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Research Article
Inhibitory Action of Date Palm (*Phoenix dactylifera* L.) Leaf Extract on Pancreatic Lipase and α-Amylase Activities

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Abstract

Background and Objective: Cardiovascular Diseases (CVDs) remain the main cause of mortality globally. High cholesterol levels (hypercholesterolemia) and high blood glucose (diabetes) are among the factors that increase the risk for CVDs. Application of inhibitors for the digestive enzymes accountable for the macronutrient hydrolysis, such as carbohydrates and fats, is one of the prevalent approaches in the development of medications against CVDs. The present study was performed to examine, *in vitro*, the lipase and amylase inhibitory potential of phenolic rich extract of leaves of four date palm cultivars. **Materials and Methods:** In the current study, the research investigated the potentiality of phytochemicals extracted from leaves of four date palm cultivars (Rawthan, Rabeaa, Barny and Ajwa), collected from Al-Madinah Governorate as lipase and amylase inhibitors and as antioxidants. Moreover, the total contents of flavonoids and phenolics were assessed. **Results:** The results revealed that all the tested cultivars showed promising lipase and amylase inhibition and antioxidants capacities. However, Rawthan and Ajwa were the most powerful cultivars. **Conclusion:** Therefore, the results presented herein suggest as the earliest report, the potential use of date palm leaves as a potential source for lipase and amylase inhibitors as an approach to decrease the risk for CVDs.

Key words: Cardiovascular diseases, date palm, leaf extract, lipase, α-amylase, hypercholesterolemia, maltose

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Cardiovascular Diseases (CVDs) primarily consist of Coronary Heart Disease (CHD), Rheumatic Heart Disease (RHD), stroke and cardiomyopathy are accountable for the principal cause of mortality worldwide[1]. The modern routine of populations backs the expansion of risk factors for CVD, such as hypertension, diabetes, smoking and hypercholesterolemia[2]. at the beginning of the twentieth century, CVD considered accountable for fewer than 10% of all mortality globally, however it summed up to 30% by 2017. By 2020, CVD will turn out to be the main cause of mortality and disability in low and middle-income countries[3]. Probable risk factors for CVD include hypertension, tobacco, physical inactivity, higher low-density lipoprotein cholesterol, diabetes and a cluster of interconnected metabolic risk factors[4].

The reports of the World Health Organization (WHO) and Ministry of Health Portal in the Kingdom of Saudi Arabia (MOH) show that people can decrease the risk of CVDs by actively being involved in regular physical activities, avoiding tobacco, selecting a diet rich in fruit and vegetables and staying away from foods with high amounts of fat, sugar and salt, besides keeping a healthy weight[5]. MOH Statistical Yearbook CVDs remain the reason for 42% of the Kingdom's non-infectious diseases deaths in 2010[5].

Cholesterol is produced nearly in all cells and significant concentrations of it can be absorbed from the diet[6]. Low-Density Lipoprotein (LDL) is considered the "bad" cholesterol type, attaching to the inner walls of the blood vessels, clogging them, causing heart issues for instance stroke, atherosclerosis and Coronary Heart Disease (CHD)[7]. Based on the lipid hypothesis, remarkably high amounts of cholesterol (hypercholesterolemia) or elevated levels of LDL cholesterol are considered the major lipid risk factors for CVDs[8]. Triglyceride (TG) is an ester made from glycerol, that later gets broken down by digestion. It is not possible for lipids to be absorbed by the duodenum in their intact form and they can be absorbed as fatty acids, monoglycerides and diglycerides. When the TG has been digested in the human body, elevated amounts of TG in the bloodstream are related to atherosclerosis and CHD[9]. Numerous observational studies and investigations reported in the previous year's mainly suggesting that TG is an independent risk factor for CHD[10]. Moreover, Risks for entire forms of CVD have risen considerably in patients with type 1 and type 2 diabetes[11]. The death rate in non-diabetic patients was lower in comparison to the diabetic patients who experienced CHD[12].

One of the most common methodologies in the drug development against the CVD is the application of inhibitors for the digestive enzymes accountable for the hydrolysis and absorption of macronutrients, such as carbohydrates and triglycerides[6]. Thus, inhibition of the digestive enzymes could improve glycemic index imbalance and glucose intolerance and decrease the levels of LDL cholesterol. The majority of the synthetic inhibitors in the market, for instance, acarbose, contain robust amylase and glucosidase inhibitory properties, leading to excessive inhibition of amylase activity, subsequently abnormal fermentation of undigested saccharides in the colon[13]. Thus, searching for new sources of digestive enzymes inhibitors is of immense importance.

Pancreatic lipase (EC 3.1.1.3) is considered a crucial enzyme in triacylglycerol absorption, causing hydrolyzation of triacylglycerol to 2-monoacylglycerol as well as fatty acids. It is common knowledge that dietary fat cannot be directly absorbed from the intestine unless it has been treated by pancreatic lipase[14]. α-Amylases (E.C.3.2.1.1.), starch-degrading enzymes, catalyze the hydrolysis of internal α-1,4-O-glycosidic bonds in polysaccharides with the retention of α-anomeric arrangement in low molecular weight products, such as glucose, maltose and maltotriose units[15]. Two major isoforms of human amylases exist, pancreatic amylase and salivary amylase[16]. Pancreatic α-amylase stands a crucial enzyme in the digestive system and catalyzes the first step in starch hydrolysis[17]. Amylase amounts are conversely connected to a lot of cardiometabolic risk factors and might echo metabolic abnormalities and abnormal glucose metabolism[18].

Pancreatic lipase (EC 3.1.1.3) and α-amylase which were produced and secreted by the pancreas have an important role in the principal lipolytic and glycosides chain hydrolysis to efficiently digest triglycerides, starch and glycogen. Thus, molecules that revealed pancreatic and α-amylase inhibitory activities decrease energy absorption and intake and contain a potential application for the treatment of obesity and CDVs[19].

Date palm (*Phoenix dactylifera* L.) is very important and widely ranged in the Middle East[20]. It is used usually for consumption by human, for clinical use and its chemical properties[21]. Date palm's fruits have 88% of sugar, therefore they are considered a high-energy source[22]. There is big value importance in Date palm's therapeutic and nutritional point of view[23]. It is a valuable source for minerals, sugars, fibres, essential amino acids, unsaturated fatty acids and secondary metabolites. The leaves of the Date palm also have antimutagenic, antifungal and antibacterial properties[24].

Moreover, the extracts of Date palm's leaf have natural antioxidant properties, which can be used for health care and medical care[25]. A strong correlation has been reported between the polyphenolic and flavonoids content of Date palm extract and its free radical scavenging ability[26].

Numerous publications have highlighted the inhibitory potential of plant phenolic acids and flavonoids against pancreatic lipase and amylase[6,14]. Therefore, owing to its valuable content of phenolic acids and flavonoids, date palm leaves could be a candidate source for lipase and amylase inhibitors. To our knowledge, the ability of date palm leaves to inhibit pancreatic lipase and amylase has not been investigated. Consequently, the present project was performed to examine, *in vitro*, the lipase and amylase inhibitory potential of phenolic rich extract of leaves of four local cultivars of date palm (Rawthan, Rabeaa, Barny and Ajwa). Moreover, the total content of phenolic acids and flavonoids and the total antioxidant capacity of the extracts were tested.

MATERIALS AND METHODS

Study area: This research was conducted from February-July 2020, at the Department of Biology, Faculty of Science, Taibah University, Saudi Arabia.

Plant Materials: Leaves of four date palm cultivars (Rawthan, Rabeaa, Barny and Ajwa) were collected from local farms in Al-Madinah. The leaves were thoroughly washed to remove all traces of dust, dried and ground to fine chops.

Extraction of phytochemicals: Phytochemicals were extracted in 50% aqueous methanol. Briefly, dried powdered plant materials were extracted by infusion in the solvent at the rate of 10% (w/v) for 24 hrs, at ambient temperature. Afterthought, each extract centrifuged for 15 min at 3000 g and the residue washed with the same solvent and centrifuged once again. The clear supernatants obtained from each plant were combined and evaporated at 50°C. The final volume in each extract was adjusted to 1/10 of its original volume to obtain a concentration of 100%, w/v. The extracts were stored in the refrigerator until the time of the proceeding analyses.

Determination of phenolic compounds: The Folin-Ciocalteu phenol method[27] was utilized for phenolic aglycone determination. One mL of the diluted extract was combined with 1 mL of 10% Folin-Ciocalteu phenol reagent and 1 mL of 20% anhydrous sodium carbonate and levelled up to a known volume by addition of distilled water. The blue colour absorbance was measured after 30 min at 650 nm versus a water reagent blank. The phenolic amount was measured from the catechol standard curve and assessed as mg phenolic g^{-1} dry weight. Subtraction of phenolic amount before and after acid hydrolysis resulted in the number of phenolic glycosides.

Flavonoid's determination: Total flavonoids were determined in the diluted extract by using the protocol reported by Sakanaka *et al.*[28]. A pre-determined volume of the extract (0.25 mL) was combined with 1.25 mL of distilled water inside a test tube, subsequently 75 µL of 5% (w/v) sodium nitrite solution was added. After 6 min of incubation, 150 µL of 10% (w/v) aluminium chloride solution was added, then the mixture was set aside for an additional 5 min before adding 0.5 mL of 1 M sodium hydroxide. The mixture was levelled up to 2.5 mL with the addition of distilled water and combined well. The absorbance was assessed promptly at 510 nm. The concentration of total flavonoids was estimated using the catechin standard curve and stated as mg catechin g^{-1} fresh weight.

Determination of total antioxidant activity: The total antioxidant activity of the plant extracts was determined according to the method outlined in Mohamed *et al.*[29] and calculated as Trolox equivalent using a standard curve for Trolox.

Evaluation of the lipase and amylase inhibition capacities: Lyophilized Lipase and α-amylase (FLUKA) were dissolved in distilled water at the rate of 10 mg mL^{-1}. After centrifuging at 5,000 rpm for 10 min at 4°C, the supernatants were used in the experiments.

The inhibitory activity of each extract versus α-amylase was assessed using starch as the substrate, following the protocol described in Herrera *et al.*[6] with some modifications. Succinctly, the reaction mix contained 1 mL of extract solution at different concentrations plus 1 mL of α-amylase solution at 10 mg mL^{-1} in a buffer. This mix was positioned in an incubator at 37°C for 10 min. Following pre-incubation, the reaction started by adding 1 mL of potato starch solution (0.5% in water heated at 100°C for 15 min). After incubation (30 min at 37°C) the reaction was completed by adding 0.1 mL of 50 mM $HgCl_2$. The formed reducing sugars were determined

calorimetrically using Nelson's method adopted by Clark and Switzer[30]. To eliminate the error due to the endogenous reducing sugars in the extract, a negative control was conducted in the same manner described above but with a boiled enzyme.

For lipase inhibition assay the method reported by Abd-Elhakeem *et al.*[31] was followed up using phenylacetate as a substrate. Succinctly, the reaction mix contained 1 mL of extract solution at different concentrations and 1 mL of lipase solution at 10 mg mL^{-1} in a buffer. This mix was positioned in an incubator at 37°C for 10 min. Following pre-incubation, the reaction started by adding 1 mL of 150 µM phenylacetate. After incubation (30 min at 37°C) the reaction was completed by adding 0.1 mL of 50 mM $HgCl_2$. Thereafter, the reduction in the substrate concentration was assessed by spectrophotometry using the Folin-Ciocalteu phenol method described above[27].

The inhibition of enzyme activities was calculated as follows:

$$\text{Inhibition (\%)} = 100 - \frac{A_{\text{substrate control}} - (A_{\text{extract sample}} - A_{\text{extract control}})}{A_{\text{substrate control}} - A_{\text{control sample}}} \times 100$$

RESULTS

Phenolic contents of date palm leaves: The result of Fig. 1 shows the concentrations of phenolic compounds extracted from the leaves of four date palm cultivars. The concentration phenolic ranged from 5.54-7.71 mg g^{-1} dry weight. The Higher concentration of phenolic was detected in the leaves of Rawthana, while the lowest one detected in the Barny cultivar.

Flavonoids contents of date palm leaves: The contents of flavonoids in the leaves of the tested cultivars are represented in Fig. 2. The levels of flavonoids varied between 1.02-2.06 mg g^{-1} dry weight. The concentrations of flavonoids in date Palm leaves extracts followed the sequence Rawthan>Ajwa>Rabeaa>Barny.

Total antioxidant capacity: The result of Fig. 3 demonstrates the Total Antioxidant Capacity (TAC) of the tested date palm cultivars. TAC of the leaf extracts varied between 0.2-0.35 mM Trolox equivalent mL^{-1} extract. The higher antioxidant capacity was detected in extracts of Rawthan while the lowest ones detected in Barny.

Lipase inhibition capacity of date palm leaves: The *in vitro* lipase inhibition capacity of the date palm leaves was determined (Fig. 4). IC_{50} of the tested leaf extracts ranged from 6.85-8.25 (mg mL^{-1}). The highest inhibition capacity was detected for Rawthan and Ajwa cultivars, where they showed the lowest IC_{50} (6.85 and 7.13, respectively). On the other hand, the Barny cultivar showed the lowest inhibitory action versus lipase activity.

Amylase inhibition capacity of date palm leaves: α-Amylase inhibition capacities of the four cultivars of date palm were evaluated and graphically represented in Fig. 5. The IC_{50} of the tested cultivar ranged from 9.12-26.54 (mg mL^{-1}). Rawthan and Ajwa cultivars showed much higher α-amylase inhibition capacity (IC_{50} = 9.12 and 9.60 mg mL^{-1}, respectively) than those exerted by Rabeaa and Barny (IC_{50} = 22.11 and 26.54 mg mL^{-1}, respectively).

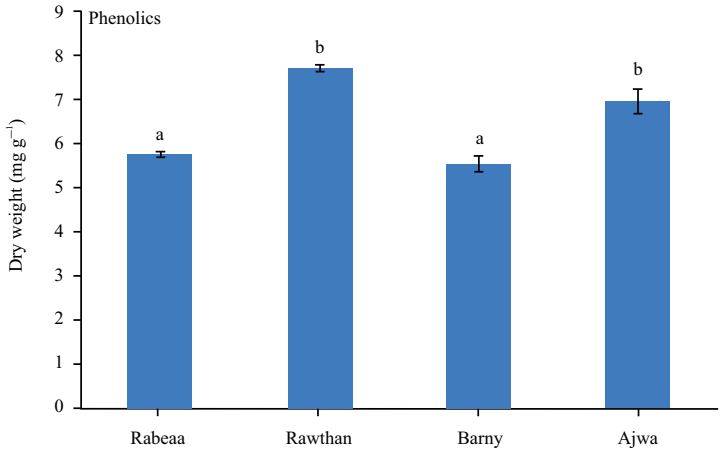

Fig. 1: Total phenolic amounts in the leaves of date palm cultivars

Reported numbers are the average of 3 trials (Mean±SE). The letters a and b show significant differences among the cultivars (Duncan's test $p<0.05$, n = 3)

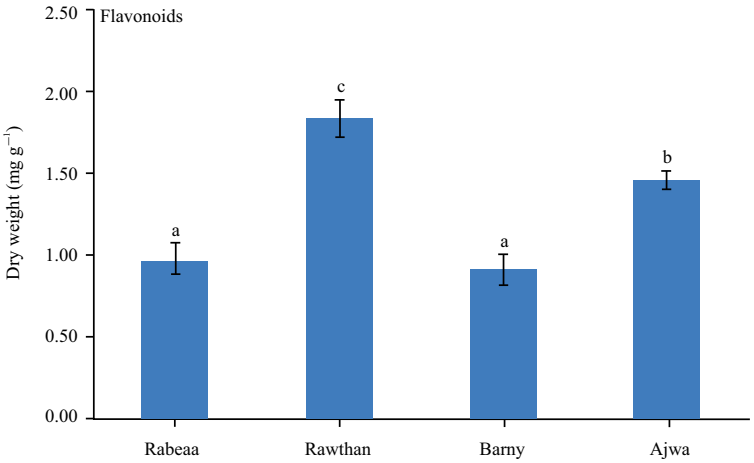

Fig. 2: Total flavonoid contents in the leaves of date palm cultivars

Reported numbers are the average of 3 trials (Mean±SE). The letters a, b and c show significant differences among the cultivars (Duncan's test p<0.05, n = 3)

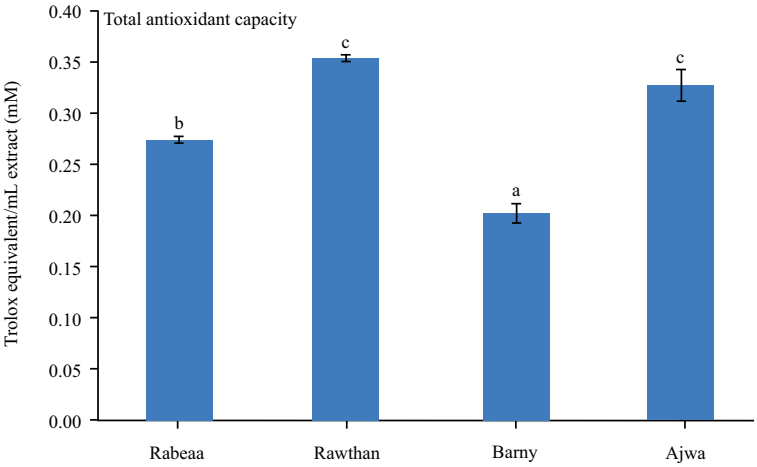

Fig. 3: Total antioxidant capacity of the extracts o date palm leaves

Reported numbers are the average of 3 trials (Mean±SE). The letters a, b and c show significant differences among the cultivars (Duncan's test p<0.05, n = 3)

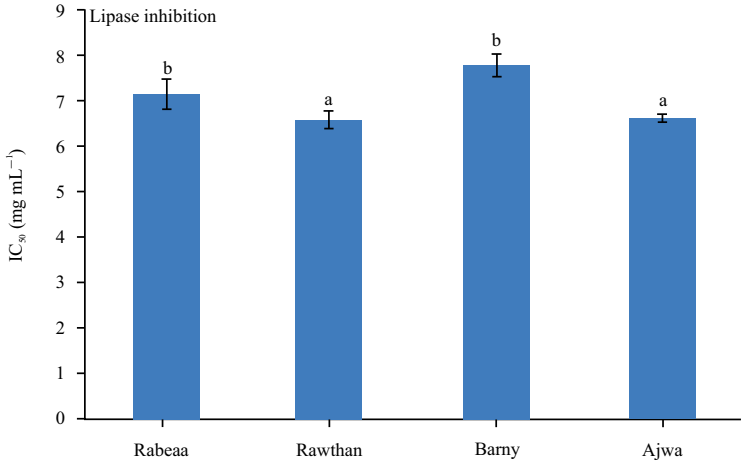

Fig. 4: Lipase inhibition capacity of the extracts o date palm leaves

Reported numbers are the average of 3 trials (Mean±SE). The letters a and b show significant differences among the cultivars (Duncan's test p<0.05, n = 3)

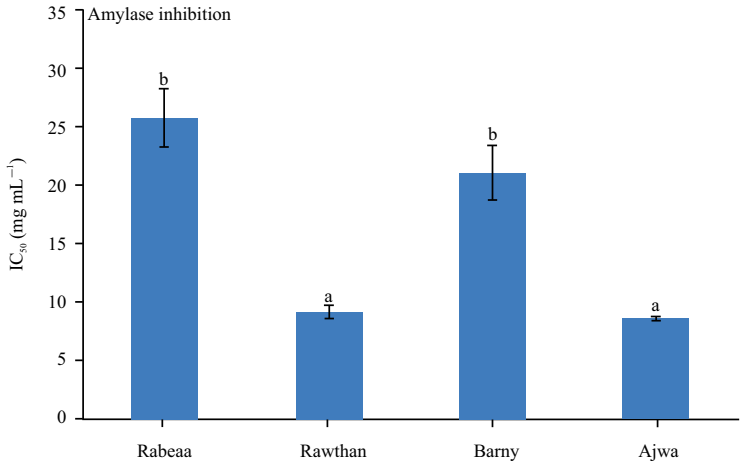

Fig. 5: Amylase inhibition capacity of the extracts o date palm leaves

Reported numbers are the average of 3 trials (Mean±SE). The letters a, b and c show significant differences among the cultivars (Duncan's test $p<0.05$, $n = 3$)

DISCUSSION

In the current study, we have explored the potentiality of phytochemicals extracted from leaves of four date palm cultivars (Rawthan, Rabeaa, Barny and Ajwa) as lipase and amylase inhibitors. The results revealed that all the tested cultivars are promising sources for lipase and amylase inhibitors, however, Rawthan and Ajwa were the most powerful. One of the common methodologies in the drug development against CVD is the application of inhibitors for the digestive enzymes accountable for the hydrolysis and absorption of macronutrients, such as carbohydrates and triglycerides[6]. In this regard, extracts from several plant species, such as fenugreek, *Opuntia ficus-indica*, berry and quinoa, were reported for their lipase and amylase inhibitory activity[6,14,32].

Reactive oxygen species such as free radicals are recognized as major reasons for chronic diseases. A key factor that governs the medicinal worth of plants is their antioxidant capacities[33]. In the present study, leaf extracts of the tested date palm cultivars showed considerable antioxidant potentials. TAC of the leaf extracts was dependent on the tested cultivar. Similarly, previous studies reported that fruits of the different date palm cultivars collected from local markets in Saudi Arabia showed variation in their antioxidant capacity[26]. They reported that among the studied cultivars Khlas El shiokh, Khlas Al Ahsa, Khlas Al Kharj showed the strongest DPPH scavenging capacity.

The biological activities of plants, including hypocholesterolemic, antidiabetic and antioxidants, are mainly ascribed to their valuable contents of secondary metabolites such as phenolic acids, flavonoids, saponins and alkaloids[34-37]. Moreover, it is understood that qualitative and quantitative differences in the production of secondary metabolites, containing phenolic acids and flavonoids, are affected by genetic, morphological and environmental factors[38,39]. In the present study, the total contents of flavonoids and phenolics in the leaf extracts of the tested date palm cultivars and their potential as inhibitors for lipase and amylase were evaluated. The results revealed that all the tested extracts inhibited the activities of lipase and amylase and showed considerable TAC, whereas a positive relationship between the levels of total flavonoids and phenolics on one side and the biological activities (amylase and lipase inhibition and TAC). These results point to a significant role for polyphenols as antioxidants and hypocholesterolemic agents, through inhibiting the activities of digestive enzymes. In this regard, Hozzein *et al.*[40] reported that CO_2 enrichment could improve the anti-lipase activity of fenugreek seeds by improving the accumulation of phenolic acids and flavonoids. Further, Mohamed *et al.*[29] stated that there were strong positive correlations between the total antioxidant capacity of several plant species and the levels of their phenolics and flavonoids. The strong relationship between phenolics content and TAC is reasonable as phenolic molecules are popular for their free radical scavenging activity[41]. Therefore, the present study suggests the potential use of date palm leaves as a cheap and safe source for lipase and amylase inhibitors, an approach to decrease the risk for CVD. As far as we know, this is the initial study dealing with the lipase and amylase inhibitory action of date palm leaves.

CONCLUSION

This study points to the anti-glycemic and hypocholesterolemic potential of date palm leaves. Such beneficial role of date palm leaves could be attributed to the

biologically active phytochemicals, such as polyphenols, that showed a significant tendency to inhibit the activities of pancreatic lipase activity. Therefore, these phytochemicals could help reduce the absorption of macronutrients such as carbohydrates and triglycerides in the small intestine, providing cheap and safe protective agents to decrease the risk for CVD.

SIGNIFICANCE STATEMENT

Bioprospecting for biologically active phytochemicals provides a promising approach to avoid the side effects of synthetic pharmaceuticals. Herein, the potential role of phytochemicals extracted from date pam leaves as inhibitors for digestive enzymes, such as pancreatic lipase and amylase was investigated. This study will help the researcher in developing plant-based pharmaceuticals to decrease the risk for CVD.

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