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Anti Lipase Activity of Rosa damascena Extracts

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OBESITY is a global health concern associated with high morbidity and mortality. One of the strategies in prevention or treatment of obesity is inhibition of pancreatic lipase responsible of absorption of fats. The aim of this study was to evaluate the anti-lipase activity of *Rosa damascena* Mill. Petal extracts (hydroalcoholic extract, aqueous extract, essential oil and hydrosol). Extracts were assayed for their *in vitro* activity against PPL (porcine pancreatic lipase) by using spectrophotometry with 2, 4-dinitrophenyl butyrate as substrate. extracts exhibited different anti-lipase activity, alcoholic extract had the highest (62%) anti-lipase activity and is equivalent to 0.11 µg orlistat/mL. followed by essential oil, aquose extract and rose water with) 55%, 46 and 20% inhibition), respectively. This study supports investigation of effectiveness of these extracts *in vivo* to be safe and cheap natural medical replacement in the future.

Keywords: Anti lipase activity, Pancreatic lipase, Rosa damascena Mill.

Introduction

Rosa damascena is a small plant belonging to the Rosaceae family. It is a small plant with aromatic light pink flowers, which appear in spring [1] and today are highly cultivated all over the world, In addition to its perfuming effect, flowers of *Rosa damascena* are used for medical purposes. It has been used as cardio tonic [2], mild laxative [3], anti-inflammatory [4], cough suppressant [5] and also for the treatment of menstrual bleeding and digestive problems [6]. Recent studies demonstrated anti-HIV [7], ant conflict [8], antibacterial [9], antitussive [10] and respiratory smooth muscle relaxant [11] properties for this plant.

Obesity in addition to be a health problem is a social one, It leads to diabetes, cardiovascular disorders, musculoskeletal disorders and some types of cancer [12] and it is also ranking fifth for deaths worldwide [13]. The number of people suffering from this disease is increasing rapidly especially children and adults. Recent researches demonstrated the potential of natural products to counteract obesity. Pancreatic lipase is the main lipid-digesting enzyme. Inhibition of pancreatic lipase is an attractive targeted approach for the treatment of obesity [14]. For instance, orlistat, which is obtained from Streptomyces toxytricini, is the only pancreatic lipase inhibitor currently approved for long-term treatment of obesity. Its use can result in up to 10% weight loss when used in combination with dietary, behavioral and exercise therapy [15] but it also has unpleasant and non-negligible side effects [16]. Therefore, there is a need for more lipase inhibitors or medicinal products that are safe and effective. Many plants have been studied for their antilipase activity, including alcoholic extract of Rosa damascena [17]. In this study we investigate anti lipase activity of different extracts of Roas damascena (hydroalcoholic extract, aqueous extract, essential oil and hydrosol) and compare between them.

<u>Experimental</u>

Materials

Rosa damascena flowers were collected in Syria –Marah village – al Nabek district, and it has been authenticated at Damascus University Pharmacognosy and Medical Plants Department. Lipase (Type II; from Porcine pancreas), orlistat, and p-nitrophenyl butyrate were purchased from Sigma Chemical Co. (St.Louis, MO, USA). All reagents were of biochemical grade

Establishment of calibration curve

Stock solution of the orlistat (positive control) were freshly prepared with 1000 μ g/mL by dissolving (0.01 g) of orlistat in 0.1 ml of DMSO then was completed with 9.9 ml of phosphate buffer to get 1000 μ g/mL stock solution.

Standard series were made by gradual dilution with phosphate buffer to the required concentration (200, 100, 50, 25 and 10).

Preparation of plant extracts Hydro alcoholic extract

Flower petals were air dried and grounded into fine powder. The Powdered material (500 g) was extracted with (50 ml) methanol and water (80:20) for 24 hr. The suspensions were filtered then the solvent was evaporated under low temperature and low pressure to give gummy residue (crude extract). This crude was stored at -20 °C until use.

Aqueous extract

Petals were boiled with water for 30 min and the water extract was dried under reduced pressure at 60°C.

Isolation of the essential oil

The essential oil was isolated by hydro distillation of the air-dried petals according to the method recommended in European Pharmacopoeia. 145 g of rose petals were hydrodistilled in a Clevenger apparatus for 3 hr to obtain the essential oil and hydrosol (18). The hydrosol and essential oil obtained in this way were stored at -5°C prior to use.

Hydrosol preparation

Hydro distillation of petals produces essential oil besides to hydrosol or (rose water).

Pancreatic lipase inhibition assay

Porcine pancreatic lipase (PPL, type II) activity was measured using p-nitro phenyl butyrate (p-NPB) as a substrate (19). PPL stock solutions (1 mg/mL) were prepared in a 0.1 mM potassium phosphate buffer (pH 6.0) and the solutions were stored at -20° C. To determine the lipase inhibitory activity, the extracts (final

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concentrations(200, 100, 50, 25, 10, 5 µg/mL) or Orlistat (at same concentrations) as a positive control were pre-incubated with PPL for 1 hr in a potassium phosphate buffer (0.1 mM, pH 7.2, 0.1% Tween 80) at 30°C before assaying the PPL activity. The reaction was then started by adding 0.1 µL NPB as a substrate, all in a final volume of 100 µL. After incubation at 30°C for 5 min, the amount of p-nitro phenol released in the reaction was measured at 405 nm using a UV-Visible spectrophotometer. The activity of the negative control was also examined with and without an inhibitor; DMSO was used as negative control.

The inhibitory activity (I) was calculated according to the following formula:

Inhibitory activity (I %)= $100 - ((B - b)/(A - a) \times 100)$

Where, A is the activity without inhibitor, a is the negative control without inhibitor and B is the activity with inhibitor, b is the negative control with inhibitor.

Results and Discussion

Obesity is one of the biggest complications to global health nowadays. Pancreatic lipase inhibitors which help to limit intestinal fat absorption have been proved as useful medications and great promise as anti-obesity agents. Presence of pancreatic lipase inhibitors has been reported in some natural resources, but due to the problem related to these extracts, more investigations for finding new and better pancreatic lipase inhibitors in nature is a necessity. Rosa damascena Mill L. commonly known as rose having several pharmacological properties including anti-HIV, antibacterial, antioxidant, antitussive, hypnotic and anti-diabetic (20). As part of the continuing search for biologically benefits of Rosa damascena Mill, different Rosa damascena petals extracts (hydro alcoholic extract, aqueous extract essential oil, hydrosol) were tested at different concentrations (200, 100, 50, 25 and 10 µg/mL) for PPL inhibition (Table 1).

With weaker effects than the positive control (orlistat) at each concentration, all extracts exhibited increasing inhibitory activities as concentration rose from 10 to $200 \,\mu\text{g/mL}$ (Fig. 1).

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Plant extract	Inhibition (%)
Alcoholic extract	62%
Aqueous extract	46%
Essential oil	55%
Hydrosol	20%
Orlistat	93%

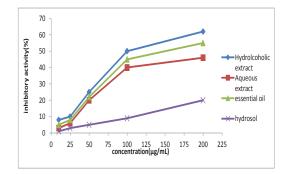


Fig. 1. Porcine pancreatic lipase inhibitory activities of different *Rosa damascena* extracts.

All the extracts showed an inhibitory effect and it ranged from 62% to 20%. Hydro alcoholic extract had the highest lipase inhibitory effect 62% at 200 μ g/mL, while hydrosol had the least one 20%, Aqueous extract showed 46% inhibition of PPL, essential oil showed 55% inhibition (Fig. 2).

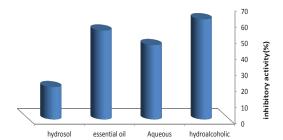


Fig. 2. Comparison between inhibitory activity of different *Rosa damascena* extracts.

The anti-lipase activities are also expressed as percent (%) inhibition relative to that of orlistat (i.e., orlistat equivalents) (Table 2), as orlistat is the only pancreatic lipase inhibitor currently approved for a long-term treatment of obesity.

The inhibitory curve for orlistat is shown in Fig. 3, where 200 μ g/mL of orlistat resulted in 93% inhibition of pancreatic lipase activity.

 TABLE 2. Orlistat equivalent of Rosa damascena extracts.

Diant antra at	Orlistat Equiv.		
Plant extract	(µg/mL)		
Hydrolcoholic extract	8,8		
Aqueous extract	6,5		
Essential oil	7,8		
Hydrosol	2,8		

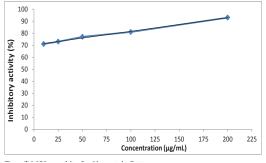


Fig. 3. Inhibitory activity of positive control orlistat.

Conclusions

Natural products are in increasing demand from the manufacturers of foods, cosmetics and pharmaceuticals that explains importance of studies and research on medical plants.

This study shows that *Rosa damascena* extracts are capable of inhibiting lipase activity, hydro alcoholic extract is considered very cheap compared with essential oil, and it has higher PPL inhibition, that's why research should be focused on it especially that, majority of studies are on essential oil, and attempt should be made to isolate active components of this extract to be used as anti-obesity agents in the future.

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الفعالية المضادة لأنزيم ليباز البنكرياس لخلاصات الوردة الدمشقية

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تعتبر البدانة داء العصر وهي متعلقة بنسب إمر اضيات ووفيات مرتفعة. إحدى الإستر اتيجيات المتبعة في الوقاية و علاج البدانة هي تثبيط أنزيم ليباز الببكرياس المسؤول عن امتصاص الدسم.

يهدف البحث إلى تقييم الفعالية المضادة لأنزيم ليباز البنكرياس لخلاصات الوردة الدمشقية وهي (الخلاصة الكحولية، الخلاصة المائية، الزيت العطري ماء الورد).

تم قياس الفعالية المضادة لأنزيم ليباز البنكرياس في الزجاج باستخدام المعايرة اللونية و مركب -4 2, 4 معايرة اللونية و مركب -4 2, 4

النتائج: أبدت الخلاصات قيم مختلفة للفعالية المضادة لأنزيم الليباز، حيث امتلكت الخلاصة الكحولية للوردة الدمشقية أعلى قيمة مضادة لأنزيم الليباز (٦٢٪)، يليها الزيت العطري للوردة (٥٥٪) ومن ثم الخلاصة المائية بنسبة تثبيط (٤٦٪) و أخيراً ماء الورد(٢٠٪).

الاستنتاجات: تمتلك الخلاصة الكحولية للوردة الدمشقية فعالية مضادة لأنزيم ليباز البنكرياس، وتدعم هذه الدراسة إجراء اختبارات سريرية على فعالية الخلاصة حيوياً داخل جسم الإنسان لتكون علاج فعال و آمن في معالجة البدانة مستقبلاً.