

Inhibitory Effects of Edible and Medicinal Plant Extracts on the Enzymatic Activity of Pancreatic Lipase

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Abstract. Plants with a strong activity to reduce the digestion of lipids from the diet are a possible way to prevent and combat obesity. This study evaluated 37 ethanol extracts of plants, some edible, medicinal, or belonging to a family that has the inhibitory activity of pancreatic lipase, aimed at looking for a new anti-obesity agent. Inhibition of pancreatic lipase (PL) was measured *in vitro* and *in vivo* assay. The plasma triacylglycerol levels after 1, 2, and 3 h in fasted male Wistar rats fed, by oral administration, with a lipid emulsion were measured. The antioxidant activities were evaluated using DPPH (1,1-diphenyl-2-picrylhydrazyl radical scavenging activity), FRAP (ferric reducing antioxidant power) and ABTS (2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid), and the total phenol and flavonoid content were determined. Nine plants exhibited low inhibition (<41 %), nine showed medium inhibition (41-50 %), eight demonstrated high inhibition (51-60%) and 11 plants had the highest inhibition (≥ 61 %). *Hibiscus rosa-sinensis*, dried leaves extract displayed the highest inhibitory activity of pancreatic lipase 71.90 % at 400 $\mu\text{g/mL}$, with a dose-dependent inhibition against PL similar to orlistat. The kinetic study showed uncompetitive inhibition. Moreover, *H. rosa sinensis* extract also reduced the elevation of plasma triacylglycerol levels after lipid emulsion administration similar as orlistat did. *H. rosa-sinensis* dried leaves had higher antioxidant activity and total phenolic compounds than fresh leaves. *H. rosa-sinensis* presented the strongest anti-lipase activity and could be used as an anti-obesogenic agent or as a food additive to reduce the absorption of fats from the diet.

Keywords: Obesity; lipase; triacylglycerol; inhibition; antioxidant capacity.

Resumen. Las plantas con una fuerte actividad para reducir la digestión de los lípidos de la dieta son una posible forma de prevenir y combatir la obesidad. Este estudio evaluó 37 extractos etanólicos de plantas, algunas comestibles, medicinales o pertenecientes a una familia que tiene una actividad inhibitoria de la lipasa pancreática, con el objetivo de buscar un nuevo agente anti-obesogénico. Se midió la inhibición de la lipasa pancreática (PL) *in vitro* e *in vivo*, se midieron los niveles de triacilglicerol en plasma 1,2 y 3 h después de la administración oral de una emulsión lipídica a ratas Wistar machos en ayunas. Las actividades antioxidantes se evaluaron utilizando DPPH (1,1-diphenyl-2-picrylhydrazyl actividad atrapadora de radical), FRAP (poder antioxidante reductor del hierro) y ABTS ácido (2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphónico), también se midió el contenido total de fenoles y flavonoides. Nueve plantas exhibieron una inhibición baja (<41 %), otras nueve con una inhibición media (41-50 %), ocho demostraron una inhibición alta (51-60 %) y once plantas mostraron la inhibición más alta (≥ 61 %). El extracto de hojas secas de *Hibiscus rosa-sinensis*, mostró la mayor actividad inhibitoria de la PL con un 71.90 % a 400 $\mu\text{g/mL}$, con una inhibición dependiente de la dosis contra PL similar a orlistat. El estudio cinético mostró una inhibición acompetitiva. Además, el extracto de *H. rosa-sinensis* también redujo la elevación de los niveles de triacilglicerol en plasma después de la administración de una emulsión de lípidos similar a como lo hace orlistat. Las hojas secas de *H. rosea-sinensis* tuvieron mayor actividad antioxidante y cantidad de compuestos

fenólicos totales que las hojas frescas. *H. rosa-sinensis* presentó la mayor actividad anti-lipasa y podría usarse como un agente anti-obesogénico o como un aditivo alimentario para reducir la absorción de grasas de la dieta.

Palabras clave: Obesidad; lipasa; triacilglicerol; inhibición; capacidad antioxidante.

Introduction

Some strategies to prevent obesity are control food intake or alter lipids metabolism by inhibiting fat absorption [1]. Pancreatic lipase (triacylglycerol acylhydrolase) is the most important enzyme in the digestion of 50-70 % of the fat diet and, it is responsible for the hydrolysis of triacylglycerols (triglycerides) to monoacylglycerols and free fatty acids [2].

Tetrahyrilipstatin (Orlistat TM), a derivative of lipstatin obtained from *Streptomyces toxytricini*, is the only pancreatic lipase inhibitor currently approved for the treatment of long-term obesity. Its use can result in up to 10 % weight loss when used in combination with diet and physical activity. However, this drug can cause adverse liver effects (cholelithiasis, colostatichepatitis, and sub-acute liver failure) and troublesome gastrointestinal symptoms [3]. The inhibition of pancreatic lipase in many plants has been studied for its anti-obesogenic potential. In addition, the use of natural products to control obesity increased the interest in finding inhibitors of pancreatic lipase without the orlistat side effects [4].

Many medicinal, herbal, and edible plant extracts have been reported with *in vitro* inhibitory activity against pancreatic lipase, mainly in plants of eastern countries e.g., Malaysia, China, Jordan, and Korea [5,6,7]. One of the studies with the highest number of plants (400 plants) searching to inhibit pancreatic lipase activity was reported by Roh and Jung [8]. Mexico is a country with a high biodiversity of plants. However, there are only two studies focused on searching for a plant with a high inhibitory activity of pancreatic lipase. Ramirez *et al.* [9] reported that among 23 medicinal plants used in traditional medicine for diabetes, only two plants had 31.4 % and 27.2 % of lipase inhibitory activity. The second study [10] reported four plants with pancreatic lipase inhibition ≥ 60 % by analyzing a suite of 30 medicinal plants from Oaxaca State. This study aims to analyze 37 ethanol extracts from different parts of plants, easy to obtain, to assess their anti-lipase activity.

Experimental

Plant materials

Table 1 shows the plants and the parts tested. Some plants are edible, medicinal, or belong to a family that has the inhibitory activity of pancreatic lipase. The parts of the plants used in this study were based on the lack of studies in these tissues. The compound's composition changes with the use of fresh or dry material and for this reason, both were studied [11,12]. Some species were obtained from a local supermarket and others were acquired from a local greenhouse in Puruándiro, Michoacán, Mexico. The plant identity was confirmed by the taxonomist Patricia Silva of the Department of Botany of the University of Michoacán, and a voucher specimen was deposited in the herbarium of the University of Michoacán (EBUM). The voucher number of *Hibiscus rosa-sinensis* is EBUM240774.

Plant extractions

Leaves, fruits, seeds, roots, peel, *Opuntia* sp. cladode, strawberry sepal, and rhizomes were washed with water. Some leaves were dried in the convection oven at a temperature of 40 °C for two weeks. Dried and fresh samples were grounded into fine powder. Three grams of the sample were placed in 30 mL of 98 % ethanol for seven days at room temperature. The solvent was evaporated under 45 °C and low pressure. The dried extracts were dissolved in 1 % of dimethyl sulfoxide (DMSO).

Pancreatic lipase inhibition

The plant extracts obtained at 1 mg/mL were diluted in 1 % DMSO at 50, 100, 200, 300, and 400 µg/mL to perform an inhibition curve. 0.2 mL of the different concentrations were added to 0.1 mL of a pancreatic lipase solution (1 mg/mL); then, Tris-HCl buffer pH 7.4 was added to up to 1 mL and incubated for 15 min at 37 °C. After that, 0.1 mL of p-nitrophenyl-butyrate (100 mM) was added and incubated for 30 min at 37 °C; then, the samples were read at 405 nm. Orlistat was used as a reference control [13]. IC₅₀ is the concentration that gives 50 % lipase inhibition.

Antioxidant activity determinations

Radical-scavenging activity (RSA) assay

Free radical scavenging capacity was analyzed by the 2, 2-diphenyl-1 picryl hydrazyl (DPPH) assay according to the method of Hatano *et al.* [14]. Trolox was used as standard in a range of 25-800 µM.

Ferric reducing antioxidant power (FRAP) assay

The FRAP assay was performed using the method described by Thaipong *et al.* [15].

Trolox equivalent antioxidant capacity (TEAC)

The assay was performed as Rufino *et al.* [16] with modifications that reduced the time to obtain the ABTS⁺ radical cations to two hours. It was produced by mixing an equal volume of 2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) liquid substrate system (ready-to-use) trade Sigma, and 2.4 mM potassium persulfate. The mixture was shaken for 2 h at room temperature in the dark. At this time the absorbance reached a value of 3.0; the ABTS⁺ solution was diluted with ethanol to get an absorbance of 0.70 ± 0.02 at 734 nm. Fresh ABTS⁺ solution was prepared for each assay. A sample (0.05 mL) was added to ABTS solution (0.95 mL) after mixing, and the reaction was monitored for 7 minutes. Trolox standard was in the range of 25 - 600 µM. TEAC values were expressed as Trolox equivalents (TE)/g fresh mass.

Total phenolic content

The amount of the total phenolic was determined with the Folin-Ciocalteu reagent using the method of Pripdeevech *et al.* [17]. Gallic acid was in the range (0.01-0.4 mM). The results were expressed as mg of gallic acid equivalent (GAE)/g fresh mass.

Total flavonoid content

The amount of the total flavonoid was determined using the method of Chang *et al.* [18]. Rutin acid was used to calculate the standard curve (0.025 to 0.5 mg/mL).

Animals and experimental design

Male Wistar rats from 8-12 weeks old (200–300 g), were housed at a room temperature of 24 °C, with a 12 h light/dark cycle, fed with chow standard diets and water ad libitum. The rats were from the animal house of the Universidad Michoacana de San Nicolás de Hidalgo (UMSNH). The protocol for these experiments was approved by the Institutional Committee for Use of Animals of the UMSNH and followed the recommendations of the regulatory standard for the use of animals issued by the Mexican Ministry of Agriculture in its Federal Regulations for the Use and Care of Animals (NOM062-ZOO-1999). Thirty male Wistar rats that had fasted overnight were divided into five groups: one control group and the rest four groups were administered with oral gavage of 3 mL of a lipid emulsion, (6 mL corn oil, cholic acid 80 mg, 5 mL egg yolk and 6 mL of saline solution), with slight modifications [19]. Groups 2, 3, and 4 were administered orally with dried leaves extract of *Hibiscus rosa-sinensis* at a concentration of 62.5, 125, and 250 mg/kg respectively, and the 5th group with orlistat (50 mg/kg p.o.), as a positive control. Blood samples were collected from the tail every hour for three hours. Triacylglycerol concentrations were determined using Spinreact Kit.

Statistical Analysis

Pancreatic lipase inhibition activity and antioxidant assay results were expressed as mean \pm standard deviation (SD) (n=4). Group differences were analyzed by one-way ANOVA. Values were considered to be indicative of statistical significance *p \leq 0.05.

Results

Pancreatic lipase (PL) inhibition activity

37 plant extracts were tested at the concentrations of 50, 100, 200, 300, and 400 $\mu\text{g/mL}$ against the pancreatic lipase activity. Nine plants had a low percentage of inhibition of lipase activity < 41 %; nine extracts displayed a medium percentage of inhibition (41-50 %); eight extracts presented high inhibition in the range of range 51-60 %, and 11 extracts produced the highest percentage of lipase inhibition, \geq 61 %. Table 1 shows that some extract plants had IC₅₀ values up to 400 $\mu\text{g/mL}$. Although this study worked with a few specimens (37 plant extracts), 19 plants showed high inhibitory pancreatic lipase activity which represents 51.3 % of the plants. Moreover, all plant extracts presented inhibition against pancreatic lipase (Fig. 1).

Table 1. Inhibitory concentration (IC₅₀) of pancreatic lipase activity of the plant extracts.

Scientific Name	Family	Part Used	IC ₅₀ ($\mu\text{g/mL}$)
<i>Cucumis sativus</i>	Curcubitaceae	P	>400
<i>Heliopsis longipes</i>	Asteraceae	Rt	>400
<i>Nelumbo nucifera</i>	Nelumbonaceae	FL	>400
<i>Rubus sp.</i>	Rosaceae	FR	>400
<i>Salvia hispánica</i>	Lamiaceae	FL	>400
<i>Solanum melongena</i>	Solanaceae	FR	>400
<i>Tamarindus indica</i>	Fabaceae	FR	>400
<i>Vaccinium corymbosum</i>	Ericaceae	FR	>400
<i>Vitis sp.</i>	Vitaceae	FL	>400
<i>Amorophallus konjac</i>	Araceae	FRh	>400
<i>Fragaria vesca.</i>	Rosaceae	S	>400
<i>Heidichium coronarium</i>	Zingiberaceae	FRh	>400
<i>Hibiscus rosa-sinensis</i>	Malvaceae	FL	>400
<i>Lavandula</i>	Lamiaceae	DL	>400
<i>Opuntia sp.</i>	Cactaceae	C	>400
<i>Pistia stratiotes</i>	Araceae	DL	>400
<i>Tigridia pavonia</i>	Iridaceae	B	>400
<i>Vitis sp.</i>	Vitaceae	DL	>400
<i>Annona muricata</i>	Annonaceae	FL	324.37
<i>Callistemon citrinus</i>	Myrtaceae	DL	383.46
<i>Cucumis melo</i>	Curcubitaceae	Se	235.44
<i>Morus sp.</i>	Moraceae	FL	249.06
<i>Morus sp.</i>	Moraceae	DL	391.06
<i>Salvia hispánica</i>	Lamiaceae	Se	334.06
<i>Spinacia oleracea</i>	Amaranthaceae	DL	219.83
<i>Syzygium jambos</i>	Myrtaceae	FL	262.19
<i>Amorophallus konjac</i>	Araceae	DRh	218.21
<i>Annona comosus</i>	Annonaceae	FR	39.53
<i>Byrsonima crassifolia</i>	Malpighiaceae	FR	163.93
<i>Capsicum sp.</i>	Solanaceae	FL	278.03

<i>Carica papaya</i>	Caricaceae	Se	44.63
<i>Fragaria</i> sp.	Rosaceae	FR	45.35
<i>Hibiscus rosa-sinensis</i>	Malvaceae	DL	73.82
<i>Hibiscus sabdariffa</i> var. <i>rubra</i>	Malvaceae	FL	44.73
<i>Lavandula</i>	Lamiaceae	FL	3
<i>Passiflora</i> sp.	Passifloraceae	FL	121.93
<i>Syzygium jambos</i>	Myrtaceae	DL	207.27

Plant parts: FL: Fresh Leaves, Se: Seeds, DL: Dry Leaves, FR: Fruits, Rt: Roots, P: Peel, FRh: Fresh Rhizome, DRh: Dry Rhizome, S: Sepal of a flower, C: Cladode, B: Bulb. The results are the mean \pm SD (n = 4).

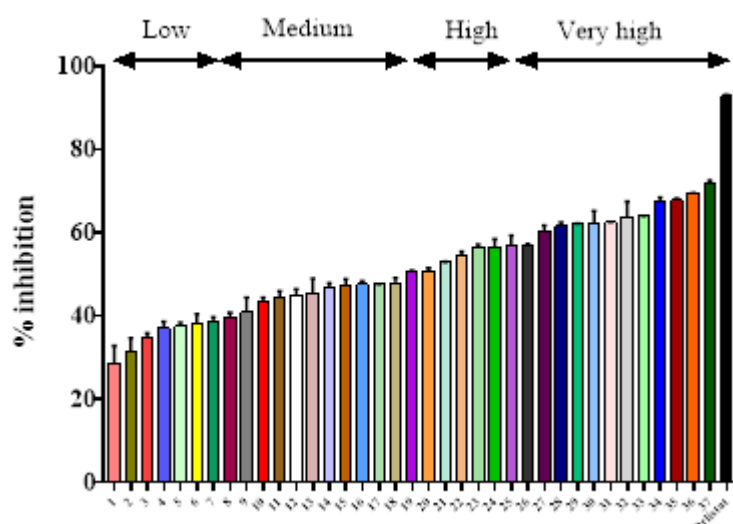


Fig. 1. The inhibitory effect of 37 ethanolic extracts on pancreatic lipase activity is divided into four groups: low (<41%), medium (41-50%), high (51-60%), and very high (\geq 61%). The values are the mean \pm SD (n = 4). 1. *Heliopsis longipes*; 2. *Cucumis sativus*; 3. *Rubus* sp.; 4. *Tamarindus indica*; 5. *Vaccinium corymbosum*; 6. *Salvia hispánica* leaf; 7. *Solanum melongena*; 8. *Vitis vinifera* fresh leaf; 9. *Nelumbo nucifera*; 10. *Amorphophallus konjac* fresh bulb; 11. *Pistia stratiotes*; 12. *Tigridia pavonia*; 13. *Vitis vinifera* fresh leaf; 14. *Opuntia* sp.; 15. *Lavandula* sp. dry leaf; 16. *Hibiscus rosa-sinensis* fresh leaf; 17. *Fragaria* sp. sepal; 18. *Heidichium coronarium*; 19. *Morus* sp. dry leaf; 20. *Callistemon citrinus*; 21. *Salvia hispánica* seeds; 22. *Morus* sp. fresh leaf; 23. *Syzygium jambos* fresh leaf; 24. *Annona muricata*; 25. *Spinacia oleracea*; 26. *Cucumis melo* seeds; 27. *Amorphophallus konjac* dry bulb; 28. *Capsicum* sp.; 29. *Byrsonima crassifolia*; 30. *Annona comosus*; 31. *Lavandula* sp. fresh leaf; 32. *Hibiscus sabdariffa*; 33. *Carica papaya*; 34. *Fragaria* sp.; 35. *Passiflora* sp.; 36. *Syzygium jambos* dry leaf; 37. *Hibiscus rosa-sinensis* dry leaf.

Hibiscus rosa-sinensis (Malvaceae), commonly known as China rose. The dried leaf extract displayed the highest inhibitory activity of pancreatic lipase, 71.90% at 400 μ g/mL, very similar to orlistat 70% at 50 μ g/mL. The inhibitory action of *H. rosa-sinensis* dried leaf extract was dose-dependent in a range of inhibitor concentrations (50-400 μ g/mL). The enzyme kinetics of *H. rosa-sinensis*, using the graphical representation of the Lineweaver-Burk, showed an uncompetitive inhibition with K_m of 11.11 μ M and V_{max} value of 10 μ mol/min, whereas K_m value of orlistat for pancreatic lipase was of 28.5 μ M and the V_{max} was 16.6 μ mol/min, also showed an uncompetitive inhibition (Fig 2).

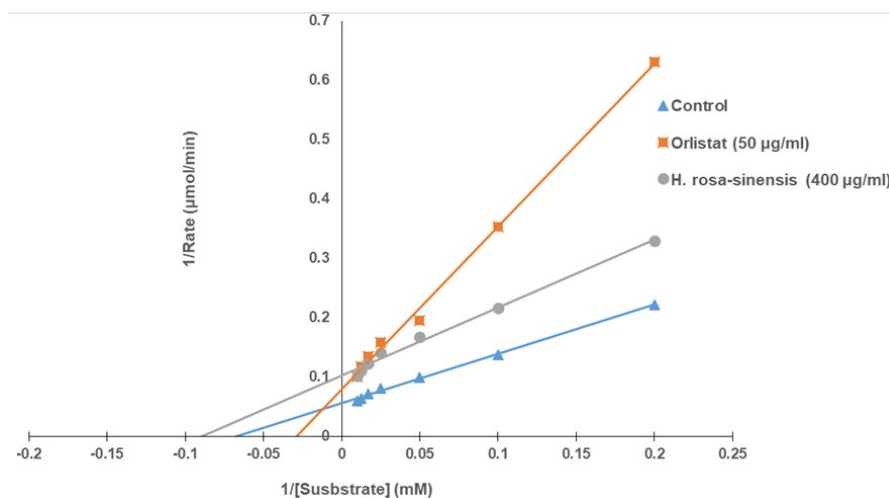


Fig. 2. Lineweaver-Burk plot of orlistat and *H. rosa-sinensis* against porcine lipase pancreatic at different concentrations of p-nitrophenyl butyrate as substrate.

Antioxidant activity

Hibiscus rosa-sinensis showed the highest PL inhibitory activity; as a result, this plant was selected to evaluate the antioxidant capacity using the DPPH, FRAP, and ABTS assays. In all assays, *H. rosa-sinensis* dried leaves had higher antioxidant activity and total phenolic compounds than fresh leaves. Conversely, fresh leaves contained a higher number of flavonoids than dried leaves (Table 2).

Table 2. Antioxidant activity and the total amount of phenolic and flavonoids of ethanol extracts of *Hibiscus rosa-sinensis* leaves

	Dry	Fresh
DPPH (μM Trolox/mL)	500.03 \pm 1.50	356.76 \pm 8.08
EC ₅₀ DPPH	293.23 \pm 10.57	380.40 \pm 0.38
FRAP (μM Trolox/mL)	646.62 \pm 1.84	432.55 \pm 1.55
EC ₅₀ FRAP	175 \pm 0.98	360 \pm 0.87
ABTS (μM Trolox/mL)	443.86 \pm 2.64	241.66 \pm 3.24
Phenolics GAE (mg/g f.w)	26.91 \pm 0.82	13.15 \pm 0.80
Flavonoids Rutin (mg/g f.w)	30.33 \pm 0.65	44.57 \pm 1.65

DPPH: EC₅₀ ($\mu\text{g}/\text{mL}$) effective concentration at which 50 % of DPPH radicals are scavenged, Trolox 150 $\mu\text{g}/\text{mL}$.
 FRAP: EC₅₀ ($\mu\text{g}/\text{mL}$) effective concentration at which the absorbance is 0.5, Trolox 75 $\mu\text{g}/\text{mL}$ (Values are given as mean \pm SD (n = 4).

Oral administration of lipid emulsion in rats

Fig. 3 shows the reduced absorption of lipids effect of *H. rosa-sinensis* dried leaves *in vivo*. Plasma triacylglycerol levels were measured after oral administration of lipid emulsion. 125 mg/kg of *H. rosa-sinensis* significantly reduced the postprandial levels of triacylglycerol, after 2 h of oil emulsion administration, similar to orlistat. Meanwhile, triacylglycerol levels increased in the other groups.

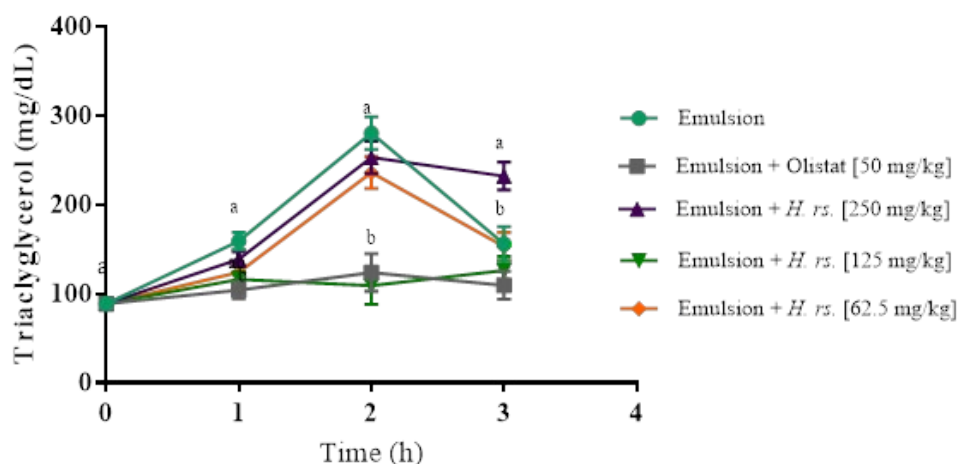


Fig. 3. Time course of the serum triacylglycerol levels after oral administration of lipid emulsion in rats, with *H. rosa-sinensis* dried leaves extract and orlistat. Values are expressed as means \pm SD ($n = 4$; values statistically different (^{a,b}) among groups ($p \leq 0.05$) according to Tukey test).

Discussion

This study tested 37 plant extracts, with different uses in Mexico, to evaluate their anti-lipase activity. Pancreatic lipase is the main enzyme responsible for the digestion of dietary triacylglycerols [2]. Therefore, the inhibition of enzymes involved in the assimilation of lipids is an important issue to prevent obesity.

11 plants presented inhibition $\geq 61\%$; in this group, dried rhizome and leaves of *Amorphophallus konjac*, *Hibiscus rosa-sinensis*, and *Syzygium jambos* presented higher inhibitory activity of pancreatic lipase, than the leaves in a fresh state. However, the opposite occurred in *Lavandula spp.*, where the fresh leaves had a higher inhibitory activity of pancreatic lipase than dried leaves. These results could be explained by considering that in some species the drying process can alter the phytoconstituents (phenolics and flavonoids) [20,21]. Moreover, as reported by Dewi *et al.* [22] in *Rubus fraxinifolius* Hayata, fresh leaves had a higher content of phenolics and flavonoids and antioxidant capacity than dry leaves, but the α -glucosidase inhibitory activity was lower. However, the opposite occurred in dry leaves. *Morus sp.* did not show a difference in pancreatic lipase inhibition between fresh and dried leaves; both presented a high anti-lipase activity. Some plant components that have been reported for pancreatic lipase inhibitory activity are flavonoids, lignans, polyphenols, terpenes, and saponins [23].

Hibiscus rosa-sinensis dried leaves extract showed the highest pancreatic lipases inhibitor activity, presenting a dose-response curve. It is a glabrous shrub widely cultivated in the tropics and has several forms with varying colors of flowers. One of the first properties of flowers and leaves was hair growth-promoting. Other uses are to treat arterial hypertension, hypoglycemic, laxative, and oral contraceptives [24]. In some Asia countries and even in Mexico, leaves have been used for dysentery, diarrhea, and as analgesic [25]. In Nigeria, the young leaves of *H. rosa-sinensis* is eaten [26]. Rios-Chavez *et al.* [27], reported as the major constituents of *H. rosa-sinensis* leave extract: fructose (18.60%), linoleic acid (9,12-octadecadienoic acid 18:2 *n*-6) (10.36%), α -linolenic acid (9,12,15-octadecatrienoic acid 18:3 *n*-3, ALA) (7.67%) and glycerol (6.18%). Seeds of *H. rosa-sinensis* are rich in unsaturated fatty acids and high content of ALA; however, *H. rosa-sinensis* leaves have an abundant composition of linoleic and α -linolenic acids. These compounds are important as a dietary component.

Linoleic acid and ALA are unsaturated fatty acids that humans cannot synthesize. These fatty acids are beneficial to health, besides being a precursor of arachidonic acid and having antioxidant properties, and anticarcinogenic and antiatherogenic effects [28].

This is the first study reporting the pancreatic lipase inhibitory activity of *H. rosa-sinensis*. In the genus *Hibiscus*, Buchholz and Melzig [29] showed that the methanol and aqueous extract of *H. sabdariffa* flowers has an inhibitory activity of pancreatic lipase of 100 % at a concentration of 2500 µg/mL. However, our study showed 70 % of inhibition of lipase activity at a concentration of 400 µg/mL. Moreover, Kumar *et al.* [30] found a hypolipidemic activity of *H. rosa-sinensis* roots ethanol extract, when used in a high-fat diet in rats.

Fig. 2 shows the inhibition mode of PL by *H. rosa-sinensis* at 400 µg/mL using a Lineweaver-Burk plot. The kinetic study revealed that *H. rosa-sinensis* extract decreases the K_m and V_{max} values, implying that it has uncompetitive inhibition of pancreatic lipase activity. In this type of inhibition, the inhibitor binds to the enzyme-substrate complex, and not to the enzyme, preventing the product formation, and making this mode of action useful for enzyme-target drug design [31].

Most of the studies of the antioxidant capacity in *Hibiscus rosa-sinensis* do not mention the flower color; this characteristic, as reported by Patel *et al.* [32] is important. These authors studied the antioxidant properties of five cultivars (red, yellow, orange, pink, and white) of *H. rosa-sinensis*, finding differences in their DPPH radical scavenging activities. The leaf extract of the red cultivar showed EC_{50} 389.2 µg/mL. This finding was consistent with our results that showed an EC_{50} 380.40 and 293.20 µg/mL in fresh and dried leaf extracts of the red cultivar of *H. rosa-sinensis*.

There are few reports about the reducing power of *H. rosa-sinensis*. Patel *et al.* [32] found that the yellow cultivar presented the highest reducing power at 1000 µg/mL and the lowest reducing power was found in the white cultivar. Divya *et al.* [33] reported a high ferric reducing power in methanol extract of leaves using 95 µg/mL. Our study showed EC_{50} values of 360 and 175 µg/mL in fresh and dry leaves of *H. rosa-sinensis*, respectively. Most of the studies use dry tissues because some conditions as storage, time, and temperature could affect antioxidant activity [14,34]. Conversely, there are reports showing that drying alters the phytoconstituents as phenolic, flavonoids, total antioxidant capacity, and some enzymatic activities [18,19,20].

In this study, the total phenolic and flavonoid contents were similar as reported Ghaffar and El-Elaimy [35]. Moreover, Wong *et al.* [36] screening six *Hibiscus* species in Malaysia and found that antioxidant properties change as a function of the environmental conditions. The total phenolic content in the leaves and flowers of *H. rosa-sinensis* was lower than our results. In addition, a study on the leaves and flowers of *Hibiscus roseus* presented lower phenolic and flavonoid content. However, phenolic compounds have potential applications in the skin [37].

Our study showed, for the first time, that dried leaves of *H. rosa-sinensis* presented higher antioxidant capacity, phenolic content, and inhibitory pancreatic lipase activity than fresh leaves and revealed a positive correlation between the dried state and biological activities.

Dietary fat promotes more body fat storage than dietary carbohydrate causing obesity, hyperglycemia, hypertension, and atherosclerosis, pathologies included in the metabolic syndrome [38]. Therefore, plants that possess the capacity to inhibit digestion and absorption of dietary fat are a good alternative to treating obesity. Tetrahydrolipostatin (Orlistat TM) is a pancreatic lipase inhibitor that does not affect the nervous system or enter the bloodstream [39]. However, it has many gastrointestinal side effects [40]. Few works measured plasma triacylglycerol levels after oral administration of a lipid emulsion in rats. As shown in Fig. 3, 125 mg/kg of *H. rosea-sinensis* leaf extract presented a reduction in triacylglycerol levels, similar to orlistat. Li *et al.* [41] reported that sesame meal presented pancreatic lipase inhibitory activity and the major compounds were linoleic acid and oleic acid. They also showed that these two fatty acids reduced fat digestion. As mentioned above, linoleic acid is one of the major compounds in *H. rosa-sinensis*, so its anti-lipase activity may be produced by this compound. The finding that *H. rosa-sinensis* reduced the elevation of plasma triacylglycerol in a similar way to orlistat did, suggests a relationship with the inhibitory pancreatic lipase activity. *H. rosa-sinensis* dried leaf extract may be useful for body weight control.

Conclusions

Based on the analysis of 37 plants, *H. rosa-sinensis* showed the highest inhibition of pancreatic lipase activity that displayed a positive correlation with the antioxidant capacity. Consequently, *H. rosa-sinensis* can

be used to reduce body weight. Further studies are required to identify the active compound that shows inhibitory pancreatic lipase activity.

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