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Original Article

Potential Anti-obesity Effects of some Medicinal Herb: *In vitro* α-Amylase, α-Glucosidase and Lipase Inhibitory Activity

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In this study, *in vitro* inhibitory activity of methanolic and chloroform extracts of some medicinal plants including *C. wightii*, *T. ammi*, *N. sativa*, *C. arabica*, *L. usitatissimum*, *C. cyminum*, and *R. Graveolens* was evaluated toward α-glucosidase, α-amylase, and lipase comparing with acarbose and orlistat as the standard inhibitors. Our results revealed that both methanolic and chloroform extracts of *C.wightii* depicted high activity toward α-glucosidase (IC_{50}s = 100.2 and 110.3 µg/mL, respectively) while methanolic and chloroform extracts of *R.graveolens* as well as chloroform extract of *C.arabica* showed good to moderate inhibitory activity (IC_{50}s = 281.0, 460.5, and 280.0 µg/mL, respectively). Among the evaluated extracts, methanolic extract of *R.graveolens* and chloroform extract of *C.arabica* were found to be potent inhibitors toward α-amylase (IC_{50}s = 215.0 and 180.0 µg/mL, respectively). However, moderate activity was obtained by methanolic and chloroform extracts of *C. wightii* and chloroform extract of *R.graveolens* (IC_{50} = 273.5, 358.5, and 479.0 µg/mL). It should be noted that all extracts demonstrated no significant inhibitory activity against lipase.

Key words: α-amylase, obesity, α-glucosidase, lipase, medicinal plants

Obesity is characterized by the accumulation of excess body fat which has been considered as a serious health issue in recent decades. According to an estimate provided by

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Table 1. Medicinal plants investigated for their α-glucosidase, α-amylase, and lipase inhibitory activity

Scientific name	Family	Local name	Part used
C. wightii	Burseraceae	Luke-e-Maghsool	Gum
T.ammi	Apiaceae	Zenyan	Seeds
N. sativa	Ranunculaceae	Siah-daneh	Seeds
C. arabica	Rubiaceae	Ghahve-ye-Sabz	Seeds
L. usitatissimum	Linaceae	Bazr-e-Katan	Seeds
C. cyminum	Apiaceae	Zire-ye-Sabz	Seeds
R. graveolens	Rutaceae	Sodab	Leaves

World Health Organization (WHO) in 2016, 39% of adults were overweight and 13% suffered from obesity worldwide (1). Obesity is considered as the main contributor to the burden of non-communicable diseases such as cardiovascular diseases, atherosclerosis, type 2 diabetes, non-alcoholic fatty liver disease and some types of cancer (2).

Adherence to low-calorie diets, changing dietary habits, increasing physical activity levels and taking anti-obesity supplements and medications are the major approaches for the treatment of obesity (3, 4). Anti-obesity medications are mainly described via three potent mechanisms including enhancing energy expenditure, suppression appetite and inhibition of gastrointestinal enzymes involved in weight loss (3, 5). In this respect, development of efficient agents for inhibiting the metabolism of carbohydrates and fat digestion and absorption leads to the reduction of calorie intake, promotion of weight loss, and management of possible complications of obesity. However, current available synthetic drugs such as acarbose, orlistat and miglitol have been associated with some gastrointestinal problems including bloating, diarrhea, flatulence and abdominal discomfort (6).

Most dietary patterns consider carbohydrates as the main macronutrients for energy intake. They must be broken down into monosaccharides using two main enzymes namely α-amylase and α-glucosidase to be used by tissues and cells (7, 8). Therefore, inhibition of related enzymes causes the decrease of calorie intake to promote weight loss (7, 9). Additionally, human pancreatic lipase plays a promising role in absorption of dietary fat especially triglyceride. As lipids are well-known to generate more energy than sugars and proteins, they need also significant attention as a versatile approach to treat obesity which can be achieved by exact control of digestion of dietary lipids (10).

Recently, medicinal plants possessing versatile biological activities have attracted lots of attention due to their positive effect on losing weight along with fewer side effects. Although several studies have indicated the enzyme inhibitory potential of medicinal plants (11-14), development of efficient natural products for the treatment of obesity is still in high demand. In this regard, examining the potential anti-obesity activity of medicinal plants, *C.wightii*, *T. ammi*, *N.sativa*, *C. arabica L.*, *L.usitatissimum*, *C.cyminum*, and *R. graveolens* (Table 1), via enzyme inhibition can be helpful.

Materials and methods

Plant materials

All plants were obtained from Faculty of traditional medicine, Tehran University of Medical Sciences, Tehran, Iran. They were identified and deposited in the herbarium of Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran.

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Plant extract preparation

A suspension of each plant powder (20 g) in chloroform (150 mL) was settled in an ultrasonic bath for 20 min at 40 °C. Then, it was stirred on a shaker for 24 h. The mixture was filtered off and the residue was mixed with the same amount of chloroform and extracted in the same manner. The filtrate was evaporated under reduced pressure. The remained material was extracted with methanol exactly according to the above mentioned procedure.

α-Glucosidase inhibitory activity

The glucosidase inhibition assay was performed spectrophotometrically using *p*-nitrophenyl*α*-D-glucopyranoside as a substrate according to the literature (15). *α*-Glucosidase from *Saccharomyces cerevisiae* (1 U/mL) was prepared in potassium phosphate buffer (pH 6.8, 50 mM), and plant extracts were dissolved in DMSO (10 % final concentration). After 10 min incubation, the enzyme (20 µL), various concentrations of the plant extract (20 µL), and buffer (135 µL) at 37 °C, *p*-nitrophenyl-α-D-glucopyranoside (25 µL, 4 mM, Sigma Aldrich, USA) was added and followed by incubation at 37 °C for 20 min. The change in absorbance of the solution was recorded at 405 nm. For the control experiment, plant extract was replaced with DMSO (10% final concentration). All experiments were performed in triplicate and the results were expressed as mean ± SD. Acarbose (Sigma Aldrich, USA) was used as a standard inhibitor. The percentage of inhibition was calculated as follows: α-glucosidase inhibition (%) = $[(Abs_{control} - Abs_{sample})/Abs_{control}] \times 100$.

α-Amylase inhibitory activity

α-Amylase inhibitory activity was determined spectrophotometrically based on the literature (12, 16). Plant extracts were dissolved in DMSO (10 % final concentration). The reaction mixture containing 50 µL enzyme (2.5 U), 50 µL of 0.5 % (w/v) soluble starch in phosphate buffer (pH 6.8, 50 mM), and 100 µL of the plant extract were incubated at 37 ºC for 30 min. The amount of reducing sugar was quantified by 3, 5-dinitrosalcylic acid (DNS). The percentage of inhibition was calculated using the following equation: $[(Abs_{control}-Abs_{sample})/Abs_{control}]\times100$. All experiments were performed in triplicate and the results were expressed as mean ± SD.

Lipase inhibitory activity

Lipase inhibitory activity was measured using 4-nitrophenyl butyrate as a substrate according to the reported method (13). Porcine pancreas lipase (PPL) stock solution (5 mg/mL) was prepared in potassium phosphate buffer (pH 7.0, 0.1 mM). To determine lipase inhibitory activity, 35 µL of the enzyme, 5 µL of 4-nitrophenyl butyrate (15 mM), 5 µL of the plant extract, and 220 µL of potassium phosphate buffer were incubated at 37 ºC for 10 min. The amount of 4-nitrophenol released in the reaction was measured at 405 nm. The percentage of inhibition was calculated as follows: $[(Abs_{control}-Abs_{sample})/Abs_{control}] \times100$. All experiments were performed in triplicate and the results were expressed as mean ± SD.

Kinetic study

Kinetic study of α-glucosidase

The mode of inhibition of methanolic and chloroform extracts of *C. wightii* was investigated against α-glucosidase with different concentrations of *p*-nitrophenyl-*α*-D-glucopyranoside (1–10 mM) as substrate in the absence and presence of those extracts at different final concentrations (0, 45,65,100 µg/mL for methanolic extract and 0, 60,80,110 µg/mL for chloroform extract). A Lineweaver–Burk plot was generated to identify the type of inhibition and the Michaelis–Menten constant (K_m) value was determined from the plot between reciprocal of the substrate concentration (1/[S]) and reciprocal of enzyme rate (1/V) over various inhibitor concentrations. Experimental inhibitor constant (K_i) value was constructed by the secondary plots of the inhibitor concentration [I] versus K_m.

Kinetic study of α-amylase

The mode of inhibition of chloroform extract

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C. arabica and methanolic extract of *R. graveolens* was studied at different concentrations of starch (0.5–2%) as substrate in the absence and presence of those extracts at different final concentrations (0, 100, 130, 180 µg/mL for *C. Arabica* and 0, 70, 140, 215 µg/mL for *R. graveolens*). A Line weaver–Burk plot was generated to identify the type of inhibition, and the Michaelis–Menten constant (K_m) value was determined from the plot between reciprocal of the substrate concentration (1/[S]) and reciprocal of enzyme rate (1/V) over various inhibitor concentrations. Experimental inhibitor constant (K_i) value was constructed by secondary plots of the inhibitor concentration [I] versus K_m.

Results

Biological activity

In the present study, methanolic and chloroform extracts of seven medicinal plants including *Commiphora wightii*, *Trachyspermum-ammi*, *Nigella sativa*, *Coffea arabica*, *Linum usitatissimum*, *Cuminum cyminum*, and *Ruta graveolens* which have been recommended in Iranian traditional medicine for their anti-obesity activity were evaluated against α-glucosidase, α-amylase, and lipase in comparison to acarbose and orlistat as the reference drugs (Table 2 and Table 3).

Our results revealed that both methanolic and chloroform extracts of *C. wightii* depicted high activity toward α-glucosidase by percent inhibition of 91.0% (IC_{50}s = 100.2 and 110.3 µg/mL, respectively) while methanolic and chloroform extracts of *R. graveolens* as well as chloroform extract of *C. Arabica* were found to be good to moderate inhibitors by percent inhibition of 71.0% (IC_{50} = 281.0 µg/mL), 54.0% (IC_{50} = 460.5 µg/mL), and 65.0% (IC_{50} = 280.0 µg/mL), respectively. The other plants showed low anti-α-glucosidase activity.

In the case of α-amylase inhibitory activity, the best activity was related to the chloroform extract of green coffee (*C. arabica*) by percent inhibition of 78.0% (IC_{50} = 180.0 µg/mL). Besides, methanolic extract of *R. graveolens* and chloroform extract of *C. wightii* were found to be highly active toward α-amylase by percent inhibition of 73.0% (IC_{50} = 215.0 µg/mL) and 65.5% (IC_{50} = 358.5 µg/mL), respectively. It should be noted that methanolic extracts of *C. wightii* and *T. ammi* as well as chloroform extract of *R. graveolens* demonstrated good anti-α-amylase activity (54.5%, 53.0%, and 55%). However, moderate activity was obtained by the chloroform extract of *N. sativa* (45.0%) and the other plants depicted low inhibitory activity toward α-amylase.

According to data reported in Table 2, all

Table 2. hibitory activity of methanol and chloroform extracts of some plants at the concentration of 500 µg/mL toward α-glucosidase, α-amylase, and lipase

Plant	α-Glucosidase inhibition (%)		α-Amylase inhibition (%)		Lipase inhibition (%)	
	Methanol extract	Chloroform extract	Methanol extract	Chloroform extract	Methanol extract	Chloroform extract
C.wightii	91.0± 3.0	91.0± 1.5	54.5± 1.5	65.5± 0.5	3.0± 1.0	13.0± 2.0
T. ammi	16.0± 2.0	20.0± 2.5	53.0± 1.6	0.0± 0.0	0.0± 0.0	7.0± 1.0
N. sativa	3.0± 0.5	30.0± 5.7	13.0± 1.0	45.0± 1.0	0.0± 0.0	6.0± 1.0
C.arabica	14.0± 3.0	65.0± 6.2	32.5± 0.5	78.0± 3.2	10.0± 2.0	12.0± 1.0
L. usitatissimum	15.0± 3.0	20.0± 2.6	10.0± 1.5	33.0± 1.0	0.0± 0.0	10.0±1.0
C. cyminum	35.0 ± 2.0	20.0± 3.5	45.5± 0.5	6.0± 1.5	27.0± 1.0	17.0± 1.0
R. graveolens	71.0 ± 7.0	54.0± 4.0	73.0± 3.0	55.0± 3.0	29.0± 1.0	0.0± 0.0

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Table 3. Inhibitory activity of selected extracts against α-glucosidaseand α-amylase

Plant	IC_{50} (µg/mL)	IC_{50} (µg/mL)
C.wightii(methanolic extract)	100.2±2.0	273.5±12.0
C.wightii(chloroform extract)	110.3±2.0	358.5±3.2
C. Arabica (chloroform extract)	280.0±3.8	180.0±2.6
R. graveolens(methanolic extract)	281.0±8.4	215.0±3.2
R. graveolens(chloroform extract)	460.5±12.4	479.0±10.1

Standard inhibitors: Acarbose (for alpha-glucosidase inhibition): 484.2± 10.4 µg/mL; Acarbose (for alpha-amylase inhibition): 69.7± 3.3 µg/mL; Orlistat (for lipase inhibition): 0.32± 0.02 µM µg/mL.

plants showed low to poor activity toward lipase and among them, the highest activity was obtained by the methanolic extracts of *R. graveolens* (29.0%) and *C. cyminum* (27.0%).

As described above, two extract solvents methanol (polar) and chloroform (nonpolar) were used to identify appropriate solvent to obtain effective constituents possessing desired inhibitory activities. As can be seen in Table 2, both methanol and chloroform were suitable solvents for active anti-α-glucosidase and anti-α-amylase extracts, however, there is no definite description for favourite solvent since related extracts showed no activity toward lipase due to the lack of desired constituents in methanolic and chloroform extracts.

Kinetic study

The kinetic studies of α-glucosidase and α-amylase inhibition were conducted for different

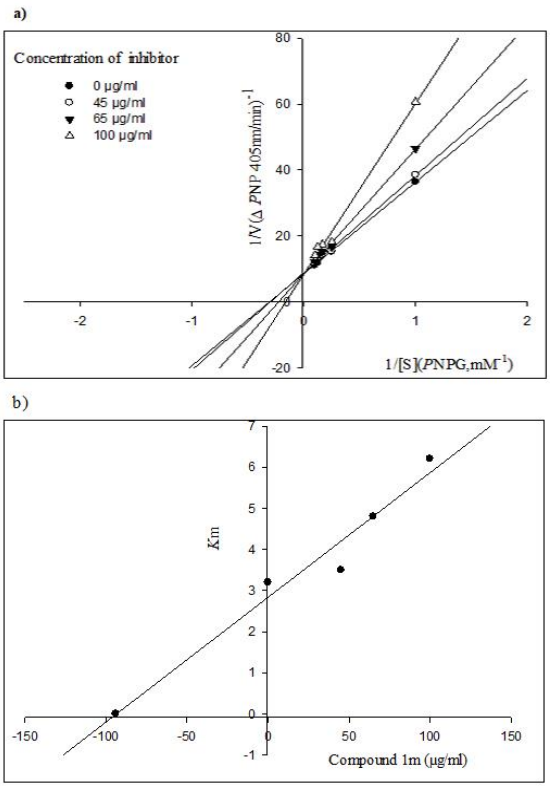


Figure 1. Kinetics of α-glucosidase inhibition by methanolic extract of *C. wightii.* (a): the line weaver–Burk plot in the absence and presence of different concentrations of extract; (b): the secondary plot between K_m and various concentrations of extract.

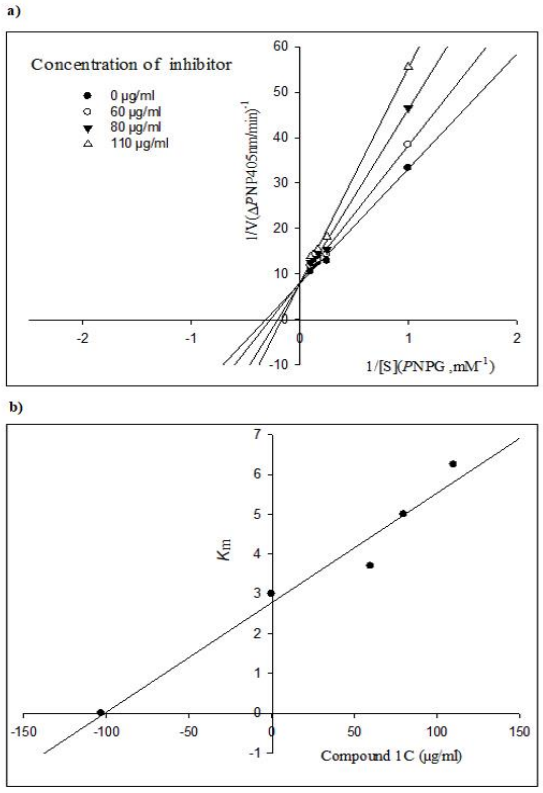


Figure 2. Kinetics of α-gluosidase inhibition by chloroform extract of *C. wightii.* (a): the line weaver–Burk plot in the absence and presence of different concentrations of extract; (b): the secondary plot between Km and various concentrations of extract.

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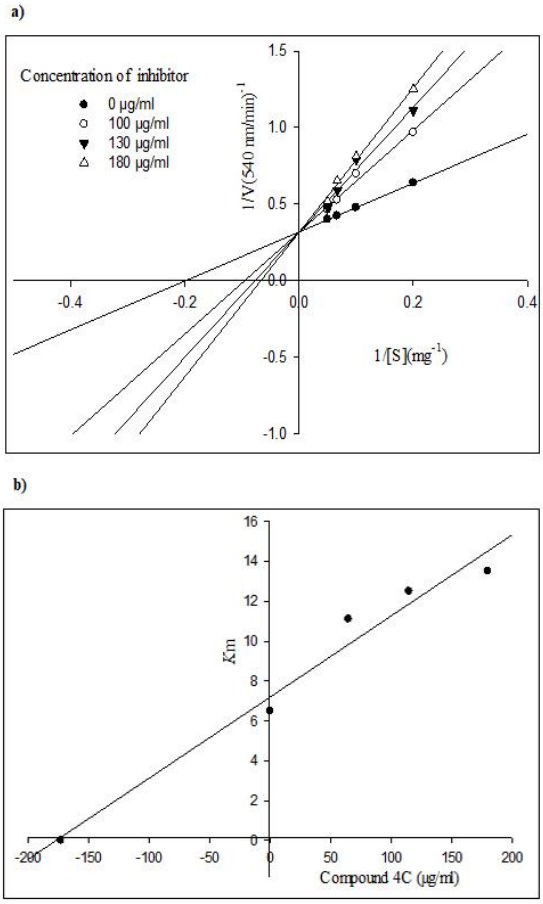


Figure 3. Kinetics of α-amylase inhibition by chloroform extract of *C. arabica*. (a): the line weaver–Burk plot in the absence and presence of different concentrations of extract; (b): the secondary plot between Km and various concentrations of extract.

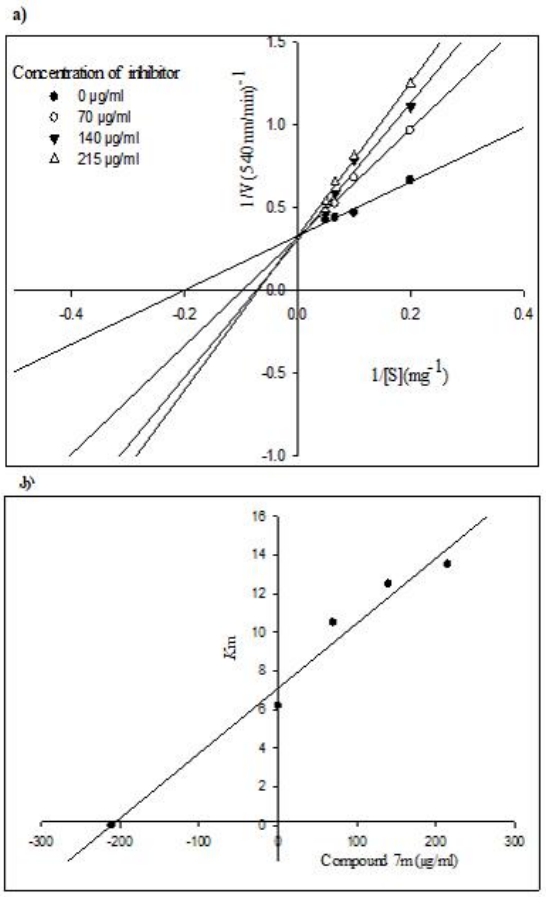


Figure 4. Kinetics of α-amylase inhibition by methanolic extract of *R. graveolens*. (a): the line weaver–Burk plot in the absence and presence of different concentrations of extract; (b): the secondary plot between Km and various concentrations of extract.

extracts of *C. wightii*, *C. arabica*, and *R. graveolens*to get better insight to inhibition pattern (Figures 1-4). According to Line weaver-Burk plots (Figures 1-4a), methanolic and chloroform extracts of *C. wightii*, chloroform extract of *C. arabica*, and methanolic extract of *R. graveolens* showed competitive inhibition since the K_m gradually increased and V_{max} remained unchanged with increasing inhibitor concentration. It seems that bioactive compounds present in those extracts could bind to the active site of enzymes in a competitive manner. Also, plot of the K_m versus different concentration of inhibitor gave an estimate of the inhibition constant, K_i for the corresponding extracts. In this regard, K_i values were calculated as 94, 103, 173, and 211µg/mL for methanolic extract of *C.wightii*, chloroform extract of *C. wightii*, chloroform extract of *C. arabica*, and methanolic extract of *R. graveolens*, respectively (Figures 1-4b).

Discussion

C. wightii was found as the most potent α-glucosidase and α-amylase inhibitor. However, *C.arabica* and *R. Graveolens* also showed good inhibitory activities. The inhibitory activity of C. wightii, R. graveolens, and C. arabica against α-glucosidase and α-amylase made them suitable for further studies on drug formulations for the treatment of obesity and diabetes mellitus. C. wightii (Muqil) is a type of gum commonly used in Unani and Iranian traditional medicine. It contains guggulsterone which can inhibit adipogenesis and induce apoptosis of adipocytes leading to weight management. Additionally, C.

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wightii can improve the ability of thyroid to absorb iodine, regulate metabolism, improve lipid profile and is used for atherosclerosis and rheumatism (14). It is also known for anti-oxidative and anti-inflammatory properties (11, 17) and in this study, it showed good anti-α-amylase and anti-α-glucosidase activity. However, there was no significant inhibitory activity toward lipase.

R. graveolens (Rue) has been frequently used in Iranian traditional medicine and its biological properties such as anti-inflammatory, anti-oxidative, anti-carcinogenic, anti-microbial, and anti-spasmodic activities have been efficiently provedin the literature (15, 18,19). It possesses flavonoids, lignans, glycosides, coumarins and quinoline alkaloids (20). It was found that methanol extract of R. graveolens containing polar constituents exhibited remarkable inhibitory activity against α-glucosidase. However, it showed moderate inhibitory activity toward α-amylase.

Chlorogenic acid is the main polyphenolic derivative found in green coffee (C. arabica) known as the most probable compound responsible for the medicinal properties of green coffee. In several animal (21-23) and human studies (24, 25), anti-obesity properties of green coffee has been reported. It inhibits fat absorption and activates fat metabolism in the liver (23). As reported here, only chloroform extract showed inhibitory effects against enzymes involved in carbohydrate digestion. However, it depicted no significant inhibitory activity toward lipase.

Lipase inhibitors are generally classified into three main groups: (i) pseudosaccharides such as acarbose, (ii) proteinaceous inhibitors and (iii) polyphenolic inhibitors. Polyphenolic compounds are widely found in plants absorbing lots of attention due to their inhibitory activity against digestive enzymes which is associated with their structures, number and position of hydroxyl groups (7). Their α-glucosidase inhibitory activity is probably achieved via hydrogen scavenging by providing hydrogen needed for hydrolysis of α-(1,4)-glucosidic linkage (26). In this way, the inhibitor prevents the hydrogen ion from binding to the catalytic sites of the enzyme. For instance, acarbose mimics enzyme substrate and inhibit the enzyme activity (27). In the case of α-amylase inhibition, two mechanistic pathways are suggested: (i) formation of a complex between enzyme and inhibitor, and (ii) reducing the rate of glucose diffusion from the active site by slowing the digestion and absorption of carbohydrates and viscous water-soluble dietary fibers (28, 29). As all medicinal plants investigated in this study are rich in polyphenols, their inhibitory activities can be related to this class of oxygen-containing compounds. However, further experiments are required to investigate the accuracy of this hypothesis. It should be noted that all plants showed no remarkable anti-lipase activity. It seems that complementary studies are needed to develop drug formulations for the treatment of obesity based on the desired medicinal plants.

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Conflict of interest

The authors declared no conflict of interest.

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