An-Najah National University

Faculty of Graduate Studies

Medicinal Plants as a Source of Inhibitors of the Digestive Enzymes: Alpha-glucosidase, Alpha-amylase and Pancreatic Lipase

By

Raghad Albzoor

Supervisor

Dr. Awni Abu-Hijleh

Co-supervisor

Prof. Hilal Zaid

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This thesis was defended Successfully on 21 / 3 /2019 and approved by:

Defense Committee Members	**Signature**
1. Dr. Awni Abu-Hijleh / Supervisor	**……**
2. Prof. Hilal Zaid / Co-supervisor	**……**
3. Dr. Siba Shanak / External Examiner	**……**
4. Dr. Nidal Jaradat / Internal Examiner	**……**

Dedication

I dedicate this thesis to my husband Abdalraheem and my children Alma and Obay, my parents, sisters, brothers, all the family members and friends.

Acknowledgements

First of all, my immeasurable thanks to my God, who has enabled me to accomplish this work.

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I would thank my husband, my mother, father, sisters, brothers and friends for their continuous love and support in my decisions and my life.

Thank you all …

الاقرار

أنا الموقعة أدناه موقعة الرسالة التي تحمل العنوان:

Medicinal Plants as a Source of Inhibitors of the Digestive Enzymes: Alpha-glucosidase, Alpha-amylase and Pancreatic Lipase

أقر بأن ما اشتملت عليه الرسالة هو نتاج جهدي الخاص، باستثناء ما تمت الاشارة اليه حيثما ورد، وأن الرسالة ككل، أو أي جزء منها لم يقدم من قبل لنيل أي درجة أو لقب علمي أو بحثي لدى أي مؤسسة تعليمية أو بحثية أخرى.

Declaration

The work provided in this thesis, unless otherwise referenced, is the researchers own work, and has not been submitted elsewhere for any other degree or qualification.

Students Name: **إسم الطالب:**

Signature: **التوقيع:**

Date: **التاريخ:**

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List of Abbreviations

A. cepa	*Allium cepa*
A. halimus	*Atriplex halimus*
A. sativum	*Allium sativum*
Abs	Absorbance
BMI	Body Mass Index
C. cassia	*Cinnamomum cassia*
DM	Diabetes Mellitus
GDM	Gestational Diabetes
GRAS	generally recognized as safe
J. regia	*Juglans regia*
N. sativa	*Nigella sativa*
O. europaea	*Olea europaea*
PL	Pancreatic Lipase
p-NPB	paranitrophenylbutylrate
SE	standard error
STZ	Streptozotocin
T. foenum-graecum	*Trigonella foenum-graecum*
T1DM	Type 1 Diabetes Mellitus
T2DM	Type 2 Diabetes
U. dioica	*Urtica dioica*
WHO	World Health Organization

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Abstract

Obesity and diabetes are reaching epidemic proportions all over the world in the twenty-first century. Herbal medicine has been suggested as an alternative source of potentially useful antihyperlipidemic and antihyperglycemic agents. The objective of this study was to assess in vitro the inhibitory activity of selected local anti-diabetic and anti-obesity medicinal plants on carbohydrate and lipid digestive enzymes namely, α-glucosidase, pancreatic α-amylase, and pancreatic lipase. The inhibitory activities of ethanol: water (50%:50%) six herbal extracts (namely: *Allium sativum*, *Allium cepa*, *Atriplex halimus*, *Cinnamomum cassia*, *Olea europaea* and *Trigonella foenum-graecum*) were evaluated using the enzymatic colorimetric assays. Among the six herbal extracts (at a concentration of 200 µg/ml), only *A. sativum* (bulb) showed an inhibitory activity against the intestinal sucrase (α-glucosidase) with a percentage inhibition of 46.74±11.55% ($p<0.01$). For pancreatic lipase only *C. cassia* (bark) showed an inhibitory activity with a percentage inhibition of 14.33±4.98% ($p<0.05$). The highest inhibitory percentage was received by *C. cassia* (bark) and *O. europaea* (leaves) on pancreatic α-amylase with IC_{50} value 24±3.01 and 192.94±6.4 µg/mL, respectively. Taken together,

these results indicate that the above mentioned plants can be potentially useful to treat diabetes or obesity. Further studies are needed for identification of the chemical composition and therapeutic effect of these plants and to identify the active ingredients responsible for these activities. *In vivo* tests are also required to examine other mechanisms responsible for the activity of these plants as antiobesity and antidiabetic agents.

Chapter One
Introduction

1.1 Background

The modern lifestyle and increase of consumption of high fat and high carbohydrate diets has led to an observed prevalence of obesity and diabetes worldwide. Hyperglycemia and hyperlipidemia are the major metabolic disorders associated with diabetes, overweight and obesity (Ogden *et al*., 2006; Zaid *et al*., 2015).

About 80% of the world's population still utilize herbal-based therapies as the main form of drugs, and about 25% of the currently used modern drugs conventional drugs contain at least one active phytochemical. In addition, about 75% of plants that provide active ingredients for prescription drugs came to the attention of researchers because of their use in traditional medicine (Saad *et al*., 2017).

Many medicinal plants have been reported to be useful in diabetes and have been used worldwide as antihyperglycemia and antihyperlipidemic remedies. More than 400 plant species having hypoglycemic activity have been available in literature. However, searching for new antidiabetic drugs from medicinal plants is still attractive because they contain substances which demonstrate alternative and safe effects on diabetes mellitus (Malviya *et al*., 2010).

1.1.1 Diabetes and its types

Diabetes or diabetes mellitus (DM) is a chronic disorder that can affect the metabolism of carbohydrates, fats and proteins. It is characterized by hyperglycemia, which is a consequence of defects in insulin secretion, insulin action, or both. Insulin is a hormone secreted from pancreatic beta cells and triggers some of the body organs (muscle, liver, and fat) to uptake more glucose from the bloodstream when glucose is elevated after a carbohydrate-rich meal (Zaid *et al.*, 2008). Several symptoms are associated with diabetes including but not limited to increased hunger, thirst and frequent urination. Untreated diabetes may cause several acute complications including ketoacidosis, kidney failure, stroke, heart disorders, eye damage, impotence, foot ulcer and death. The main characterized types of diabetes mellitus are: type 1 DM (T1DM), type 2 DM (T2DM) and gestational diabetes mellitus (GDM) (Saad *et al.*, 2017).

Type 1 Diabetes (which results from β-cells destruction, usually leading to absolute insulin deficiency) or Immune-Mediated Diabetes, is a form of diabetes, which accounts for only 5–10% of the characterized diabetes cases. This form was previously known as insulin-dependent diabetes or juvenile onset diabetes, and it results from the destruction of beta cells by a beta cell-specific autoimmune process. Autoimmune destruction of β-cells has multiple genetic predispositions and is also related to environmental factors that are still poorly defined (American Diabetes Association, 2014; Yoon & Jun, 2005).

Type 2 Diabetes accounts for 90–95% of the defined diabetic cases. Previously referred to as non–insulin dependent diabetes or adult-onset diabetes. This form varies from a predominant insulin resistance with relative insulin deficiency to a predominant insulin secretory defect with insulin resistance (American Diabetes Association, 2014; Kharroubi & Darwish, 2015).

Gestational diabetes (GDM) is defined as the diabetes form being diagnosed in the second or third trimester of pregnancy that is not clearly overt diabetes. The prevalence of GDM is increasing worldwide and is the most common metabolic disorder during pregnancy. It occurs in about 2–10% of all pregnancies and may disappear after delivery. It resembles type 2 DM in several respects, involving a combination of relatively inadequate insulin responsiveness and secretion. After delivery, approximately 5–10% of women with GDM are found to have type 2 DM (Santangelo *et al*., 2016; Saad *et al*., 2017).

1.1.2 Obesity

Obesity is the main risk factor for diabetes and other chronic diseases. According to the World Health Organization (WHO), obesity is defined as a condition of excessive or abnormal fat accumulation in adipose tissues, thus leading to health impairment. The body mass index (BMI) is commonly used as an indicator of obesity and overweight in adults. BMI is calculated through dividing body weight in kilograms by height in meters squared. The relationship between obesity and diabetes, particularly

T2DM, is known as diabesity (World Health Organization, 2000; Saad *et al*., 2017).

1.1.3 Carbohydrate and lipid digestive enzymes

Carbohydrate and lipid digestive enzymes (mainly pancreatic α-amylase, α-glucosidase and pancreatic lipase) play central roles in regulating body intake of fatty acids and sugars. α-Amylase represents the best known amylolytic enzyme. It catalyzes the hydrolysis of α-1,4-glucosidic bonds in starch and related α-glucans. It is an enzyme with a broad substrate preference and product specificity and one of the most frequently occurring glycoside hydrolases (Janecek *et al*., 2014).

Moreover, α-Glucosidase (α-1,4-glucoside glucohydrolase) is an enzyme being widely distributed in microorganisms, animals, and plants, and it catalyzes the liberation of α-glucose from the non-reducing end of the substrate. It is a membrane-bound enzyme in the epithelium of the small intestine, which works together with α-amylase to facilitate the absorption of glucose by the small intestine through catalyzing the hydrolytic cleavage of oligosaccharides into absorbable monosaccharides (Kumar *et al*., 2011). α-Glucosidase and α-amylase inhibitors such as acarbose are known to function by modulating the blood glucose level after a meal by interfering with the activity of carbohydrate-digesting enzymes and delaying glucose absorption. This mechanism is indeed employed in the current treatment of T2DM (Kato *et al*., 2016; Thilagam *et al*., 2013).

Lipases are enzymes that digest lipids. Pancreatic lipase (triacylglycerol acyl hydrolase), the lipolytic enzyme synthesized and secreted by the pancreas, plays a key role in the efficient digestion of triglycerides. It removes fatty acids from the 1 and 3 positions of dietary triglycerides, yielding β-monoglycerides and a long chain of saturated and polyunsaturated fatty acids as the lipolytic products (Birari & Bhutani, 2007; Shi & Burn, 2004). Orlistat is an inhibitor of the pancreatic lipases in the lumen of the gastrointestinal tract, and as such it interferes with the systemic absorption of dietary fat (Heck *et al*, 2000).

Plant-based medicine has gained enormous publicity in the world over the past three decades because of their safety (especially the 'GRAS' herbs, generally recognized as safe), effectiveness and availability (Zaid *et al*., 2016). Scientists have investigated many plants for the development of newer therapeutics for biologically active antihyperlipidemic and antihyperglycemic agents from natural resources (Seyedan *et al*., 2015; Zaid *et al*., 2015). Nevertheless, studies addressing the inhibition of these herbal medicines on the key enzymes of carbohydrate and lipid digestion, which include α-amylase, α-glucosidases and lipase, are still in their infancy (Sompong *et al*., 2016).

1.2 Literature Review

Diabetes mellitus is currently a growing global health concern. The estimated number of 171 million (2.8%) diabetics worldwide in 2000 is expected to increase to at least 366 million (4.4%) by the year 2030 (Wild

et al., 2004). The global prevalence of diabetes among adults over 18 years of age has risen from 4.7% in 1980 to 8.5% in 2014. The World Health Organization (WHO) projects that diabetes will be the seventh leading cause of death in 2030 (Mathers & Loncar, 2006; World Health Organization, 2016b).

The prevalence of obesity is also increasing at an alarming rate. In 2014, according to WHO, more than 1.9 billion adults aged 18 years and older were overweight (BMI $\geq$ 25 kg/m^2). Of these, over 600 million adults were obese (BMI $\geq$30 kg/m^2). Overall, about 13% of the world's adult population (11% of men and 15% of women) were obese in 2014, and 39% of adults aged 18 years and over (38% of men and 40% of women) were overweight. The worldwide prevalence of obesity more than doubled between 1980 and 2014 (World Health Organization, 2016a). Nonetheless, only a few medications are currently available. Newer approaches for the treatment of obesity have included inhibition of dietary triglyceride absorption via the inhibition of pancreatic lipase (PL), as this is the major source of excess calories. Natural products provide a vast pool of PL inhibitors that can possibly be developed into clinical products (Birari & Bhutani, 2007).

The current treatment of diabetes depends on suppressing and controlling blood glucose to the normal levels. Conventional drugs along with lifestyle management and weight control are being used to control diabetes. Regrettably, anti-diabetic drugs cause diverse side effects and are not entirely effective. Indeed, no cases have been reported to have recovered

totally from diabetes (Li *et al*., 2004). Several medicinal plants with certain degree of antidiabetic activity by different mechanisms of action have been reported. These include *Allium sativum* (garlic), *Allium cepa* (onion), *Atriplex halimus L.* (salt bush), *Cinnamomum cassia* (cinnamon), *Juglans regia L.* (walnut), *Nigella sativa* (black seed), *Ocimum basilicum L.* (basil), *Olea europaea L.* (olive), *Teucrium polium* (felty germander), *Trigonella foenum-graecum* (fenugreek), and *Urtica dioica L.* (nettle) (Kadan *et al*., 2013; Saad *et al*., 2017).

A study on the use of medicinal herbs by diabetic Jordanian patients was conducted through interviewing 310 diabetic patients who visited two medical centers in Jordan: The Medical Center of Jordan University of Science and Technology and Sarih Medical Center, between December 2003 and August 2004. Researchers found that 31% of the interviewed patients used herbal products (96 patients). The most commonly used herbs were *T. foenumgraecum* (22.9%), *Lupinus albus* (14.6%), *A. sativum* (11.5%), *Coriandrum sativum* (10.4%), *Cumminum cyminum* (9.4%), *Eucalyptus globules LA* (9.4%), *U. dioica L. (*8.3%), *N. sativa* (7.3%), *Zea mays L.* (6.3%), *A. cepa* (5.2%), *O. europea L.* (3.1%), *Salvia officinalis L.* (3.1%), and *Tilia cordata* (1%). The side effects were reported by 36.5% of the patients and included headache, nausea, dizziness, itching, palpitation, and sweating. Among the patients, 72.9% used the herbs as therapy along with their anti-diabetic drugs (Otoom *et al*., 2006).

The parts of plants that possess active compounds for the treatment of diabetes were studied. In some cases, the active ingredients are scattered all over the plants, and the entire plants were prepared and extracted for the desired ingredient. Generally, leaves are the favorable storage sites for desired compounds, and more than 35% of the plants extractions for diabetic treatment can be obtained from leaves. Fruits also contain substantial amounts of active ingredients. Therapeutic compounds can be extracted from other parts of plants such as root, aerial parts, flowers, seeds, stem barks, etc. (Chan *et al*., 2012).

Garlic cloves and onion bulbs are effective in the treatment and prevention of diabetes. They have many similar active compounds such as allyl propyl and diallyl sulfide. They increase insulin secretion from the pancreas. Nonetheless, the excessive consumption might lead to harmful effects on the body (Zaid & Saad, 2013). Aqueous garlic extracts (10% v/v) promoted glucose-induced insulin secretion on the isolated pancreas (Mustafa *et al*., 2007). It was reported that Garlic and onion decrease blood glucose levels by normalizing liver hexokinase and glucose-6- phosphatase activities (Sheela *et al*., 1995). In another study, daily oral feeding of garlic extracts at 100 mg/kg decreased plasma glucose levels and increased plasma insulin levels (Grover *et al*., 2002). Garlic ethanol extract, introduced orally to normal and alloxan-induced diabetic rats and rabbit, lowered blood glucose levels and increased insulin secretion (Chauhan *et al*., 2010).

A. halimus (Saltbush) and *O. europaea* (Olive) are extensively used to treat diabetes. A study of antidiabetic activity of aqueous leaf extract of *A. halimus* in streptozotocin–induced diabetic rats suggest that the aqueous leaf extract of *A. halimus* has beneficial effects in reducing the elevated blood glucose level in streptozotocin-induced diabetic rats (Chikhi *et al*., 2014). Powdered olive leaf (in a mixture with J. regia, U. dioica, and A. halimus) decreased glucose absorption from the intestine and lowered blood glucose levels in rats and diabetic subjects (Said *et al*., 2008).

T. foenum-graecum (fenugreek) has been used to treat a number of conditions including diabetes. Alkaloid extracts of fenugreek dried seeds was tested in streptozotocin induced hyperglycemic rats. It was administered orally for 21 days. Fenugreek extracts effect on blood glucose, serum insulin, and lipids was studied in diabetic rats. Result suggest that the mode of action of fenugreek may be caused by their contents of alkaloids through reducing the increased blood glucose level, thereby preventing hyperglycemia during diabetes and reducing lipid profile to almost normal (El-Soud *et al*., 2007).

C. cassia (also known as *Cinnamomum arromaticum*, Chinese cinnamon or Chinese cassia) is one of the well-known and oldest spices. There is an on-going debate whether *C. cassia* possesses an anti-diabetic effect. While some studies have shown no beneficial effect, others have indicated improvements in cholesterol levels, insulin sensitivity and postprandial glucose levels with cinnamon (Rafehi *et al.*, 2012). A study demonstrated that the intake of 1, 3, or 6 g of cinnamon (finely ground and capsulated

bark) per day by people with type 2 diabetes reduces serum glucose after 40 days (Khan *et al*., 2003). Studies supporting the anti-diabetic properties of cinnamon have proposed several mechanisms. A study compared *C. cassia* with acarbose, as a potential inhibitor of α-glucosidase, in streptozotocin- (STZ) nourished mice. Cinnamon reduced maltose induced post-prandial glucose spike by 86.3% (600 mg/kg body wt.) as compared to control 54.2% (5 mg/kg body wt.) ($P < 0.001$ vs. control). The reduction of sucrose induced post-prandial glucose spike was also significant with a reduction of 67.58% for cinnamon (600 mg/kg body wt.) against 70.71% for control (5 mg/kg body wt.) (Shihabudeen *et al*., 2011).

A study of inhibitory effects of 90% ethanol extracts of six allium species on α-Amylase found that both *A. sativum* (garlic) and *A. cepa* (onion) bulbs have a favorable α-amylase inhibitory activity (Nickavar & Yousefian, 2010). Garlic and onion water bulbs extracts also inhibited α -amylase and α–glucosidase (Wongsa *et al*., 2012). Garlic and onion bulbs extracts were prepared sequentially with petroleum ether, hexane, chloroform, ethanol and water were subjected to in vitro α-amylase inhibitory assay. Petroleum ether, hexane, chloroform and aqueous extracts showed no inhibitory effects however ethanol extracts showed minimal inhibitory activity (Kumar *et al*., 2010). α-Amylase inhibitory activity of onion bulb methanol extract was also documented (Jaiswal & Rizvi, 2017). Methanol extracts of *A.sativum* bulb exhibited inhibitory activities for sucrase 24.11±1.45% and for maltase 19.37±1.81% (Moradabadi *et al*., 2013).

Water, methanol, isopropanol, acetone, methyl-butyl-tertiary ether and cyclohexane were used in sequential order to make extracts of *T. foenum-graceum* seeds and *A. sativum* rhizomes. All *T. foenum-graceum* seeds extracts and none of *A. sativum* rhizomes extracts showed potential in vitro alpha amylase inhibitory activity (Sudha *et al*., 2011). Methanol and water extracts of *T. foenum-graecum* seeds, *A. sativum* bulb, *O. europaea* leaves displayed potentials for invoking amylase and lipase inhibitor activity (Buchholz & Melzig, 2016). In a distinct study, ten selected medicinal plants were screened for α-amylase inhibitory activities. Among the tested samples, *T. foenum-graecum* seeds revealed appreciable α-amylase inhibitory activities in a concentration-dependent manner (Nickavar & Yousefian, 2011). Results obtained from study of the enzymatic activities of phenolic extracts of *A. halimus* have shown that dichloromethane fraction of *A. halimus* has a powerful inhibition percentage on α-amylase activity, while *A. halimus* butanolic fraction has low percentage of inhibition (Tahar *et al*., 2017). Ganeshpurka and colleagues showed that ethyl acetate and water extracts of *T. foenum-graecum* leaves revealed inhibitory potential of *T. foenum-graecum* on α-amylase and α-glucosidase activity (Ganeshpurkar *et al*., 2013).

1.3 Aim of study

T. foenum-graecum (seeds), *A. halimus* (leaves and stem), *O. europaea* (leaves), *A. sativum* (bulb), *A. cepa* (bulb) and *C. cassia* (bark) were tested at the co-supervisor laboratory for anti-diabetic activity in a selected model for glucose transporter activity (Kadan *et al*., 2013). Different research

studies have been conducted to test the effect of some of the above mentioned plants on one or two of the digestive enzymes: α-amylase, α-glucosidase and lipase mostly in water and methanol extracts. However, to the best of our knowledge the effect of water/ethanol extracts of these plants (in the selected parts) on all of the above enzymes was not reported yet (February 2019). The aim of this study is to evaluate the potential inhibitory levels of 50% ethanol (in water) extracts from the aforementioned plants on the intestinal α-glucosidases, pancreatic α-amylase and pancreatic lipase, and to compare the inhibitory effects of the different extracts on the three enzymes.

Chapter Two
Materials and Methods

2.1 Materials

Rat intestinal acetone powder, acarbose, sucrose, glucose oxidase kit, porcine pancreatic α-amylase, starch, 3,5- Dinitrosalicylic acid, p-nitrophenylbutylrate (*p*-NPB), porcine pancreatic lipase, orlistat and acetonitrile were purchased from Sigma-Aldrich Company. All other chemical reagents used in this study were of analytical grade.

2.2 Methods

2.2.1 Plants collection

All information about plant collection including scientific name, common name, plant part and source is presented in table 2.1 below.

Table 2.1 List of plants used in this study and the part of the plants from which the extracts have been prepared

Scientific name	Common name	Plant part	Source
Trigonella foenum-graecum	Fenugreek	Seed	Local store
Atriplex halimus	Salt bush	Leaves and stem	Tantora beach
Olea europaea	Olive	Leaves	Tubas
Allium sativum	Garlic	Bulb	Local store
Allium cepa	Onion	Bulb	Local store
Cinnamomum cassia	Cinnamon	Bark	Local store

2.2.2 Plant extracts preparation.

The selected plants were extracted according to Kadan *et al*. (2013) with 50% ethanol (in water). Fifteen grams of grinded plant material were added to 100mL of the solvent and heated for 15 minutes at 60◦C under stirring. Extract supernatants obtained were passed through a 0.2μm filter and stored in aliquots at -80◦C for further experimental work.

2.2.3 Enzyme assays

2.2.3.1 Intestinal α-glucosidase inhibitory activity

The assessment of intestinal α-glucosidase inhibitory activity was performed according to Adisakwattana *et al*. (2012) using acarbose as a positive control. 100 mg of rat intestinal acetone powder were homogenized in 3 ml of 0.9% NaCl solution. The solution was centrifuged at 12,000x g for 30 min, and the supernatant was used for the enzyme assay.

The crude enzyme solution, 150 µl for sucrase assay, was incubated with 200 µg/ml of the different plants extracts for 15 min, followed by the addition of 100 µl of 0.1 M phosphate buffer, pH 6.9 and 150 µl sucrose (400 mM). Acarbose was used as a positive control. The reaction was incubated at 37°C for 60 min. Thereafter, the mixtures were suspended in boiling water for 10 min to stop the reaction. The released concentrations of glucose from the reaction mixtures were determined by glucose oxidase method and the product color density was measured in spectrophotometer

at 450 nm. Intestinal α-glucosidase inhibitory activity was expressed as percentage inhibition using the following formula:

$$\%Inhibition = \frac{Abs_{control} - Abs_{sample}}{Abs_{control}} \times 100\%$$

Where $Abs_{control}$ is the absorbance without any inhibitor or extract (control), and Abs_{sample} is the absorbance of the sample with the extract.

2.2.3.2 Pancreatic α-amylase inhibitory activity

The pancreatic α-amylase inhibition assay was performed according to Adisakwattana *et al*. (2012) using acarbose as a positive control. Porcine pancreatic α-amylase (4 units/ml) was dissolved in 0.1 M sodium phosphate buffer, pH 6.9.

The various plant extracts, or acarbose as a positive control (200 µg/ml), were preincubated with 250 µl of the enzyme solution at 37°C for 10 min. The reaction was initiated by adding 500 µl of the substrate solution (1% starch in 0.1 M sodium phosphate buffer, pH 6.9). After 5 min of incubation, the reaction was stopped by adding 1 ml of 96 mM 3,5-Dinitrosalicylic acid solution to the reaction mixture. The mixtures were heated at 100°C for 10 min in order to stop the reaction and then cooled to room temperature in a cold water bath. Subsequently, the reaction mixtures were diluted 10 times with distilled water. The absorbance was recorded at 540 nm using a spectrophotometer. Pancreatic α-amylase inhibitory activity was expressed as percentage inhibition using the following formula:

$$\%Inhibition = \frac{Abs_{control} - Abs_{sample}}{Abs_{control}} \times 100\%$$

Where $Abs_{control}$ is the absorbance without any inhibitor or extract (control), and $Abs_{samples}$ is the absorbance of the sample with the extract.

2.2.3.3 Pancreatic lipase inhibitory activity

The assessment of pancreatic lipase inhibitory activity was performed according to Bustanji *et al.* (2011) using orlistat as a positive control and *p*-nitrophenyl butyrate (*p*-NPB) as a substrate. The enzyme solutions were prepared immediately before use. Crude porcine pancreatic lipase (PL) was suspended in a buffer solution (20 mM Tris-HCl, 1.3 mM $CaCl_2$, 150 mM NaCl, pH= 8.0) at a concentration of 10 mg/mL and incubated at 37°C for 10 min. Subsequently, the solution was centrifuged for 10 min at 1500x g. The supernatant was used as the enzyme source for the ensuing experiments.

For each experiment, the PL was combined with the different extracts (final concentrations 200µg/ml) or with orlistat as a positive control. The mixture was then incubated at 37°C for 15 min. The volume was brought to 950 µl using Tris–HCl buffer. After that, the reaction was initiated via the addition of 50 µl *p*-NPB (10mM in acetonitrile). The absorbance of the solution was measured spectrophotometrically at 410 nm for 5 min. The PL activity is related to the rate of *p*-nitrophenol release, which is measured as the increase in absorbance at 410 nm against blank using denatured enzyme. It was estimated from the slope of the linear segment of absorbance vs. time.

Percentage of inhibitory activity was calculated using the following formula:

$$\%Inhibition = \frac{\Delta Abs_{control} - \Delta Abs_{sample}}{\Delta Abs_{control}} \times 100\%$$

Where $\Delta Abs_{control}$ is the enzyme activity without extract or inhibitor (control), and $\Delta Abs_{samples}$ is the enzyme activity of the sample with extract.

2.2.4 Data analysis

The data was expressed as mean ± standard error (SE) for n=3. The IC_{50} values were calculated from plots of inhibitor concentration versus percentage inhibition curves. Student's t-test was used for the statistical analysis and significance measures ($P<0.05$) was considered to be statistically significant.

Chapter Three
Results

3.1 Intestinal α-glucosidase inhibitory activity

Water-ethanol extracts of six plants (*T. foenum-graecum*, *A. halimus*, *O. europaea*, *A. sativum A. cepa* and *C. cassia*) were tested for their α-glucosidase (sucrase) inhibitory activity, at a concentration of 0.2 mg/ml. The results are shown in Figure 3.1.

At a concentration of 0.2 mg/ml, among the six herbal extracts, only *Allium sativum* showed an inhibitory activity against the intestinal sucrase with a percentage inhibition of 46.74±11.55%. Furthermore, acarbose (the positive control, tested at 0.2 mg/mL) markedly inhibited intestinal sucrase with 98.26±1.52%. On the other hand, other extracts promoted the activity of intestinal sucrase. *T. foenum-graecum*, *A. halimus*, *O. europaea*, *A. cepa* and *C. cassia* had increased the enzyme activity by 139.72±29.98%, 14.46±9.26%, 102.9±12.32%, 22.31±25.97% and 88.87±6.92%, respectively.

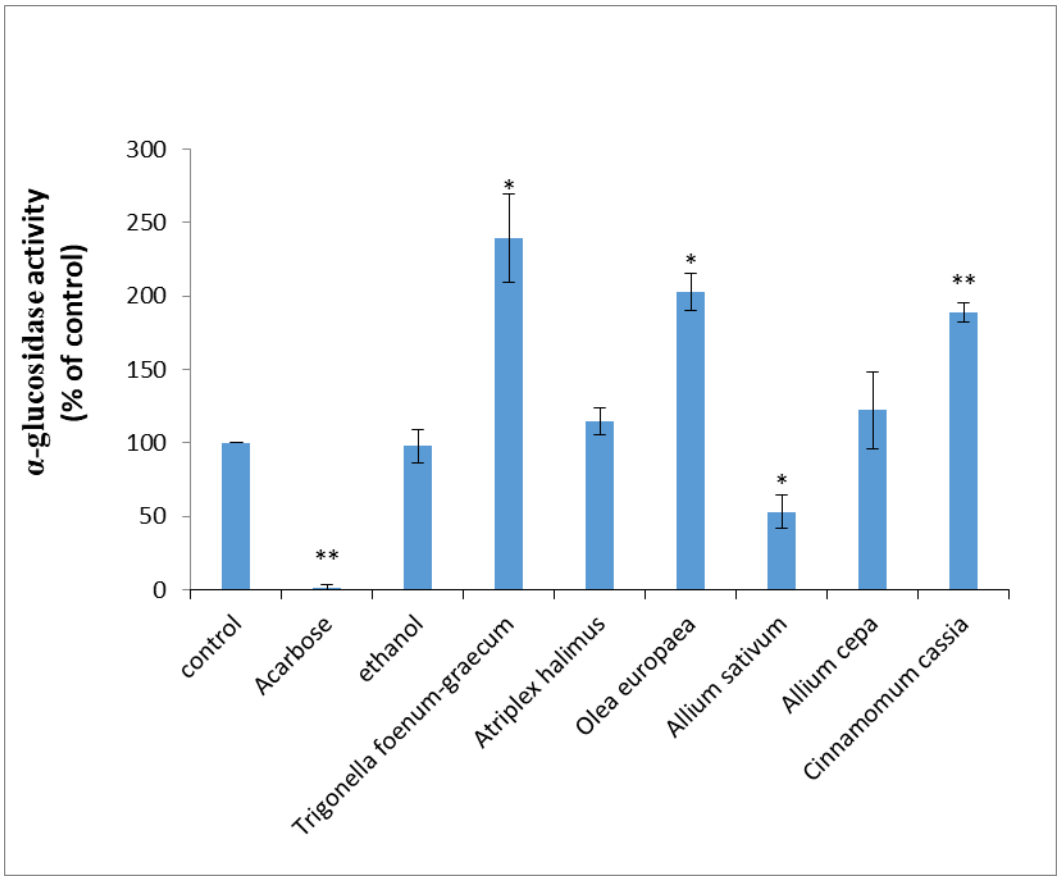

Figure 3.1 The effects of plant extracts on the activity of intestinal α-glucosidase. Data are expressed as mean ± SD (n=3). Data with different asterisks *,** shows significant difference. (*) denoting significant value (P≤0.05), (**) significant value (P≤0.01), two-tailed student t-test.

3.2 Pancreatic lipase inhibitory activity

Pancreatic lipase inhibitory activity of the previously mentioned plant extracts was tested using *P*-NPB as a substrate as described in the materials and methods section. The results are shown in Figure 3.2. The figure depicts that the inhibitory activity of the six tested plant extracts (0.2 mg/mL) was not significant except for *C. cassia*. Indeed, only *C. cassia* showed an inhibitory activity with a percentage inhibition of 14.33±4.98%. *T. foenum-graecum*, *A. halimus*, *O. europaea*, *A. sativum* and *A. cepa* showed no significant effect on lipase activity. In contrast, *O. europaea* had increased the enzyme activity by 27.70±12.52%. For comparison of the

inhibitory activity, the irreversible lipase drug inhibitor, orlistat, was tested (0.2 mg/mL), which markedly inhibited pancreatic lipase with 45.8±4.52%.

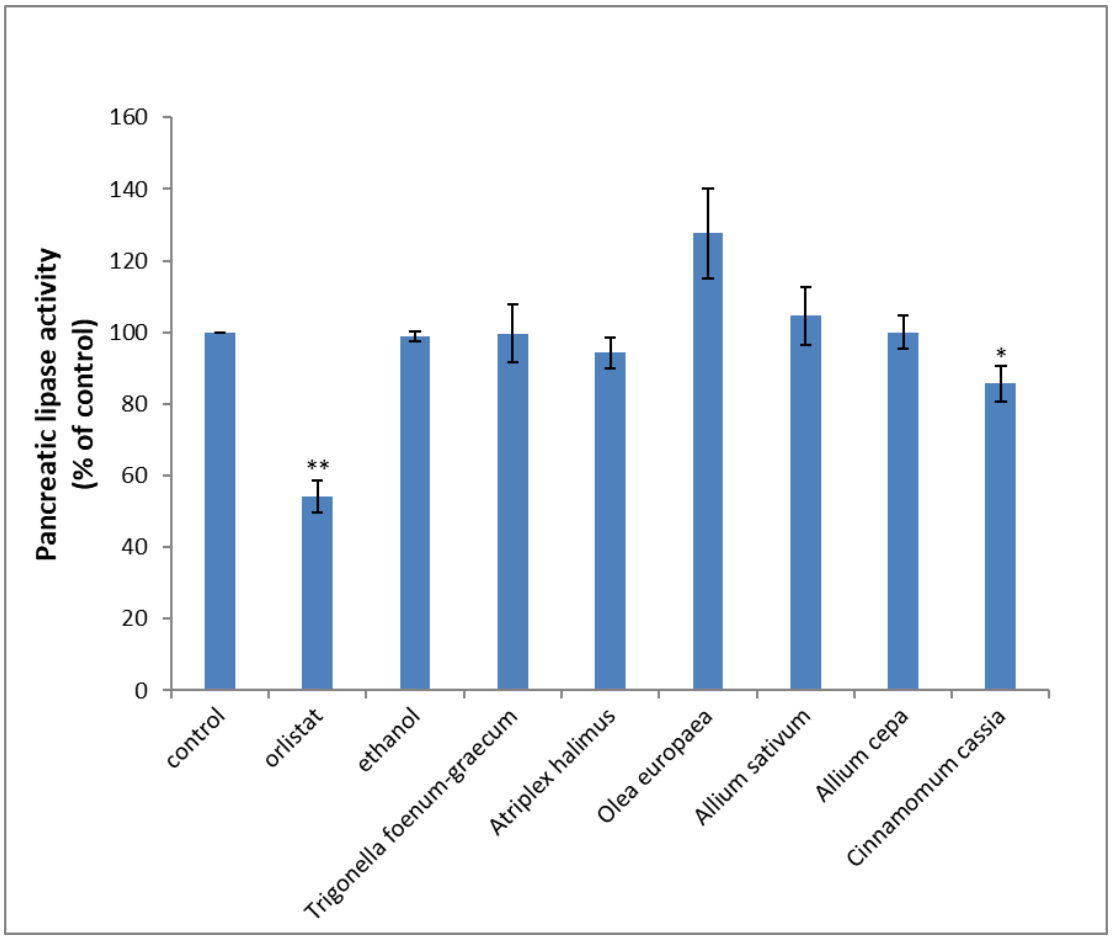

Figure 3.2 The effects of plants extract on the activity of Pancreatic lipase. Data are expressed as mean ± SD (n=3). (*) denoting significant value (P≤0.05), (**) significant value (P≤0.01), two-tailed student t-test.

3.3 Pancreatic α-amylase inhibitory activity

The results of pancreatic α-amylase inhibition by the various plants are summarized in figure 3.3. The highest inhibitory percentage was received by 0.2 mg/ml of *C. cassia* (82.29±7.6%), followed by *O. europaea* (51.76±20.57%). On the other hand, other extracts promoted the activity

of pancreatic α-amylase. Acarbose was used as a positive control (pancreatic α-amylase inhibitory activity of 94.9±5.1% at 0.2 mg/mL).

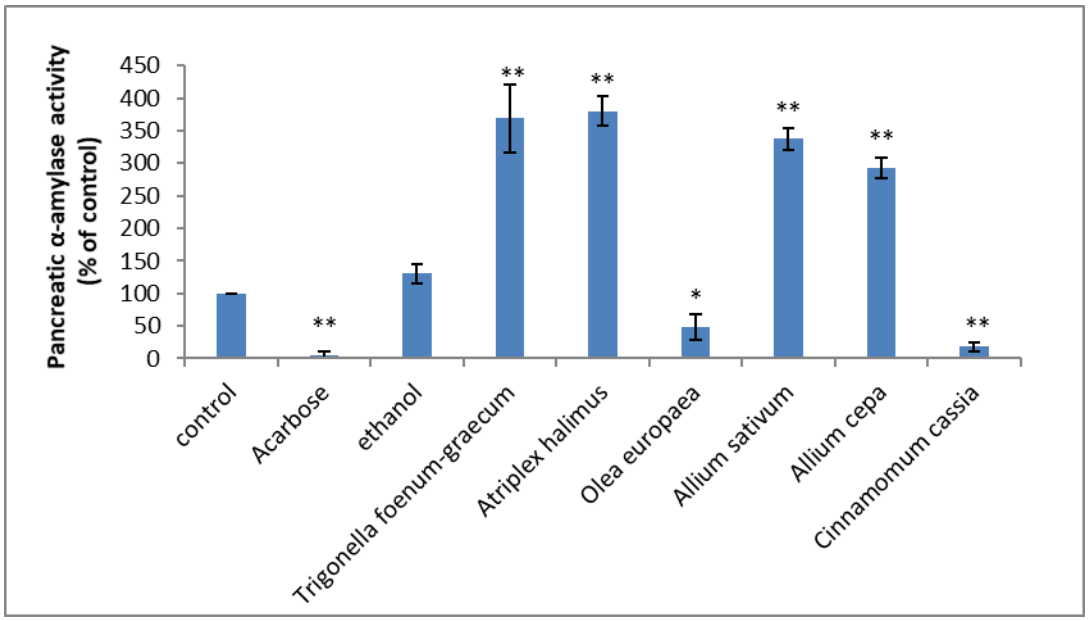

Figure 3.3 The effects of plants extract on the activity of pancreatic α-amylase. Data are expressed as mean ± SD (n=3). Data with different asterisks *,** shows significant difference with * denoting significant value (P≤0.05),** significant value (P≤0.01), two-tailed student t-test.

Figure 3.4 and figure 3.5 show the inhibitory profiles of the two most potent plant extracts (*C.cassia and O.europaea*). Both extracts exhibited a dose-dependent pancreatic α-amylase inhibitory effect using a concentration range of 0-1000 µg/mL. The percent inhibition was plotted against the extract concentrations to determine the IC_{50} value. For *C.cassia,* the IC_{50} value was 24±3.01 µg/mL. For *O.europaea,* it was 192.94±6.4 µg/mL (table 3.1).

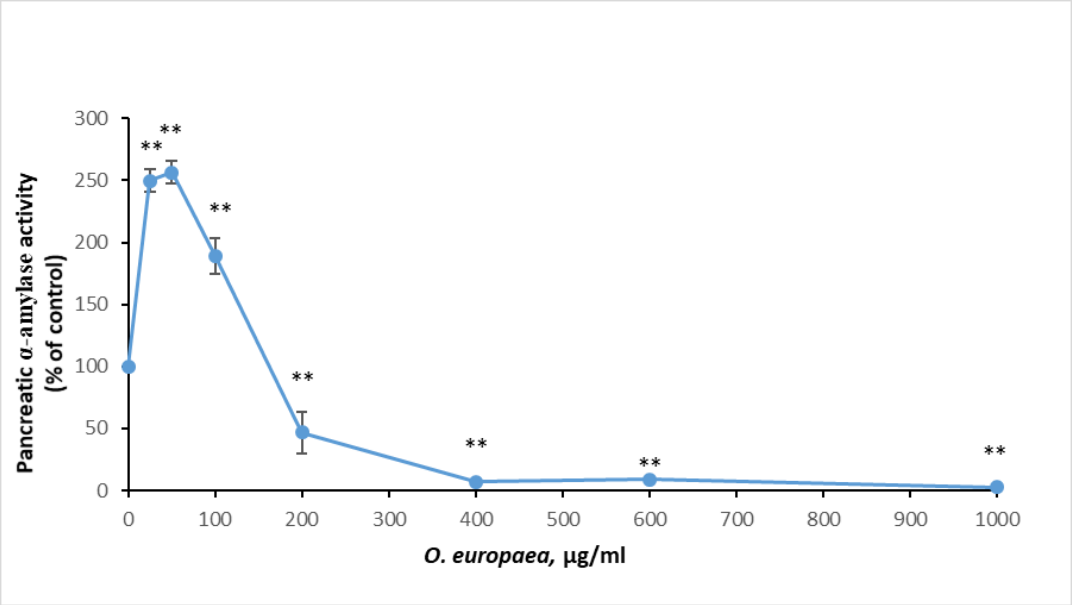

Figure 3. 4 Dose dependent inhibitory activity of *O. europaea* extract on the pancreatic α-amylase. Data with ** denoting significant value (P≤0.01), two-tailed student t-test.

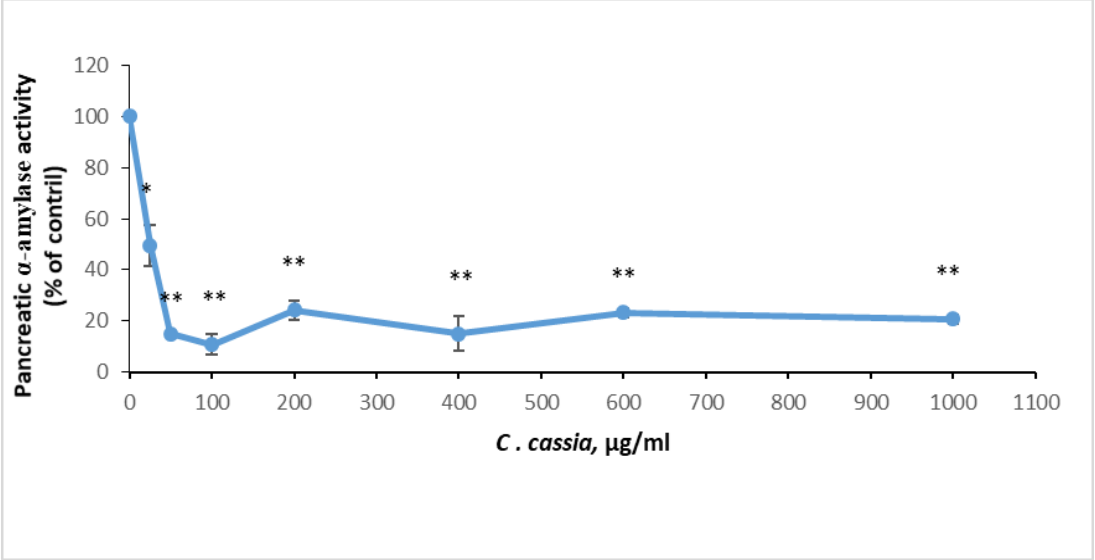

Figure 3. 5 Dose dependent inhibitory activity of *C. cassia* extract on the pancreatic α-amylase. Data with ** denoting significant value (P≤0.01), two-tailed student t-test.

Table 3.1: IC_{50} value for two medicinal plants on pancreatic α-amylase

Plant extract	**IC_{50} value (µg/mL)**
O. europaea	192.94±6.4
C. cassia	24±3.01

Chapter Four
Discussion and Conclusions

4.1 Discussion

In the last few decades and nowadays, the prevalence of obesity and diabetes is considered one of the major public health problems. On the other hand, many medicinal plants possess anti diabetic and/or anti-obesity activity. These plants might have different mechanisms of action, including but not limited to enhancing insulin secretion, stimulating glucose disposal and inhibition of digestive enzymes of carbohydrates and lipids and thus diminish the sugars and lipids entry into the blood.

In the present study, the *in vitro* inhibitory activity of water-ethanol extracts of *T. foenum-graecum* (seeds), *A. halimus* (leaves and stem), *O. europaea* (leaves), *A. sativum* (bulb), *A. cepa* (bulb), and *C. cassia* (bark) on α-glucosidase, pancreatic α-amylase, and pancreatic lipase were evaluated at nontoxic concentrations as was reperted by Kadan *et al.* (2013). Results are summarized in table 3.

The present study evaluated the inhibitory activities using water-ethanol extract. The extraction method differs from previous studies, which may result in various biological activities. In addition, many studies test the inhibitory activities at a high concentration of plant extracts without considering the toxicity of these concentrations.

Table 3.1 Inhibitory percentage of herbal extracts (200 µg/mL) against pancreatic lipase, intestinal α-glucosidase, and pancreatic α-amylase.

Plant samples	% Inhibition against Pancreatic lipase	% Inhibition against Intestinal α-glucosidase	% Inhibition against pancreatic α-amylase
T. foenum-graecum	0.27 ±8.06	-139.72±29.98*	-268.71±53.04*
A. halimus	5.71±4.25	-14.46±9.26	-280.32±23.5**
O. europaea	-27.70±12.52	-102.9±12.32**	51.76±20.57*
A. sativum	-4.62±8.18	46.74±11.55**	-237.28±16.92**
A. cepa	-0.02±4.54	-22.31±25.97	-192.5±16.4**
C. cassia	14.33±4.98*	-88.87±6.92**	82.29±7.6**

Data are expressed as mean ± SD (n=3). Data with different asterisks *, ** shows significant difference with * denoting significant value (P≤0.05),** significant value (P≤0.01), two-tailed student t-test (the minus indicates activation). (-) indicates activation.

Among the six plant extracts examined (50% ethanol), some showed a high inhibitory activity at a concentration of 0.2 mg/mL. This activity was shown by the bark extract of *C. cassia*, which has a significant inhibition with a percentage of 82.29±7.6 against pancreatic α-amylase, as compared with the negative control; and an IC_{50} of 24±3.01 µg/ml. On the other hand, the extract slightly inhibited pancreatic lipase and exhibited no inhibitory activity against intestinal sucrase. Several studies have demonstrated that extracts of cinnamon species can have antidiabetic activities, and have investigated their mechanisms of action (Chen *et al*., 2012; Verspohl *et al*., 2005). Water extracts of several Cinnamomum species have been reported to have pancreatic α-amylase inhibitory activities. *C. cassia* has a pancreatic α-amylase inhibitory activity with an IC_{50} of 1.77±0.05 mg/ml (Adisakwattana *et al.*, 2011)

The results of this study showed that at a concentration of 200 µg/ml, 50% ethanol extract of *O. europea* leaves has an inhibitory activity only against pancreatic α-amylase with 51.76±20.57% and an IC_{50} of 192.94±6.4 µg/ml. A previous study revealed that aqueous extracts of *O. europaea* leaves have inhibitory activity with IC_{50} of 990±120 µg/ml (Bechiri *et al*., 2015). 70% ethanol extract of *O. europaea* was reported to have an anti-lipase activity of 36.8±1.3 % at a concentration of 5 mg/ml (Jamous *et al*., 2018).

The inhibitory activity of the leaf extracts of *O. europaea* was attributed to both hydroxytyrosol and the oleuropein that are involved in the antidiabetic effect. The inhibitory activity of these two active compounds against α-amylase and α-glucosidase was reported by Hadrich *et al.* (2015). Results show that hydroxytyrosol had the strongest α-glucosidase inhibitory effect with an IC_{50} value of 150 µM with mild inhibition against α-amylase.

A. sativum and A. cepa were also examined for their inhibitory activities on the above mentioned enzymes. The inhibitory activity has been reported here only for *A. sativum* on rat intestinal sucrase with a percentage inhibition of 46.74±11.55. In a different study, ethanol extracts of *A. sativum* and *A. cepa* exhibited α-amylase inhibitory effects with high IC_{50} values of 17.95 and 16.36 mg/ml, respectively (Nickavar & Yousefian, 2010). Methanolic extracts of *A. sativum* bulb showed an inhibitory activity of 24.11±1.45% for rat intestinal sucrase (Moradabadi *et al*., 2013).

Although *T. foenum-graecum* is known to have anti-diabetic effects, it showed no inhibitory activity against the three enzymes at the tested concentrations. Damani *et al*. (2018) found that aqueous extract of T. *foenum-graecum* exhibited maltase inhibitory activity with IC_{50} value of 1.05%. *T. foenum-graecum* seeds revealed appreciable α-amylase inhibitory activities with IC_{50}=1.87 mg/mL (Nickavar & Yousefian, 2011). There are several mechanisms behind the antidiabetic effect of fenugreek. One such suggested reason is the active ingredient 4-hydroxyisoleucine stimulates insulin production thereby controlling blood sugar level (Sundaram *et al*., 2018).

In vitro studies conducted by Kadan *et al*. (2013) reported that 50% ethanol extracts of *A. halimus* caused a good progress in the translocation of GLUT4 with no cytotoxic side effects. *A. halimus* in mixture with other plants is effective in lowering blood glucose levels in diabetic patients and facilitates glucose entry into yeast cells during anaerobic fermentation. This observation was attributed to an effect of *A. halimus* content in the mixture (Said *et al*., 2008)

4.2 Conclusions

The results of our study suggest natural resources that possess pancreatic lipase, pancreatic α-amylase and Intestinal α-glucosidase inhibitory activities with potential applications in the prevention and treatment of diabetes and obesity. Extracts of *A. halimus* (leaves and stem), *O. europaea* (leaves), *A. sativum* (bulb) and *C. cassia* (bark) have shown significant

inhibitory activities on one or more of these enzymes. However, future studies are needed for in-depth phytochemical screening and clinical, identification of the constituents responsible for this activity. Further screening for distinct potential mechanisms for the antidiabitic and antiobesity activities of our selected plants is also appreciated.

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جامعة النجاح الوطنية

كلية الدراسات العليا

النباتات الطبية كمصدر لتثبيط فعالية الانزيمات الهاضمة: الفا- جلوكوزيداز، الفا- اميلاز وليباز

إعداد

رغد البزور

إشراف

د. عوني أبو حجلة

أ. د. هلال زيد

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إعداد

رغد البزور

إشراف

د. عوني أبو حجلة

أ. د. هلال زيد

الملخص

وصلت السمنة والسكري إلى مستويات وبائية في جميع أنحاء العالم في القرن الواحد والعشرين. وقد اقترح طب الأعشاب كمصدر بديل لخافضات ارتفاع دهون الدم وارتفاع سكر الدم. الهدف من هذه الدراسة هو التقييم المخبري للنشاط المثبط لمجموعة مختارة من النباتات الطبية المحلية (الحلبة، الرغل الملحي، الزيتون، الثوم، البصل و القرفة) المستخدمة لمكافحة السكري ومكافحة السمنة على الانزيمات الهاضمة للكربوهيدرات والدهون وهي، الفا جلوكوزيداز، الفا–اميلاز وليباز. تم تقييم الأنشطة المثبطة لمستخلصات الإيثانول: الماء (50٪:50٪) لهذه النباتات باستخدام المقاييس اللونية الأنزيمية. عند تركيز 200 ميكروغرام/مليلتر، من بين المستخلصات العشبية الستة التي فحصت (بذور الحلبة، أوراق وساق الرغل الملحي، أوراق الزيتون، بصيلة الثوم، بصيلة البصل ولحاء القرفة). مستخلص الثوم كان الوحيد الذي اظهر نشاط مثبط ضد الفا–جلوكوزيداز المعوي بنسبة تثبيط 46,74±11,55٪. بالنسبة لليباز البنكرياس، فقد أظهر مستخلص لحاء القرفة نشاطًا مثبطًا بنسبة تثبيط 14,33±4,98٪. سجل مستخلص لحاء القرفة ومستخلص اوراق الزيتون اعلى نسبه مثبطه على الفا–اميلاز البنكرياس مع قيمة تركيز المادة الموافق للتثبيط النصفي 24±3,01 و192,94±6,4 ميكروغرام/مليلتر، على التوالي. ومع ذلك، هناك حاجة لدراسات مستقبلية لفحص التركيب الكيميائي والتأثير العلاجي لهذه النباتات، وتحديد المركبات الكيميائية المسؤولة عن هذه الفعالية البيولوجية. كما أن هنالك حاجة الى فحص آليات أخرى مسؤولة عن نشاط هذه النباتات كمضادات للسمنة والسكري خاصة على حيوانات التجارب.