fenólicos/volátiles.
Resultados: Las hojas *de E. japonica* son ricas en C y minerales como Na, K, Ca y Mg, y contienen altos niveles de flavonoides y procianidina B2. El extracto metanólico foliar (LME) inhibió eficazmente la actividad de la α-glucosidasa (86.05 ± 0.73 %) *in vitro*. Además, un extracto de éter de petróleo (LPE) de hoja contiene principalmente fitol, ácido palmítico, ácido linoleico, ácido esteárico y acetato de fitol.
Conclusiones: El extracto metanólico de la hoja exhibió potencial antidiabético debido a su potente inhibición de la α-glucosidasa y la presencia de diversos compuestos fenólicos, incluidos flavonoides y sus derivados glucósidos, y algunos ácidos grasos respaldan el uso tradicional de *E. japonica* como medicina herbaria con efectos antidiabéticos.
Palabras clave: actividad antidiabética; flavonoides; ácidos grasos; espectrometría de masas.

Diabetes mellitus, a major global public health problem, is a common metabolic disorder characterized by abnormally high plasma glucose levels, with serious consequences including retinopathy, diabetic neuropathy, and cardiovascular disease (Kashtoh & Baek 2022). One of the most effective ways to reduce postprandial hyperglycaemia in non-insulin-dependent diabetes, is to reduce the amount of glucose absorbed by inhibiting carbohydrate-hydrolyzing enzymes in the gastrointestinal tract, such as α-glucosidase and α-amylase (Proença *et al.* 2017, Pandhi *et al.* 2020). Among these, α-glucosidase, located in the brush border of jejunal enterocytes, is the most important enzyme in carbohydrate digestion. Inhibitors of α-glucosidase were first introduced in the 1970s (Proença *et al.* 2017), nowadays, approved oral drugs like voglibose, acarbose, and miglitol are employed to inhibit α-glucosidase activity (Proença *et al.* 2017, Dirir *et al.* 2022). Although these inhibitors slow down glucose absorption, undesirable gastrointestinal side effects limit their use (Dirir *et al.* 2022). Based on this, research have been looking for new inhibitors with improved efficacy and minimal side effects (Dirir *et al.* 2022, Lavanya *et al.* 2024). In recent years, many attempts have been made to discover effective α-glucosidase inhibitors from natural sources to build a physiologically functional diet or a lead compound for the treatment of diabetes (Kukavica *et al.* 2024). On this hand, plants are rich sources of secondary metabolites, many α-glucosidase inhibitors have been identified from plants, including alkaloids, flavonoids, anthocyanins, terpenoids, phenolic compounds, glycosides, and others (Kashtoh & Baek 2022). Certain phenolic compounds, particularly flavonoids, found in medicinal plants, have demonstrated the ability to inhibit α-glucosidase enzyme (Lavanya *et al.* 2024). Also, these compounds have been identified as intestinal glucosidase activity inhibitors following *in vivo* and *in vitro* assays (Pereira *et al.* 2011).

Minerals such as Na, K, Ca, Mg and Zn are crucial for human health, as they play vital roles in functions such as fluid balance, muscular function, bone health, and regulation of blood pressure (Schiefermeier-Mach *et al.* 2020). Getting minerals from edible or medicinal plant sources is critical to preventing deficiencies and maintaining a balanced intake, thus reducing the risk of cardiovascular disease, bone problems, and other nutrition-related disorders. Also, for prevention of diabetes mellitus essential vitamins, minerals and amino acids are required (Pathak 2014). This shows that some minerals contained in medicinal plants can be used to treat conditions, and its associated complication caused by diabetes mellitus (Kibiti & Afolayan 2015). For example, Mg supplementation appears to have a beneficial role, improving glucose parameters in people with insulin sensitivity parameters in people at high risk for diabetes (Veronese *et al.* 2016). In diabetes, Zn deficiency appears to be due to hypoglycemia, impaired Zn absorption, and excessive Zn excretion. Diabetic patients have significantly lower mean serum Zn levels compared with healthy controls. Zn supplementation for type-2 diabetics has beneficial effects in elevating their serum Zn level, and in improving their glycemic control (Jayawardena *et al.* 2012). It has been suggested that abnormal Zn metabolism may play a role in the pathogenesis of diabetes and/or its complications. Also, Zn increases the effectiveness of insulin *in vitro* (Shisheva *et al.* 1992). Normal K concentration is necessary for optimal insulin secretion, and deficiency of K causes diabetic acidosis (Narendhirakannan *et al.* 2005). Depletion of K can result in reduced glucose tolerance. The Na and K ions play an important role in the diseases related to renal disorders (Narendhirakannan *et al.* 2005).

Eriobotrya japonica (Thunb.) Lindl. (Rosaceae, Maloideae) is an evergreen tree commonly known as the loquat or Japanese medlar. This species is native to China, and it was later introduced to Japan (Changkui *et al.* 1995). In the eighteenth century, it was exported from those countries to northern India, the Mediterranean regions, and England. Nowadays, this species is found in more than 30 countries around the world (Dhiman *et al.* 2021). Since its introduction to the Americas, *E. japonica* has been showed to exhibit the ability to thrive in a variety of soil types and diverse environmental conditions (Deluchi & Keller 2010).

Around the world *E. japonica* is economically significant as it can be used as an ornamental tree, and its fruits are edible, including the peel (Ahumada *et al.* 2017). Additionally, its leaves, fruits, stems, and flowers have medicinal properties (De Tommasi *et al.* 1991, Banno *et al.* 2005, Lee & Kim 2009, Rashed & Butnariu 2014). The leaves have been traditionally used in Chinese medicine to treat respiratory tract inflammation (Lee & Kim 2009, Liu *et al.* 2016). Also, the leaves are used fresh or dried to relieve stomach ailments, depression, or to reduce the side effects of alcohol consumption (Liu *et al.* 2016, Maher *et al.* 2015, Ramos-Hryb *et al.* 2017). In addition, studies have indicated that leaves

possess diuretic, antitumoral and anti-inflammatory properties (Banno *et al.* 2005, Baljinder *et al.* 2010, Cha *et al.* 2011, Tan *et al.* 2017). The cell suspension culture extract, also derived from *E. japonica* leaves, has demonstrated significant anti-cancer efficacy against both androgen-sensitive and castration-resistant prostate cancer cells (Hsieh *et al.* 2021). Also, *E. japonica* is renowned for its high antioxidant (Jung *et al.* 1999, Song *et al.* 2010, Nawrot-Hadzik *et al.* 2017).

Li *et al.* (2016) reported the *in vivo* metabolism and bioavailability, synergies, and competitive effects, as well as potential toxicity of *E. japonica* extracts in animal models. Despite this plant's benefits, the medicinal properties of its fruit and leaves are not fully understood (Parrado 2021). Currently, the benefits are linked to unknown dosages of the plant, implying that it may not be appropriate for oral human consumption.

The chemical composition of leaves of this species is complex and diverse, and it has been suggested that they are rich in fiber, minerals such as Ca, K, Na, and Fe, and vitamins B2, B6 and B12 (Khouya *et al.* 2022). Hwang *et al.* (2010) reported that loquat leaves contain 8.78 % moisture, 6.74 % crude protein, 7.87 % crude fat, 6.99 % crude ash, 43.61 % dietary fiber, and 26.01 % carbohydrates. They identified 16 amino acids, diverse fatty acids such as lauric, myristic, pentadecanoic, stearic, and oleic acids. Also, the loquat leaves analyzed in this study contained 0.039 mg of vitamin A, 0.096 mg of vitamin E, and 0.575 mg of vitamin C, and the mineral content was ranked as Ca > K > Mg > Na > Fe > Mn > Zn.

Phenolics are among the most studied compounds in *E. japonica* leaves, being phenolic acids and flavonoids the most abundant (Uysal *et al.* 2016, Chen *et al.* 2017a, b, Wu *et al.* 2018, Park *et al.* 2019, Silva *et al.* 2020, Khouya *et al.* 2022). In addition, more than 164 volatile compounds have been identified in their leaves (Taniguchi *et al.* 2002, Zhu *et al.* 2022). As well, triterpenes as methyl betuliate, methyl maslinate, methyl corosolate, and oleanolic, ursolic, maslinic, corosolic, tormentic, and euscaphic acids were isolated and identified from loquat leaves (Lv *et al.* 2008). Chen *et al.* (2017a) identified some terpenoids including methoxy-euscophic acid, tormentic acid, methyl corosolate, corosolic acid, maslinic acid, oleanolic acid, ursolic acid and some phenolic/terpenoid compounds like methoxy-3-O-p-coumaroyltormenic acid, 3-O-p-coumaroyltormenic acid, methoxy-3-O-p-coumaroylmaslinic acid and 3-O-p-coumaroylmaslinic acid.

In México, *E. japonica* is an introduced species and is not extensively cultivated, but the tree can be found often in gardens, orchards, and as living fences. It is primarily grown in the states of Guerrero, Veracruz, Morelos, and Oaxaca (Juárez-Vázquez *et al.* 2019, Parrado 2021). In 2008, the total cultivated area for *E. japonica* was 59.5 hectares (Parrado 2021). During 2016, the State of Mexico was the primary producer of loquats, with a total of the 93 % fruit harvest from this species (Parrado 2021). This fruit tree shows production potential in central Mexico due to its favorable climatic conditions (Juárez-Vázquez *et al.* 2019).

Given the preliminary information on the antidiabetic activity reported in loquat leaves, and the presence of various minerals. We hypothesize that loquat plants from the state of Veracruz will be a rich source of minerals and/or secondary metabolites with antidiabetic properties. To contribute to the knowledge macronutrient content of leaves, and the phytochemistry properties of leaves of *E. japonica* from Veracruz, Mexico, the aims of this study were 1) to determine the content of carbon (C), nitrogen (N), sodium (Na), potassium (K), calcium (Ca), magnesium (Mg), and phosphorus (P), 2) to perform a phenolics/volatile-targeted metabolomic analyses, 3) to test the antidiabetic potential by the α-glucosidase *in vitro* inhibition assay.

Materials and Methods

Plant material. Eriobotrya japonica leaves (500 g, fresh weight) were obtained from the Botanical Garden "Francisco Javier Clavijero" of the Instituto de Ecología (INECOL), A.C., in Xalapa, Veracruz, Mexico, using three adult individuals of this species, the leaves were collected in the spring of 2021. A specimen was identified by curators of the herbarium XAL, and a voucher was deposited in the herbarium XAL (number XAL0106251). Leaves were washed with distilled water and were dried at 25 °C for one day. The dried leaves were then stored at -20 °C before being lyophilized for six days in a freeze dryer (FreeZone 1, Labconco, Kansas, MO, USA). After lyophilization, the leaves were ground in a mortar and stored in a plastic bag at 4 °C prior to further processing.

Total leaf content of carbon, nitrogen, and macro elements. Total C and N content from 1 g of freeze-dried leaves (n = 3) was determined by dry combustion using an auto-analyzer (TruSpec CN, LECO, Corporation, St. Joseph, MI). Quantification of phosphorus (P), sodium (Na), potassium (K), calcium (Ca), and magnesium (Mg) was performed according to Etchevers (1988). Macro elements of leaves were quantified by atomic absorption (Ca and Mg) using a fast-sequential atomic absorption spectrometer (AA240FS, Varian) and flame spectrophotometry for K and Na were analyzed using a flame photometer (410 corning). For the quantification of total P, the colorimetric method of vanadomolybdophosphoric acid was used (Etchevers 1988). The absorbance of the standard dilutions was measured with a spectrophotometer (Genesys 20, Thermo Scientific, Waltham, Massachusetts, USA). A triplicate was performed for each measurement.

Identification and quantification of phenolic compounds by UPLC-QqQ-MS analysis. The leaf methanolic extract (LME) was obtained using an accelerated solvent extraction system (ASE 350, Dionex Corporation, Sunnyvale, CA, USA) following the protocol previously described by Infante-Rodríguez *et al.* (2020). For this purpose, 3 g of plant material were mixed with 1 g of diatomaceous earth (Thermo Scientific, Waltham, MA, USA) and placed in a 34 mL cell. The method consisted of a single static cycle of 15 min at 60 °C. The methanolic extract was concentrated by rotary evaporation under reduced pressure at 40 °C (RII, Büchi, Flawil, Switzerland). The identification and quantification of phenolic compounds was performed in an ultra-performance liquid chromatograph coupled to a triple quadrupole mass spectrometer (UPLC-QqQ-MS, 1290-6460 Agilent Technologies, Santa Clara, CA, USA) using a dynamic multiple reaction monitoring (dMRM) method following the protocol previously described by Infante-Rodríguez *et al.* (2020). For this, chromatographic separations were carried out on a ZORBAX SBC18 column (1.8 µm, 2.1 × 50 mm) (Agilent Technologies) with the column temperature at 40 °C. Mobile phase consisted of (A) water containing 0.1 % formic acid and (B) acetonitrile containing 0.1 % formic acid. The gradient conditions of the mobile phase were: 0 min 1 % B, 0.1-40 min linear gradient 1-40 % B, 40.1-42 min linear gradient 40-90 % B, 42.1-44 min isocratic 90 % B isocratic, 44.1-46 min linear gradient 90-1 % B, 46.1-47 min 1 % B isocratic (total run time 47 min). The flow rate was 0.1 mL/min, and 5 µL of sample injection volume. dMRM were obtained on an Agilent 6460 Triplequadropole (QqQ) mass spectrometer. The ESI source was operated in positive and negative ionization modes, desolvation temperature of 300 °C, Cone gas (N2) flow of 5 L/min, nebulizer 45 psi, sheath gas temperature 250 °C, sheath gas flow of 11 L/min, capillary voltage (positive and negative) 3,500 V, nozzle voltage (positive and negative) 500 V. For quantitation of each phenolic compound, a calibration curve in a concentration range of 0.25 to 19 µM was constructed (R^2 values = 0.99 were considered for the linearity range) (Table 1) and quantities were established by using MassHunter Workstation Software version B.06.00 (Agilent Technologies). Results are expressed as µg/g of sample (dry weight).

Identification of volatile compounds by GC-MS analysis. The leaf petroleum ether extract (LPE) for gas chromatography coupled to mass spectrometer (GC-MS) analysis was obtained using the method of Ferguson (Borgonetti *et al.* 2020). An amount of 10 g of lyophilized vegetable powder was taken and soaked in methanol for 24 h. Subsequently, the filtrate was extracted with petroleum ether; the LPE was analyzed using a GC coupled to a single quadrupole MS (2010 Plus-QP2010 Ultra, Shimadzu, Tokyo, Japan) equipped with a ZB-5MSi column (30 m × 0.25 mm ID × 0.25 µm). Electron impact (70 eV) spectra were obtained. Helium was the carrier gas (0.8 cm^3/min, constant flow), and a split-less injector (temperature of 250 °C, split valve delay of 3 min) was used to inject the sample. The oven temperature was held at 50 °C for 2 min, then programmed to increase at a rate of 15 °C/min to 280 °C, which was held for 10 min. The ion source temperature was 250 °C. Tentative identifications were made by comparison of fragmentation patterns with those patterns available in the NIST/EPA/NIH Mass Spectral Library, NIST 11, Software version 2.0 (National Institute of Standards and Technology, www.nist.gov) using a range of 84-100 % similarity values, with the Lab solutions GC-MS solutions 2.72 software (Shimadzu, Tokyo, Japan). Some identifications were confirmed by comparison of retention times and mass spectra with commercially available standards analyzed in the same instrument and analytical conditions. The relative area was calculated integrating each compound peak area and determining the contribution of each compound to the total area (sum of the individual areas).

Table 1. Conditions and search compounds using HPLC-MS protocol operated in dynamic MRM (Multiple Reaction Monitoring).

Compound	dMRM transition			Mass spectrometric conditions			Quantification conditions		
	Precursor ion	Product ion	Retention time	Collision energy	Fragmentor	Polarity	Quantification range (µM)	Regression type	R^2
Shikimic acid	173.1	111.1	0.49	10	100	Negative	0.5 - 19	Quadratic	0.99
Gallic acid	169.0	125.2	1.4	10	100	Negative	1 - 19	Quadratic	0.99
L-Phenylalanine	166.1	131.0	1.92	10	100	Positive	0.25 - 19	Quadratic	0.99
Protocatechuic acid	153.0	109.1	2.5	10	100	Negative	0.25 - 19	Quadratic	0.99
4-Hydroxybenzoic acid	137.1	92.8	3.76	10	100	Negative	0.25 - 19	Quadratic	0.99
Gentisic acid	153.0	109.0	3.83	10	100	Negative	0.25 - 19	Quadratic	0.99
4-Hydroxyphenylacetic acid	107.1	77.0	4.72	20	140	Positive	0.25 - 19	Quadratic	0.99
(-)-Epigallocatechin	305.1	125.0	4.83	20	140	Negative	1 - 17	Quadratic	0.99
(+)-Catechin	291.0	138.9	5.07	10	100	Positive	0.5 - 19	Quadratic	0.99
Vanillic acid	169.0	93.0	5.12	10	100	Positive	0.25 - 19	Quadratic	0.99
Scopolin	355.1	193.0	5.25	20	100	Positive	0.25 - 19	Quadratic	0.99
Chlorogenic acid	355.1	163.0	5.34	10	100	Positive	0.25 - 19	Quadratic	0.99
Caffeic acid	181.0	163.	5.38	10	100	Positive	0.5 - 19	Quadratic	0.99
Malvin	655.1	331.1	5.82	40	100	Positive	0.5 - 19	Quadratic	0.99
Kuromanin	449.0	286.9	6.34	30	100	Positive	0.5 - 19	Quadratic	0.99
Procyanidin B2	577.1	425.1	6.4	10	100	Negative	1 - 19	Quadratic	0.99
Vanillin	153.0	124.9	6.52	10	100	Positive	0.25 - 19	Quadratic	0.99
Keracyanin	595.2	287.1	6.88	20	100	Positive	0.5 - 19	Quadratic	0.99
(-)-Epicatechin	291.0	138.8	6.96	10	100	Positive	0.5 - 19	Quadratic	0.99
4-Coumaric acid	165.0	147.0	7.21	10	100	Positive	0.25 - 19	Quadratic	0.99
Mangiferin	423.0	302.8	7.32	10	100	Positive	0.5 - 19	Quadratic	0.99
Umbelliferone	163.0	107.0	7.64	30	100	Positive	0.25 - 19	Quadratic	0.99
(-)-Gallocatechin gallate	458.9	139.0	7.95	20	80	Positive	1 - 19	Quadratic	0.99
Scopoletin	193.0	133.0	8.4	10	100	Positive	0.25 - 19	Quadratic	0.99
Ferulic acid	195.1	145.0	8.6	20	100	Positive	0.25 - 19	Quadratic	0.99
Quercetin 3,4-di-O-glucoside	627.0	302.9	8.77	10	100	Positive	0.5 - 19	Quadratic	0.99
3-Coumaric acid	165.05	147.04	8.81	10	100	Positive	0.5 - 19	Quadratic	0.99
Salicylic acid	137.0	93	9.15	10	100	Negative	0.5 - 19	Quadratic	0.99

Compound	dMRM transition			Mass spectrometric conditions			Quantification conditions		
	Precursor ion	Product ion	Retention time	Collision energy	Fragmentor	Polarity	Quantification range (μM)	Regression type	R^2
Sinapic acid	225.1	207.1	9.16	10	100	Positive	0.25 - 19	Quadratic	0.99
Epicatechin gallate	443.1	123.0	9.83	10	100	Positive	1 - 19	Quadratic	0.99
Ellagic acid	300.5	145.0	9.98	30	170	Negative	1 - 19	Quadratic	0.99
Myricitrin	465.0	318.9	10.03	10	100	Positive	1 – 19	Quadratic	0.99
Pelargonidin	271.1	121	10.22	20	10	Positive	1 - 19	Quadratic	0.97
Quercetin 3-D-galactoside	465.0	302.9	10.26	10	100	Positive	0.25 - 19	Quadratic	0.99
Rutin	611.0	302.9	10.35	10	100	Positive	0.25 - 19	Quadratic	0.99
p-Anisic acid	153.1	109.0	10.45	5	120	Positive	0.25 - 19	Quadratic	0.99
Quercetin 3-glucoside	465.0	303.0	10.57	10	100	Positive	0.25 - 19	Quadratic	0.99
Luteolin 7-O-glucoside	449.0	287.0	10.77	10	100	Positive	0.5 – 19	Quadratic	0.99
Malvidin	331.1	287.1	11.14	20	100	Positive	1 - 17	Quadratic	0.96
2,4-Dimethoxy-6-methylbenzoic acid	197.0	179.0	11.41	5	80	Positive	0.25 - 19	Quadratic	0.99
Penta-O-galloyl-B-D-glucose	771.1	153.0	11.68	20	100	Positive	0.5 - 19	Quadratic	0.99
Kaemperol 3-O-glucoside	449.0	286.9	11.91	10	100	Positive	0.25 - 19	Quadratic	0.99
Quercitrin	449.1	303.1	11.95	10	100	Positive	0.5 - 19	Quadratic	0.99
Naringin	273.0	153.0	12.13	10	120	Positive	0.25 - 19	Quadratic	0.99
Myricetin	317.0	179.0	12.29	10	100	Negative	0.5 - 15	Quadratic	0.99
Hesperidin	609.1	301.1	12.68	20	100	Negative	0.5 - 19	Quadratic	0.99
trans-Resveratrol	229.1	135.0	12.69	10	100	Positive	0.5 - 19	Quadratic	0.99
Rosmarinic acid	361.1	163.0	12.8	10	100	Positive	0.5 - 19	Quadratic	0.99
Secoisolariciresinol	363.2	137.1	13.02	20	100	Positive	0.5 - 19	Quadratic	0.99
Phloridzin	435.0	272.9	13.04	10	100	Negative	0.25 - 19	Quadratic	0.99
trans-Cinnamic acid	149.1	131.0	14.08	10	100	Positive	0.25 - 19	Quadratic	0.99
Psoralen	187.0	131.1	14.99	20	100	Positive	0.25 - 19	Quadratic	0.99
Quercetin	302.9	153.1	15.18	35	100	Positive	1 - 19	Quadratic	0.99
Luteolin	287.1	153.0	15.28	30	100	Positive	0.5 - 19	Quadratic	0.99
Angelicin	187.0	131.1	15.75	20	100	Positive	0.5 - 19	Quadratic	0.99
Naringenin	271.0	151	16.79	10	100	Negative	0.5 - 19	Quadratic	0.99

Compound	dMRM transition			Mass spectrometric conditions			Quantification conditions		
	Precursor ion	Product ion	Retention time	Collision energy	Fragmentor	Polarity	Quantification range (µM)	Regression type	R^2
Apigenin	271.0	153.0	17.45	30	100	Positive	0.5 - 19	Quadratic	0.99
Matairesinol	359.2	137.1	17.55	10	100	Positive	0.25 - 19	Quadratic	0.99
Kaempferol	287.1	153.0	17.81	30	100	Positive	0.25 - 19	Quadratic	0.99
Hesperetin	303.1	177.1	18.06	20	100	Positive	0.25 - 19	Quadratic	0.99
Podophyllotoxin	415.1	397.1	19.01	10	100	Positive	0.25 - 19	Quadratic	0.99
Methyl cinnamate	163.1	131.0	21.46	6	100	Positive	0.25 - 1	Quadratic	0.99

The retention time variation allowed for the search of the compounds was 2 min in each case. The fragmentor voltage was 100 V and the cell accelerator voltage was 7 V for each compound. It was made a calibration curve for each compound in a concentration range of 0.25 to 19 µM.

α-Glucosidase enzyme inhibition assay. The α-glucosidase enzyme inhibition by the LME of *E. japonica* was determined by *in vitro* enzymatic inhibitory assays according to Infante-Rodríguez *et al.* (2022). The α-glucosidase enzyme (≥ 100 U/mg protein) from the yeast *Saccharomyces cerevisiae* (Desm.) Meyen (Sigma Aldrich St. Louis, MO, USA) was diluted to 0.005 mg/mL in phosphate buffer (PB, 100 mM, pH 7.2, Sigma Aldrich, St. Louis, MO, USA). Then, 20 µL of LME dissolved at 1 mg/mL in PB was mixed with 100 µL of 4-nitrophenyl-α-D-glucopyranoside (Sigma Aldrich, St. Louis, MO, USA) at 1 mM in PB. Acarbose (Sigma Aldrich, St. Louis, MO, USA) was used as a positive control (30 mM). The reaction mixture was incubated for 5 min at 30 °C. After the incubation time, the enzyme was added to each well, and the microplate was incubated for 30 min at 30 °C. Absorbance was measured at 405 nm at the beginning and after 30 min in a microwell spectrophotometer (Multiskan FC, Thermo Scientific, Waltham, MA, USA). The inhibition percentages (PI) were calculated with the equation:

$$\mathrm{PI} = \left(\frac{\mathrm{ABScontrol} - (\mathrm{ABSextract} - \mathrm{ABSblank})}{\mathrm{ABScontrol}}\right) \times 100$$

Where $ABS_{control}$, $ABS_{extract}$, and ABS_{blank} correspond to the absorbance of the negative control, inhibitor (LME or acarbose) and PB, respectively.

Statistical analysis. The total C and N, and the quantification of P, Na, K, Ca, and Mg were expressed in percentage or cmol/Kg. Quantification of individual phenolic compounds are expressed in µg/g of dried sample. The α-glucosidase enzymatic inhibition results were analyzed with Wilcox rank sum test for group comparisons. All statistical analyses were performed with the Agricolae library (De Mendiburu 2010) in R software v. 4.1.2 (R Core Team 2020).

Results

Total leaf content of carbon, nitrogen, and macro elements. The total C and N content in *E. japonica* leaves were 49.9 ± 2.95 % and 1.77 ± 0.7 %, respectively; resulting in a C:N ratio of 28.19 ± 10.94 (Table 2). In addition, the leaves content of Na, K, Ca, and Mg were 97.96 ± 64.9 cmol/Kg, 36.73 ± 15.87 cmol/Kg, 37.29 ± 16.97 cmol/Kg, and 27.85 ± 16.41 cmol/Kg, respectively. Leaves exhibited a lower P content of 3.73 ± 0.36 cmol/Kg (Table 2).

Identification and quantification of phenolic compounds by UPLC-QqQ-MS analysis. Twenty-two phenolic compounds plus a precursor (shikimic acid) were identified and quantified in LME, mainly phenolic acids and flavonoids (Table 3). The most abundant compounds are the flavonoids rutin, kaempferol-3-O-glucoside and quercetin-3-glucoside (Table 3). In addition, the phenolic acids that exhibited the highest content are caffeic acid, 4-hydroxyphen-

ylacetic acid, and chlorogenic acid. Also, the proanthocyanidin procyanidin B2 and the dihydrochalcone glucoside phloridzin were quantified (Table 3).

Table 2. Values of C, N, C:N ratio (%) and content of leaf elements (cmol/Kg) present in *E. japonica* leaves (Average ± SD).

Element	Content
C	49.9 ± 2.95
N	1.77 ± 0.7
C:N	28.19 ± 10.94
P	3.73 ± 0.36
Na	97.96 ± 64.9
K	36.73 ± 15.87
Ca	37.29 ± 16.97
Mg	27.28 ± 16.41

Table 3. Quantification of phenolic compounds in *E. japonica* leaves.

Compound	Concentration in µg/g (Mean ± SD)
Phenolic precursor	
Shikimic acid	49.46 ± 8.59
Phenolic acids	
Caffeic acid	180.19 ± 6.68
4-Hydroxyphenylacetic acid	131.47 ± 75.49
Chlorogenic acid	74.40 ± 16.02
t-Cinnamic acid	65.88 ± 16.15
4-Coumaric acid	62.44 ± 19.19
Vanillic acid	33.75 ± 13.62
Ferulic acid	33.25 ± 10.51
4-Hydroxybenzoic acid	16.01 ± 5.78
Protocatechuic acid	15.60 ± 1.70
Flavonoids	
Rutin	558.38 ± 569.83
Kaempferol-3-O-glucoside	356.54 ± 362.21
Quercetin-3-glucoside	309.86 ± 69.31
(+)-Catechin	256.19 ± 109.83
Quercitrin	246.52 ± 115.59
Quercetin 3,4-di-O-glucoside	214.12 ± 247.10
Luteolin-7-O-glucoside	201.31 ± 92.44
Quercetin	151.47 ± 71.96
Quercetin-3-D-galactoside	132.19 ± 69.31
(-)-Epicatechin	86.97 ± 12.12
Kaempferol	80.18 ± 49.65
Proanthocyanidin	
Procyanidin B2	213.87 ± 52.58
Dihydrochalcone glucoside	
Phloridzin	29.38 ± 10.64

Identification of volatile compounds by GC-MS analysis. Nineteen compounds, including fatty acids (7), terpenes (9), and aromatic (2) and aliphatic (1) compounds were identified through GC-MS analyses in the LPE. The identified compounds with the highest relative peak area (%) were phytol and the palmitic, linoleic, and stearic acids, as well as phytol acetate (Table 4).

α-glucosidase enzyme inhibition assay. LME derived from *E. japonica* demonstrated an 86.05 ± 0.73 % inhibition of α-glucosidase enzyme *in vitro*, showing similar effectiveness to the control substance of acarbose, with an α-glucosidase inhibition of 83.43 ± 0.44 %. No significant differences in α-glucosidase inhibition were observed between the two treatments ($P = 0.1$) (Figure 1).

Table 4. Chemical composition obtained by GC-MS of LPE of *E. japonica*.

Compound	RT	RA (%)	S (%)
Fatty acids			
Palmitic acid	22.9	14.54 ± 6.36	95
Linoleic acid	24.5	12.23 ± 6.01	90
Stearic acid	24.8	6.27 ± 4.27	94
Linolenic acid, ethyl ester	24.8	4.80 ± 6.21	89
Hexadecanoic acid, ethyl ester	23.2	1.89 ± 0.61	96
Tetradecanoic acid	20.7	0.43 ± 0.02	95
Dodecanoic acid	18.4	0.21 ± 0.02	94
Terpenes			
Phytol*	24.3	27.02 ± 7.69	100
Phytol acetate*	21.6	6.49 ± 2.56	100
Phytol isomer	22.0	2.07 ± 0.81	89
Hexahydrofarnesyl acetone	21.6	0.65 ± 0.21	93
(2E,6E)-7,11-Dimethyl-2,6,10-dodecatrien-1-ol	17.0	0.48 ± 0.20	90
β-Famesene	17.1	0.35 ± 0.34	92
α-Bergamotene	17.7	0.35 ± 0.12	89
β-Bisabolene*	17.8	0.31 ± 0.05	100
β-Eudesmene	17.8	0.22 ± 0.06	92
Aromatic compounds			
Acetophenone*	11.1	1.44 ± 1.41	100
Methyl methanthranilate	16.6	1.05 ± 0.19	95
Aliphatic compounds			
3-Eicosyne	21.8	1.30 ± 0.686	90

Abbreviations represent retention time in min (RT), relative peak area (RA), and percentage of similarity (% S). RA data shows an average of triplicate samples (Mean ± Standard deviation). Symbol (*) means compounds that were corroborated with a reference standard.

Discussion

The main aims of the present study were to identify and quantify the presence of different minerals, and secondary metabolites extracted from the leaves of this plant species. In addition, to test the α-glucosidase inhibition in the LME.

Only a few studies have reported that *E. japonica* leaves contain minerals, fiber, and several vitamins. For example, Hwang *et al.* (2010) found that the mineral content of loquat leaf from a Korean population were greater in order of Ca > K > Mg > Na > Fe > Mn > Zn. An analysis of a loquat leaves from a Moroccan population made by Khouya *et al.* (2022) revealed high levels of Ca, K, and Mg with values of 267.50, 953.80, and 279.60 mg/100 g, respectively. Also, they reported a moderate content of Na (40 mg/100 g), and a relatively low amount of Fe (0.5 mg/100 g). The results of the present study showed that *E. japonica* leaves were a rich source of carbon-based compounds. Conversely, the leaves were poor in N, suggesting that the leaves have a relatively low concentration of nitrogen-containing compounds. The observed C:N ratio (28.19 cmol/Kg) indicates that C (49.9 cmol/Kg) is present in a significantly higher proportion compared to N (1.77 cmol/Kg) in our samples of *E. japonica* leaves. Furthermore, the leaves are also found to have high levels of the minerals Na, K, Ca, and Mg (Table 2). These minerals contribute to the overall nutritional profile of the leaves. However, the content of nutrients and mobile elements (such as N, P, K, and Mg) could vary significantly among seasons and loquat populations (Quiñones *et al.* 2013); these variations could be related to nutrient mobility along with individuals, high-intensity shading, soil type and soil bioavailability of nutrients, and the annual physiological cycle of each loquat cultivar; in some cases, long-term and high-intensity of shading can increase foliar mineral nutrients in this species (Shan *et al.* 2020).

Certain micronutrients play a critical role in human metabolism and are essential for maintaining optimal health and preventing some diseases (Shergill-Bonner 2013). Many diseases, including diabetes mellitus, have been experimentally shown to be controlled by medicinal plant extracts. The elements Mg, K, Ca, Mn, Fe, Zn, Br, Rb, Cr, Ti, Cu, V, Cl and Pb were identified in antidiabetic medicinal plants traditionally used in the management of diabetes mellitus (Arika *et al.* 2016, Gholamhoseinian *et al*. 2020).

Mg (that was one of major mineral found in loquat leaves) is a trace mineral of importance to human biology and health and increasing evidence suggest that play an important role in glucose metabolism (Carneiro *et al.* 2013). Also, Mg is an important ion in all living cells being a cofactor of many enzymes, especially those utilizing high energy phosphate bounds. The relationship between insulin and Mg has been recently studied and chronic Mg supplementation (3g/day for 3 weeks) can contribute to an improvement in both islet Beta-cell response and insulin action in non-insulin-dependent diabetic subjects (Paolisso *et al.* 1990). People with high blood sugar are prone to develop deficiency in some minerals like K, Zn and Mg. Low levels of insulin causes decreased utilization of glucose by body cells, increased mobilization of fats from fat storage cells and depletion of proteins in the tissues of the body, keeping the body in crisis (Pathak 2014). The functional foods of plant origin can help achieving optimal physiological metabolism and cellular functions helping the body to come out of these crises (Pathak 2014). High Mg in raw fruit peel and leaves of *P. guajava* was observed providing antidiabetic benefits in alloxan induced diabetic rats (Rai *et al.* 2007). In people at high risk of diabetes Mg supplementation significantly improved plasma glucose levels after a 2 h oral glucose tolerance test, Mg supplementation appears to have a beneficial role and improves glucose parameters in people with diabetes and improves insulin-sensitivity parameters in those at high risk of diabetes (Veronese *et al.* 2016). Minerals such as Na, K, Ca, and Mg are crucial for human health, as they play vital roles in functions such as fluid balance, muscular function, bone health, and regulation of blood pressure (Schiefermeier-Mach *et al.* 2020). A normal concentration of K is required for optimal insulin secretion, and a deficiency of K leads to diabetic acidosis (Narendhirakannan *et al.* 2005). K depletion can result in impaired glucose tolerance. In diseases associated with kidney disease (Narendhirakannan *et al.* 2005), Na and K ions play an important role. Obtaining these minerals from plant sources in the diet is critical to prevent deficiencies and maintain a balanced intake, thus reducing the risk of cardiovascular diseases, bone issues, and other nutrition-related disorders (Kear 2017). These suggest possibilities for the use of loquat leaves as a source of minerals for human health benefits.

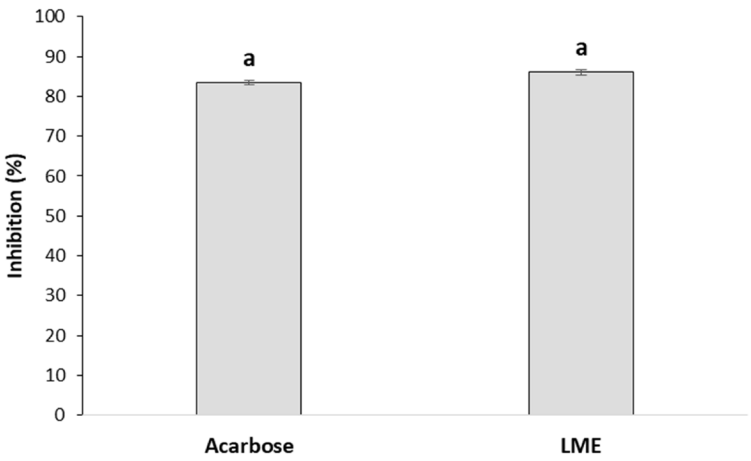

Figure 1. Inhibitory activity of the enzymes by acarbose, and leaf methanolic extract (LME) of *E. japonica* (1 mg/mL). Bars represent mean ± SD of inhibitory activity (3 mM for α-glucosidase), Wilcox rank sum test ($P > 0.05$).

Several studies have been carried out on the phytochemistry of *E. japonica* leaves, Uysal *et al.* (2016) identified and quantified 11 phenolic compounds in aqueous and methanolic extracts of loquat leaves collected in the Mediterranean region of Turkey including gallic acid, catechin, p-hydroxybenzoic acid, chlorogenic acid, p-coumaric acid, ferulic acid, and kaempferol, being the most abundant chlorogenic acid (5.79 mg/g of methanolic extract). Chen *et al.* (2017a) identified 20 compounds including phenolic acids, flavonoids, and triterpene acids in an ethanol/water (50:50) extract obtained from loquat leaves collected from China. Among the phenolic compounds identified were kaempferol xylose, chlorogenic acid, rutin, procyanidin C1, procyanidin B2, among others. Chen *et al.* (2017b) quantified gallic acid, protocatechuic acid, (+)-catechin, vanillic acid, caffeic acid, syringic acid, epicatechin, p-coumaric acid, ferulic acid, rutin, isoquercitrin, quercitrin, and quercetin in *E. japonica* leaves collected from the south of China. The most abundant compounds were (+)-catechin (883.23 µg/g of dried leaves) and epicatechin (266.49 µg/g of dried leaves). Wu *et al.* (2018) performed an HPLC-MS analysis using growing and fallen loquat leaves and report 12 compounds, including the presence of chlorogenic acid, vomifoliol-9-O-β-D-xylopyranosyl-(1→6)-β-D-glucopyranside, quercetin-3-O-galactosyl-(1→6)-glucoside, quercetin-3-O-sophoroside, quercetin-3-O-rutinoside, kaempferol-3-O-sophoroside, kaempferol-3-O-rutinoside, hyperoside, quercetin-3-O-glucoside, kaempferol-3-O-galactoside, quercetin-3-O-rhamnoside, and kaempferol-3-O-glucoside. Park *et al.* (2019) isolated and identified eight phenolic compounds from the methanolic extract of loquat leaves including chlorogenic acid, 5-O-caffeoylshikimic acid, 4-O-caffeoylshikimic acid, epicatechin, quercetin-3-O-galactoside, quercetin-3-O-glucoside, naringenin-6-C-(2”-O-acetyl)-glucoside, and naringenin-6-C-(2”,4”,6”-O-triacetyl)-glucoside. Silva *et al.* (2020) performed a chemical profiling of *E. japonica* leaves by paper spray mass spectrometry using hydroalcoholic (ethanolic and methanolic) extracts and different extraction methods. They report 49 compounds including organic acids, phenolic acids, flavonoids, sugars, quinones and terpenes. In addition, Silva *et al.* (2020) quantified the content of chlorogenic acid (159.81 - 511.04 µg/g extract), caffeic acid (25.05 - 82.01 µg/g extract), ellagic acid (0.92 - 2.73 µg/g extract), and quercetin (0.13 - 0.26 µg/g extract) in loquat levels. The best results were obtained using methanol/water (50:50) as extraction solvent and ultrasound as extraction method. The phytochemical analysis made by Khouya *et al.* (2022) revealed ten compounds in loquat leaves, consisting of protocatechuic acid, chlorogenic acid, rutin, quercetin, naringenin, epicatechin, epigallocatechin-3-gallate, kaempferol-rhamnose, and kaempferol, and a condensed tannin, procyanidin C1. The most abundant compounds were naringenin (10.93 ± 0.19 mg/g), procyanidin C1 (9.33 ± 0.16 mg/g), epicatechin (8.43 ± 0.04 mg/g) and rutin (7.55 ± 0.12 mg/g). In our study, the most abundant phenolic compounds found in MLE were flavonoids, and their glycoside derivatives included rutin, kaempferol-3-O-glucoside, quercetin-3-glucoside, (+)-catechin, quercitrin, quercetin 3,4-di-O-glucoside, and procyanidin B2. Despite the high number of chemical compounds reported in *E. japonica* leaves, few studies had performed quantitative determina-

tions and as far as we know our study reports for first time shikimic acid, quercetin 3,4-di-O-glucoside, luteolin-7-O-glucoside, and phloridzin, contributing with the phytochemical knowledge of this species. Moreover, nineteen compounds were identified using the GC-MS approach, including seven fatty acids, nine terpenes, two aromatic compounds, and one aliphatic compound. Zhou *et al.* (2019) previously reported the presence of sesquiterpenes, triterpenes, monoterpenes, and fatty acids in the low-polarity fractions of *E. japonica*. Tai *et al.* (2008) identified several main compounds in the essential oils of *E. japonica* leaves, including n-hexadecanoic acid, (E)-nerolidol, (Z,Z,Z)-9,12,15- octadecatrien -1-ol, (+)-carvone, 2-hexanoylfuran, elemicin, dihydroactinidiolide, farnesyl acetate, farnesol, and α-bisabolol. Hwang *et al.* (2010) reported that loquat leaves contain fatty acids such as lauric acid, myristic acid, pentadecanoic acid, stearic acid, and oleic acid. Hong *et al.* (2011) found 109 constituents in loquat leaves extracted with petroleum ether using capillary GC-MS, with the highest concentration of constituents being phytol. In our study, we also identified compounds such as linoleic acid, phytol isomer, and phytol in the LPE. Besides, we have reported the presence of 16 additional compounds including palmitic acid, stearic acid, linolenic acid, ethyl ester, hexadecanoic acid, ethyl ester, tetradecanoic acid, dodecanoic acid, phytol acetate, hexahydrofarnesyl acetone, (2E,6E)-7,11-dimethyl-2,6,10-dodecatrien-1-ol, β-famesene, α-bergamotene, β-bisabolene, β-eudesmene, acetophenone, methyl methanthranilate, and 3-eicosyne.

Diabetes mellitus is a metabolic disorder caused by hyperglycemia resulting from a defect in insulin secretion or action (Pandhi *et al.* 2020). Recent research suggests that certain compounds found in the leaves of *E. japonica* may have antidiabetic properties (Shih *et al.* 2010, Dhiman *et al.* 2021). These compounds include flavonoids, tannins, and triterpenoids from the leaves or seeds, which may be useful in the prevention and control of type 2 diabetes (Liu *et al.* 2016). The primary approach to mitigate the metabolic changes associated with type 2 diabetes is to inhibit the activity of the enzymes α-glucosidase and α-amylase (Proença *et al.* 2017, Infante-Rodríguez *et al.* 2022). However, the side effects of some synthetic drugs have led to great interest in nontoxic and safe natural products for the treatment of these conditions (Fu *et al.* 2019).

The LME showed *in vitro* inhibition of α-glucosidase enzyme. This enzyme plays a key role in the intestinal digestion of carbohydrates and its activity is one of the main factors affecting postprandial blood glucose (Yang *et al.* 2021). Inhibition of α-glucosidase allows the production of hydrolyzed glucose from carbohydrates to be delayed, thus controlling postprandial blood glucose levels (Zabidi *et al.* 2021). This finding could validate the use of this plant species in medicine for the treatment of diabetic patients (Dhiman *et al.* 2021).

Compounds such as cinchonain-Ib, thymosaponin, chlorogenic acid, and epicatechin, which have been identified in the leaves and seeds of *E. japonica,* can reduce blood glucose, total cholesterol, and triglycerides, improve insulin secretion and sensitivity (Ansari *et al.* 2020), and improve glucose tolerance (Ansari *et al.* 2022). Also, chlorogenic acid exhibited an in vitro IC_{50} of 9.2 µg/mL, while epicatechin exhibited an IC_{50} of 290 µg/mL with a competitive mechanism by the pocket of enzyme (Oboh *et al*. 2015b, Abioye *et al*. 2023).

Rutin, also identified in the LME, is a flavonoid found in medicinal plants with antidiabetic properties (Ansari *et al.* 2022) and has been shown to have a wide range of activities, including antihyperglycemic effects (Ghorbani 2017). Rutin exhibited an IC_{50} of 0.037 µM/mL *in vitro* against α-glucosidase enzyme (Oboh *et al*. 2015a), also, it has shown to reduce glucose absorption in the small intestine *in vivo* models by inhibiting the enzymes α-glucosidase and α-amylase involved in carbohydrate digestion (Hunyadi *et al.* 2012, Gao *et al.* 2015). Kaempferol-3-O-glucoside is another compound that has been reported to have significant antidiabetic and antioxidant effects in extracts of *Moringa oleifera* Lam. (Irfan *et al.* 2017) and *Annona squamosa* L. (Panda & Kar 2007). Extracts of the *Erica multiflora* L. plant is rich in kaempferol-3-O-glucoside and have been used to ameliorate fatty liver disease induced by a high-fat, high-fructose diet by modulating metabolic and inflammatory pathways in rats (Khlifi *et al.* 2020). In addition, this flavonoid inhibits the enzyme α-glucosidase with IC_{50} values greater than 200 µmol/L (Li *et al.* 2022). Catechin and quercitrin have also been reported to have potent antihyperglycemic activity (Dai *et al.* 2013, Mechchate *et al.* 2021).

Apart from phenolic compounds, plants can contain saturated fatty acids that exhibit strong inhibitory activity against α-glucosidase and protein tyrosine phosphatase 1B (PTP1B), which is involved in insulin receptor desensitization and has become a drug target for the treatment of type II diabetes (Rocha *et al.* 2021). By blocking the

action of PTP1B, insulin sensitivity can be improved, promoting a more effective insulin response in bodily tissues; therefore, PTP1B inhibitors have the potential to be used in the treatment of diabetes and other related metabolic disorders (Genovese *et al.* 2021). Several PTP1B inhibitors have already been found that interact with the binding site of the enzyme, surrounding the catalytic amino acid Cys215 and the adjacent region, or with the allosteric site of the enzyme (Rocha *et al.* 2021). Fatty acids, phenols, flavonoids, flavonols, flavanones, isoflavones, lignans, and phenolic acids found in plants have been reported as PTP1B inhibitors (Zhao *et al.* 2017).

According to the chemical composition of LPE, palmitic, linoleic, and stearic acids, and phytol and phytol acetate stand out compared to the other classes of compounds. Palmitic and linoleic acids are also abundant in *E. japonica* seeds (Henmi *et al.* 2019) and are the main components of the saturated fats we consume in the human diet (Trinick & Duly 2013). Palmitic acid, a saturated fatty acid, seemed the most potent inhibitor of PTP1B (IC_{50} = 45.5 μM), showing an affinity for this enzyme like some unsaturated fatty acids. Also, it has inhibitory activity towards α-glucosidase (IC_{50} = 111.51 μM) (Genovese *et al.* 2021). Palmitic acid has been reported as a compound present in leaf extracts of *Psychotria malayana* Jack, a plant used in the treatment of diabetes. *In vitro* experiments have shown an IC_{50} value of 8.04 μg/mL of palmitic acid inhibiting the enzyme α-glucosidase (Nipun *et al.* 2021).

Linoleic acid plays an important role in plant defense against abiotic stress because it can induce the transcription of genes involved in plant defense (Kessler & Baldwin 2001). It is an essential fatty acid for humans (Uysal *et al.* 2015) and its consumption has been associated with a reduced risk of type 2 diabetes, better glycemic control, and insulin sensitivity (Belury *et al.* 2018). It has also been reported that oleic and linoleic acids may contribute to high inhibition of α-glucosidase enzyme exhibiting IC_{50} values of 0.022 and 0.032 μg /mL, respectively (Su *et al.* 2013).

Stearic acid, according to Marset *et al.* (2009), is the second most consumed saturated fatty acid. When a diet is rich in this fatty acid, plasma cholesterol levels reduction is observed (Bonanome & Grundy 1988, Mensink 2005). Additionally, Habib *et al.* (1987) discovered that stearic acid inhibits tumor cells in both mice and humans. Also, it inhibits PTP1B activity, which could potentially boost insulin receptor signaling and trigger glucose uptake. In a PTP1B cell-free assay, stearic acid suppressed PTP1B activity in a dose-dependent manner ranging from 1-30 μM. At 30 μM concentration, stearic acid promoted glucose uptake into adipocytes by itself and significantly increased insulin-stimulated phosphorylation of insulin receptors at Tyr1185; however, insulin-induced phosphorylation of Akt remained unchanged (Tsuchiya *et al.* 2013).

Phytol and phytol acetate are compounds that are commonly found in the leaves of plants (Hong *et al.* 2010, Syeda & Riazunnisa 2020). Phytol has a wide range of biological activities, including anti-inflammatory, anti-allergic, immunostimulant, antinociceptive, antimicrobial, antioxidant, and antitumor effects (Pejin *et al.* 2014). Taken together, our results suggest that the flavonoids, some glycoside derivatives, and fatty acids present in LME and LPE of *E. japonica* are good candidates that could explain the antidiabetic activity observed in the leaf extracts. These compounds could exert different action mechanisms of enzyme as competitive or non-competitive inhibitors of enzymes (Zhu *et al*. 2019, Su *et al*. 2013). For example, it has been described that some flavonoids and fatty acids bind to the pocket site of the enzyme, which is considered a competitive inhibition (Zhu *et al*. 2019, Su *et al*. 2013). Also, some phenolics such as chlorogenic acid can change their action mechanism to "mixed type" in synergic to other drugs such as acarbose (Abioye *et al*. 2023). Moreover, some flavonoids may close the channel to the active center, that is usually associated with non-competitive inhibition (Zhu *et al*. 2019). Due to the high complexity of extracts and this work's objective, it is impossible to define a mechanism action at this level over the enzyme. For that reason, it is recommended for future studies isolated the active metabolites to describe their IC_{50} values, as well to study the specific actions mechanisms, and synergic effects.

In conclusion, the analysis of *Eriobotrya japonica* leaves performed on individuals collected in Veracruz, Mexico has revealed a composition of nutrients and minerals such as C, Na, K, Ca, Mg and P. Some of these minerals, such as Mg, may be important for human health, and there is increasing evidence of their importance in glucose metabolism (Carneiro *et al.* 2013. Mg supplementation may contribute to the improvement of both beta cell response and insulin action in non-insulin-dependent diabetics (Paolisso *et al.* 1990). *E. japonica* leaf extracts are a rather complex mixture of secondary metabolites and miscellaneous compounds, among which phenolic compounds and phytol, pal-

mitic acid, linoleic acid, stearic acid, and phytol acetate stand out. The LME exhibited potent α-glucosidase enzyme inhibition. The presence of various phenolic compounds, including flavonoids and their glycoside derivatives, may explain the inhibitory activity against α-glucosidase enzyme observed *in vitro* and further supports the traditional use of *E. japonica* as an herbal medicine with antidiabetic properties. Estimation of IC_{50} of LME and evaluation of α-glucosidase inhibitory potential of LPE are recommended in future studies.

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