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Phytochemical Screening, *in vitro* α-amylase and Pancreatic Lipase Inhibition Effects of *Beta vulgaris* Extracts

Mohammed Ali Ahmed Saeed [1*], Alaa Abdulsalam Mohammed[1], Abdulrahman Ahmed Bayasein[1], Ahmed Hassan Ba-saleh[1], Balqees Ali Babasil[1], Ibrahim Saleh Badakhan[1], Abdulrahman Salem AL-Amodi[1], Ahmed Sami Alammari[1], Anisa Jama Abdullahi[1], Hamdan Ali Blkhader[1], Saleh Awadh Al-Sader[1]

[1] Department of Pharmacy, Faculty of Medicine and Health Sciences, University of Science and Technology, Aden, Yemen

ABSTRACT

The use of medicinal plants for healing dates back to ancient times. *Beta vulgaris* is a medicinal plant found in Yemen. Objective: The current study aimed to screen the phytochemicals, and to determine the inhibition effect of *B. vulgaris* extracts on α-amylase and pancreatic lipase. Methods: *B. vulgaris* was extracted using different solvents (ethanol 96% ethanol 50% or water), and screened for the possible phytochemicals. Furthermore, the plants extracts were studied for the α-amylase and pancreatic lipase inhibition effects according to the protocol of Cobas Company. Results: The findings of phytochemical screening showed the presence of alkaloids, saponins, terpenoids and tannins. *B. vulgaris* extracts showed α-amylase inhibition activity (59.26-63.66%). In addition, *B. vulgaris* extracts showed inhibition effects (40.87-48.45%) on pancreatic lipase, while BV50 exhibited the highest inhibition effects on α-amylase and pancreatic lipase. Conclusion: *B. vulgaris* extracts showed potential α-amylase and pancreatic lipase inhibition effects *in vitro*, suggesting possible anti-hyperglycemic and anti-obesity properties. Further research is recommended to carry out in vivo study for the anti-hyperglycemic and anti-obesity effects, and to isolate and determine the active compounds responsible for these effects.

Keywords: *Beta vulgaris*, Beetroot, phytochemical screening, α-amylase and pancreatic lipase, anti-hyperglycemia, anti-hyperlipidemia

* *Corresponding author email:* mohali141@yahoo.com

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INTRODUCTION

A metabolic disorder called diabetes mellitus (DM) is typified by hyperglycemia brought on by abnormalities in insulin secretion and activity (1). Abnormally high blood levels of one or more lipids and/or lipoproteins are referred to as hyperlipidemia or hyperlipoproteinemia (2). To find new medications, ethnobotanical and phytochemical research is helpful. One crucial step in identifying the bioactive ingredients found in medicinal plants used in traditional medicine is phytochemical screening (3).

The AMY1 gene encodes α-amylase, an enzyme that is essential for starting the breakdown of starch in the mouth cavity. Enzymatic activity and α-amylase protein levels are positively correlated with the AMY1 gene's copy number. Numerous studies have connected metabolic diseases like insulin resistance, diabetes, and obesity to α-amylase activity. In addition, among overweight/obese adult females, there are strong correlations between α-amylase and markers of inflammation and cardiovascular disease risk (4).

Pancreatic lipase (triacylglycerol acyl hydrolase), a crucial enzyme of pancreatic juice secreted by pancreatic acinar cells, is in responsible for breaking down dietary triglycerides (roughly 50–70% of total dietary lipids) in the small intestine and is necessary for intestinal absorption and simulation. Since this enzyme is regarded as a major regulator of lipid metabolism, it may be the most effective target for the development of anti-obesity medications (5).

Beta vulgaris (beetroot) belong to Amaranthaceae family (6). Beetroot has been recognized for its wide range of medicinal properties, including its antioxidant, antidepressant, antimicrobial, anti-inflammatory, anticarcinogenic, immunomodulatory, and diuretic effects. *B. vulgaris* exhibited pharmacological effects such as antimicrobial (7), antihyperlipidemic (8), hypoglycemic, antioxidant, and anti-inflammatory effects (9), anti-atherogenic, anti-hypertensive and anti-hypercholesterolemia and increase level of HDL in rats (10), and also has anticancer effect against breast cancer cells (11-13). The current study aimed to screen the phytochemicals, and to determine the inhibition effect of *B. vulgaris* extracts on α-amylase and pancreatic lipase.

METHODS

Plant extraction

One hundred gram of *B. vulgaris* root was extracted by maceration method with 700 mL of ethanol 96%, ethanol 50%, or distilled water (D.W), respectively, in water bath at 50°C for 3 days. Extracts were filtered by filter paper separately and solvents were evaporated accordingly and extracts were stored at -5°C until further use (14).

Phytochemical screening

The *B. vulgaris* ethanol 96% extract (BV96), *B. vulgaris* ethanol 50% extract (BV50), and *B. vulgaris* water extract (BVA) were screened for the following phytochemicals (15).

- Test for Tannins

Before being filtered, 250 mg of each plant extract was separately mixed with 5 ml of distilled water, dissolved water, and extract until it was fully dissolved. Each combination was then treated with three drops of 5% ferric chloride. The precipitate's black or blue-green coloring was interpreted as a sign that tannins were present.

- Test for Saponins

To make 10 mL in a test tube, 0.5 g of plant extract was mixed separately with distilled water. The presence of saponins is indicated by the production of froth that lasts for five minutes after warming in a water bath.

- Test for Alkaloids

15 mg of each extract was mixed individually with 6 mL of 1% HCL in a water bath for 5 minutes, then filter.

Dragendorff's reagent (potassium bismuth iodide solution) (1 mL) has been added to one portion of the filtrate; the presence of alkaloids is indicated by an orange-red precipitate.

- Test for terpenoids

Salkowski test: A reddish-brown coloration of the interface shows the presence of terpenoid. The crude extract (about 100 mg) was mixed separately with

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chloroform (2 mL) and then concentrated H_2SO_4 (2 mL) was added along the test tube's wall.

- Test for steroids

Liebermann-Burchard test: 100 mg of each extract was mixed with chloroform in a test tube, followed by a few drops of acetic anhydride, boiling in a water bath, and quickly cooling in ice water. Two milliliters of concentrated H2SO4 were added to the test tube. Steroids are present when a brown ring forms at the intersection of two layers and the top layer turns green (16).

α-amylase Inhibition Effect

B. vulgaris extracts were analyzed for α-amylase inhibition effect according to the protocol of Cobas Company. The concentration of samples is 500 µg/ml. Metformin (Met) 500 µg/ml was used as a positive control.

Pancreatic Lipase Inhibition Effect

B. vulgaris extracts were analyzed for pancreatic lipase inhibition effect according to the protocol of Cobas Company. The concentrations of samples were 500 µg/ml. Orlistat (Orl) 120 µg/ml was used as a positive control.

Statistical analysis

Data are expressed as mean ± SD and analyzed using ONE WAY ANOVA Statistical Package for the Social Sciences (SPSS 26.0). Results were considered significant at $P < 0.05$.

RESULTS

The yield percentage of plant extracts are shown in Table 1. The results revealed that the highest yield percentage among BV extracts was BV96, whereas the lowest was BV50 (Table 1).

Table (1): Yield percentage of BV extracts

No.	Extracts	Yield %
1	BV96	1.38±0.12
2	BV50	0.89±0.10
3	BVA	1.14±0.14

The content of alkaloids, steroids, terpenoids, tannins, and saponins was examined in the BV96, BV50, and BVA. The qualitative findings are shown as (+) when phytochemicals are present and (-) when they are not (Table 2). BV50 showed the presence of all the tested phytochemicals except steroids compared to BV96 and BVA, and this could be due to the polarity of solvents used, as BV50 was extracted by water: ethanol 50:50.

Table (2): Phytochemical screening of *B. vulgaris* extracts

Extracts	Alkaloids	Terpenoids	Steroids	Tannins	Saponins
BV96	+	+	-	-	+
BV50	+	+	-	+	+
BVA	-	+	-	+	-

+ = presence, - = absence

All *B. vulgaris* extracts showed α-amylase inhibition effects, the highest α-amylase inhibition activity was observed for BV50. The positive control metformin (Met) showed an α-amylase inhibition activity but less activity compared to BV96, BV50 and BVA (Figure1).

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There was no significant differences between *B. vulgaris* extracts and the positive control MET.

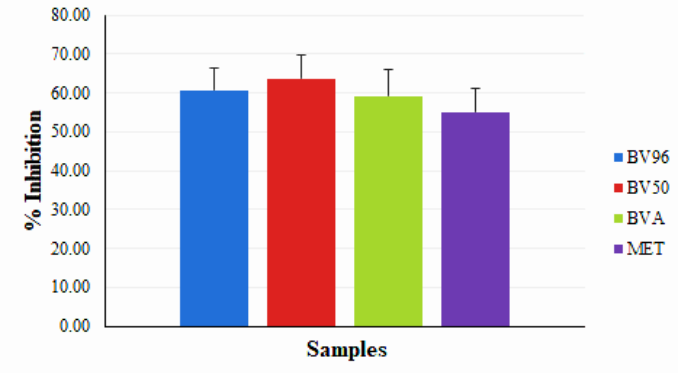

Figure 1: α-amylase inhibition effects of *B. vulgaris* extracts. There was no significant differences between *B. vulgaris* extracts and the positive control MET.

All *B. vulgaris* extracts showed inhibition effect on pancreatic lipase activity *in vitro* suggesting possible anti-obesity properties, warranting further investigation. The positive control orlistat (Orl) showed inhibition effect on pancreatic lipase, and its effect is comparable to the plant extracts. The highest inhibition activity was observed for BV50 (Figure 2). There was no significant differences between *B. vulgaris* extracts and the positive control Orl.

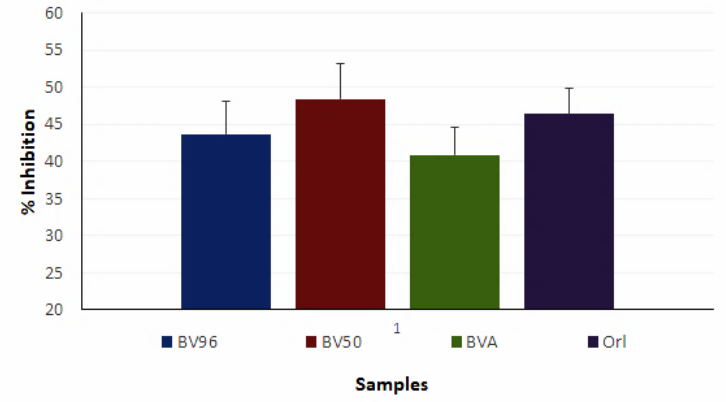

Figure 2: Pancreatic lipase Inhibition effects of *B. vulgaris* extracts. There was no significant differences between *B. vulgaris* extracts and the positive control Orl.

DISCUSSION

In this study, the phytochemical screening of plant extracts identified the presence of a few secondary metabolites, including alkaloids, tannins, terpenoids and saponins. It is recognized that the phytochemical substances found have therapeutic value. Alkaloids, for instance, have been described as potent poisons, and many of the alkaloids obtained from medicinal plants exhibit biological activities such as cytotoxicity, antispasmodic, anti-inflammatory, antimalarial, and antibacterial properties (17, 18). Likewise, plant-based steroids are known to have antibacterial and insecticidal qualities in addition to their cardiotonic impact (19).

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Because of their well-known biological functions, they are frequently employed in medications. Research has shown that tannins have antiviral, anticancer, and antibacterial properties (20-23). They function by precipitating microbial protein, which prevents them from accessing nutritious protein. The biological activities displayed by plant extracts may be caused by these phytochemical substances found in the extracts (15).

Together with lifestyle modifications, Met is the mainstay and first-line medication for controlling hyperglycemia in individuals with type 2 diabetes. Met also helps with weight loss, vascular disease prevention, and the metabolism of carbohydrates. Met can be taken alone or in combination with other medications. The decrease in vitamin B12 levels is one of its adverse effects, though. Both the diagnosis and treatment of vitamin B12 insufficiency are underdiagnosed. Peripheral neuropathy, mental-psychiatric problems, and macrocytic anemia can result from severe deficiencies, such as pernicious anemia (24). *B. vulgaris* extracts showed inhibition on α-amylase activity *in vitro* suggesting possible anti-hyperglycemic properties, warranting further investigation. The positive control MET showed comparable effects to *B. vulgaris* extracts. There was no significant differences between *B. vulgaris* extracts and the positive control MET.

Orl functions by preventing the breakdown of triglycerides (TG) in the intestine by the enzymes pancreatic and gastric lipases. Orl's lipase inhibition mechanism works by forming a covalent link with the serine in the lipase's active site. When lipase function is inhibited, dietary TG are not hydrolyzed into absorbable free fatty acids and are instead eliminated undigested. Orl has certain unpleasant gastrointestinal side effects, while being a clinically authorized pancreatic lipase inhibitor for the treatment of obesity. These adverse effects, which are caused by Orl's mode of action, include oily spotting, watery stools, and cramping in the abdomen (25). It was shown that the *B. vulgaris* extracts' percentage inhibition of pancreatic lipase activity is nearly identical to Orl's. In comparison to Orl, the plant extracts had comparatively modest concentrations of the active chemicals (26).

CONCLUSION

Alkaloids, terpenoids, tannins and saponins were found in *Beta vulgaris* extracts, and these extracts demonstrated potential α-amylase and pancreatic lipase inhibition effects *in vitro*, suggesting possible anti-hyperglycemic and anti-obesity properties. Further research is recommended to carry out *in vivo* study for the anti-hyperglycemic and anti-obesity effects, and to isolate and determine the active compounds responsible for these effects.

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CONFLICT OF INTEREST

The authors declare that no conflict of interest.

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