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Flaxseed Oil Extract and Its Medicinal Benefits

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Dedication

I dedicate my thesis to my beloved and big-hearted father for his endless love and support, who paved the way throughout my pursuit for education.

To my great and lovely mother, who has been my emotional anchor through my entire life.

To my beloved husband with special feeling of gratitude towards him for his continued support and encouragement throughout my study period.

To my wonderful brothers and sisters, who have given me the drive and determination to tackle this project with enthusiasm.

To my lovely little daughter "Salma", who filled my soul with love and my life with joy.

To those who without their love and support this project would not have been made possible.

Declaration

I certify that this thesis is submitted for the degree of master is my own research, expect where otherwise acknowledged, and that this thesis (or any part of the same) has not been submitted for higher degree to any other university or institution.

Islam Eid Jawdat Sulaiman

Signed: 

Date: 31/05/2022

Acknowledgments:

In the name of Allah the most gracious and merciful

Thanks to Allah for his grace, guidance and blessings that enabled me to successfully complete my project.

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Abstract

Flaxseed is considered a "high-value food", because it is high in omega-3, fatty acids and includes elements that are necessary for good health. There is a lot of effort these days on producing medications and looking for plant sources to lessen side effects. Extraction of natural products plays a key role in developing new medicines.

The Soxhlet extraction device was used to extract flaxseed in this study, which was able to extract practically all active chemicals from this plant.

The antioxidant activity (AA) of flaxseed extracts was determined by free radical scavenging using DPPH method. The flaxseed oil was extracted using ethanol as solvent and it resulted in (4.02±0.02mg/g), whereas when flaxseed oil was extracted using petroleum ether as solvent, the result was (1.7 ±0.2mg/g).

The total phenolic content (TPC) of flaxseed extracts was quantified using the Folin-Ciocalteu assay. A UV-Visible spectrophotometer was used to conduct all of the tests. The antioxidant properties of flaxseed, as well as their high phenolic content, were discovered. While the total phenolic content (TPC) of flaxseed oil was extracted using ethanol resulted in (230.81±0.02mg/g), which is higher than that of flaxseed oil was extracted using petroleum ether which is (53.531±0.07mg/g), where the total flavonoids content (TFC) ended with a result of (223.72±0.04 mg/g).

3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase is a crucial enzyme in the cholesterol-producing mevalonate pathway. HMG-CoA reductase lowers cholesterol production in the liver. Inhibitory impact of flaxseed oil (extracted using petroleum ether) was 56.3%.

Susceptibility of flaxseed extracts to antibacterial activity was determined in vitro using the agar disc diffusion method. The extracts from the flaxseed oil (using petroleum ether) showed antibacterial activities against MRSA, Pseudomonas and Staphylococcus, where extracted flaxseed using ethanol showed antibacterial activities against Staphylococcus only.

In vitro glucose-bovine serum albumin (BSA) assay was used to evaluate Anti-glycation formation of the end products. The study concluded that flaxseed oil has Anti-glycation activity.

The cream was made based on flaxseed oil (extracted using ethanol) had several features, including but not limited to, excellent color, smoothing, non-irritating and it's easily distributed on skin. Also, when it comes to talk about (ph) degree, the study found out that the cream has a (ph) of (5.5-6).

HPLC analysis of the standards of polyphenolic compounds and flavonoids were detected and identified by comparing the retention times of the peaks in the sample chromatogram of flaxseed oil (extracted using ethanol) with that of the standard. After conducting the HPLC analysis, the results showed Chlorogenic acid, Rutin and Trans cinnamic acid.

In the light of the previously mentioned results, flaxseed is a natural source of antioxidants and antibacterial, as well as prevent many diseases including hypercholesterolemia, skin sensitivity and diabetes.

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List of Abbreviations, Symbols and Terminology:

Abbreviation	Definition
HMG-CoA	3-hydroxy -3-methyl-coenzyme A
EtOH	Ethanol
TFC	Total Flavonoid Content
TPC	Total Phenolic Content
SDG	Secoisolariciresinol diglucoside
LA	linolenic acid
ALA	a-linolenic acid
GLA	γ-Linolenic
FA	Fatty acids
LDL	Low density liporotein
S.aureus	Staphylococcus aureus
E.coli	Escherichia coil
LPS	Lipopolysaccharides
FO	Flaxseed oil
HBA1c	Hemoglobin A1c
LDL-C	Low density liporotein cholesterol
BSA	Bovine Serum Albumin
DDPH	2,2-Diphenyl-1-picrylhydrazyl propane hydrochloride
HPLC	High-performace liquid
DW	Distilled water
KH_2PO_4	Monoptassium phosphate
$ALCL_3$	Aluminum chloride
$NaHPO_4$	Disodium phosphate

Chapter one

Introduction

1.1 Flaxseed oil

1.1.1 Introduction

Plants include a variety of biologically active chemicals that are beneficial to both people and animals. Plants have been used for medicinal purposes since antiquity, but little is known about their composition or mechanism of action. Flaxseed is one of the world's oldest crops, having been farmed for fiber and sustenance since the dawn of civilization. Flaxseed has sparked renewed attention in the field of nutrition and disease research in recent years, owing to the possible health advantages of certain of its biologically active components (B Dave Oomah, et al.,2001).

Flaxseed (also known as common flax or linseed) belongs to the Linum genus of the Linaceae family. It's one of the world's most significant oil crops, particularly in Canada and China. According to their function, flax may be split into two varieties in China: oil-flax and fibreflax. Flaxseed is one of the best plant sources of á-linolenic acid, vitamins and omega-3 fatty acids. Plant lignans are abundant in flaxseeds, making them one of the best sources. It is very high in the lignan secoisolariciresinol diglycoside (SDG) and is the greatest source of (SDG) of any food (Barbay, et al.,2010; Hanaa M, et al.,2017).

The beneficial phytochemicals lignans and phenolic acids are abundant in flaxseed after oil extraction. Seeds, oil, and seedcakes are employed in culinary, pharmaceutical, and cosmetic sectors because of their high nutritional content. The flax genome has been genetically engineered to improve the plant's disease resistance, flavor, and nutritional attributes, as well as to manufacture medicines and other substances, with the explicit goals of increasing the value of flax products (Styrchzewska, et al.,2013).

1.1.2 Phytochemical constituents of flaxseed

Flaxseed is quickly becoming one of the most important phytochemical sources. Antioxidants, these phytochemicals (phenolic acids, cinnamic acids, flavonoids, and lignins) impact cell development and survival. Flaxseed is an important source of high-quality protein and soluble fiber, and it possesses phenolic compound potential. Flaxseed contains both soluble and insoluble fiber, with the latter accounting for around a third of the overall fiber content (Amin et al.,2014).

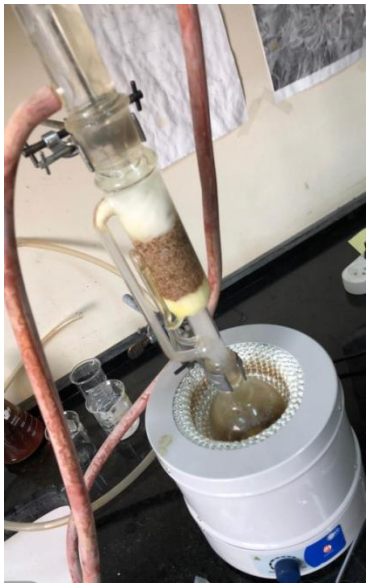
1.1.3 Extract Techniques of flaxseed

Extraction methods may differ between the brands studied, or improved performance is linked to a certain method of extraction for a given sample. As a result, many factors impacting the extraction of oils from commercial flaxseed brands, including as solvent, extraction time, temperature, sample size, and solvent volume, must be evaluated (Khattabet et al 2013).

Traditional Soxhlet Extraction. This is currently one of the most widely utilized extract processes in plant world . The principles are main but not alone in chemical analysis just too in many other

domains. Benefit of the Soxhlet technique is that the sample is constantly in touch with the solvent, which helps to shift the balance of the transfer towards the solvent. It's also a straight forward procedure that allows for more sample extraction than most recent methods (Khattab et al.,2013).

The technique is repeated in a traditional Soxhlet until full extraction is accomplished. As illustrated in Fig. 1, the sample (flaxseed is put in an extraction thimble-holder and slowly packed with condensate fresh solvent or extractant (The statement used to turn to a solvent used for extraction) from a distillation flask during operation. A siphon aspirates the contents of each thimble holder and empty it back into the distillation flask, transmitting the extracted analysts in the bulk liquid, when the liquid achieving the level of excess. Soxhlet is a hybrid continuous-discontinuous technology as a result of this execution. The assemblage can be considered a batch system since the solvent extractant is recuperated through the sample; yet, the system also has a continuing character because the solvent extractant is recuperated through the sample (Khattab et al.,2013).



(a) (b)

Figure 1.1 (a,b) Soxhlet extraction apparatus

1.2 Medical effects of flaxseed:

1.2.1 Introduction

Modern-day living presents a health problem, as contemporary society is plagued by a number of degenerative lifestyle ailments. Plant products have received much deserved attention in light of the quickly changing global health landscape and the growing recognition of the negative effects

of unregulated food processing and overmedication. Foods that can act as medicine have sparked attention as people become more conscious of the importance of nutrition and their desire for wellbeing. Foods or dietary components known as functional foods or nutraceuticals may give health benefits beyond basic nutrition. Functional foods provide a health benefit that goes beyond what is expected from their nutritious content. Flaxseed has gained a reputation as a functional food due to its nutrient content, which has beneficial effects on illness banning by delivering health-beneficial components (Katare et al.,2012).

Flaxseed is commonly used to cure a variety of ailments. Antioxidant, antibacterial, improves skin barrier function, and hypercholesterolemia characteristics have been documented. Despite various articles claiming that this herb has health advantages (Oomah et al.,2001, Barbary et al .,2010).

1.2.2 Antioxidant effect:

Antioxidants have recently been challenged for their potential harm. It is widely considered that consuming phytochemicals produced from plants, such as those found in vegetables, fruits, and nuts, on a regular basis is beneficial. Tea and herbs may help to alter the balance toward a healthy level of antioxidant. As a result, interest in natural antioxidants, particularly those derived from plants, has skyrocketed in recent years. When redox homoeostasis inside a cell is disrupted, oxidative stress ensues. This imbalance might be caused by an excess of reactive oxygen species (ROS) or a deficit in the antioxidant system. More than 100 degenerative illnesses, including cardiovascular, diabetes, cancer, atherosclerosis, neurological disorders, and arthritis, have been linked to ROS. As a result, antioxidants with free radical scavenging activity might be useful in the prevention and therapy of illnesses due to oxidants or free radicals scavenger activity (Alachaher et al .,2018,Amin et al ., 2014).

Flaxseed is quickly becoming one of the most important sources of phytochemicals in the realm of functional foods. Antioxidants, these phytochemicals (phenolic acids, cinnamic acids, flavonoids, and lignins) impact cell development and survival. It has long been used as a culinary component and is currently in great demand in the food industry. Due to its potential health advantages connected with linolenic acid (57 percent) and a significant lignan, particularly (SDG), flaxseed is a key player in the field of nutrition and disease researches (Alachaher et al .,2018,Amin et al ., 2014) .

1.2.3 Antibacterial effects:

Flaxseed oil is produced by the seeds. Solvent-processed flaxseed oil has been used as a drying oil in painting and daubing for centuries, and it is one of the oldest trading oils. The raw oil is used as an astringent in fungicidal lotions and insecticides, and it has mild insect repellent effects (The Wealth of India 2006). Unsaturated fatty acids such as oleic acid (12–30%), linoleic acid (8–29%), and linolenic acid (35–67%) are present in the oil. These fatty acids appear to provide the oil a drying effect. Acute and chronic arthritic albino rat models were used to test the therapeutic impact of flaxseed fixed oil. It has recently been found to have antiulcer efficacy in animal models. As a topical treatment, hydrolyzed linseed oil has the potential to be antimicrobial, Antibiotic-resistant staphylococcus (S. aureus) was shown to be susceptible to the hydrolyzed lipid (Kaithwas et al .,2011)

Using the agar-well diffusion method, demonstrated antibacterial activity of four different flaxseed extracts against Gram positive and Gram negative bacteria: Staphylococcus aureus, Bacillus subtilis, Klebsiela pneumonia, and Pseudomonasaerogenosa, Except for chloramphenicol, petroleum ether extract showed substantial inhibitory effects against all tested bacteria at all doses when compared to antibiotics. The clearest action was shown against K. pneumoniae at a concentration of 50 mg/cm3. The strongest inhibitory impact was obtained against using the extract concentration of 200 mg/cm, followed by aqueous extract, which demonstrated good inhibitory action against Pseudomonas aeruginose using the same concentration (Amin et al .,2014).

1.2.4 Skin sensitivity

According to epidemiological surveys, more than half of the Western population believes themselves to have sensitive skin. Sensitive skin is characterized by wetness, irritation, inflammation, and immunological reactions. Visible cutaneous indicators include dryness, scaling, erythema, calor, and changes in skin texture and structure. Patients frequently complain of itching, stinging, and burning. A new study looks at the prevalence of sensitive skin beliefs throughout the world and finds that the percentage of people who claim to have sensitive skin differs by population. Foods high in polyunsaturated fatty acids (FA) are vital for human health. Flaxseed, hempseed, and other plant oils contain linoleic acid (LA), a-linolenic acid (ALA), and γ-linolenic acid (GLA) (Keukam et al., 2011).

Nutrient supplementation or dietary intervention affects basic skin systems including the shielding barrier, water homeostasis, temperature management, and photo protection. Modulated functions are accompanied by change in cutaneous structure and texture, which impact the skin's appearance. According to research in males, increased consumption of carotenoids such as b-carotene or lycopene, carotenoid rich foods, or flavanol rich chocolate improves baseline protection against UV-induced erythema, enhances cutaneous blood flow, and changes skin

structure and hydration. A decrease in proinflammatory eicosanoids has been associated with the use of ALA-rich flaxseed oil. This study looked at the effects of 12-week flaxseed or borage oil oral supplementation on human skin features (Spirt et al .,2008).

1.2.5 Glycation of Hemoglobin in blood and type 2 diabetes

Diabetes is one of the most common non-communicable chronic illnesses in the world, and it causes a reduction in antioxidant capacity. Blood glucose level become increased in diabetes as a result of abnormalities in insulin synthesis or activity. As a result, macronutrient metabolism is disrupted, resulting in long-term health issues. Furthermore, the body's antioxidant defense mechanism is harmed by free radicals produced by long-term hyperglycemia. The majority of diabetes treatment focuses on pharmaceutical control of hypoglycemia, however this has limited success due to numerous adverse effects (Barre et al .,2008).

Mucilage (6%) and insoluble fibres (18%) are the most significant components of flaxseed, followed by proteins (25%), oils (30-40%), and -linolenic acid (50-60%) as the primary fatty acid. Its mucilage is easily extracted from the seed and has been used as a stabilizer and thickener in the food industry. In both animals and people, flaxseed meal has been shown to reduce blood cholesterol levels. The purpose of this research is to examine how flax mucilage influences blood sugar and cholesterol levels in Type 2 diabetics (Thakur et al .,2009).

1.2.6HMO-CoA reductase inhibitory activity of flaxseed oil

Hypercholesterolemia causes atherosclerosis, which is a leading reason of heart problems including myocardial infarction. Hypercholesterolemia is caused by high levels of plasma cholesterol, notably law density lipoprotein (LDL) and triglyceride levels, which can lead to obesity, diabetes, and cancer. In cholesterol biosynthesis, the (HMG-CoA) reductase catalyzes the conversion of HMG-CoA to mevalonate, which is the rate-limiting enzyme. By activating sterol regulatory element-binding protein, which upregulates (HMG-CoA) reductase and LDL receptor, inhibiting HMG-CoA reductase successfully decreases cholesterol levels in humans and most animals (Baskaran et al.,2015).

The therapeutic potential of medicinal plants for hypercholesterolemia is mostly unexplored at this time, but it might be a viable alternative to the development of effective and safe anti hypercholesterolemia drugs. This study examined the inhibitory effects of 25 medicinal plant extracts on HMG-CoA reductase. Flax seeds are high in omega-3 fatty acids, alpha-linolenic acid, dietary fiber, and natural antioxidants. The flaxseed lignan complex reduced the severity of hypercholesterolemic atherosclerosis and reduced blood cholesterol levels. Flax seeds lowered total and LDL cholesterol levels, as well as fat excretion in the blood. Flax seeds have been demonstrated to lower total and LDL cholesterol levels in people. Flaxseeds include numerous types of phenolics, which have antioxidant properties and assist to reduce the effects of free radicals (Pant et al .,2015).

1.3 Objective of this study

The main goals of this research are:

1. Studying anitglytcation assay, antibacterial effect, antioxidant activity, total phenolic and total flavonoids content of flaxseed oil.

2. Conducting HPLC analysis of flaxseed oil.

3. Investigating impact of flaxseed oil on hypercholesterolemia.

Chapter Two
Literature Review

Based on a survey of the literature, it is clear that substantial research has been done on many aspects of flaxseed oil. With this in mind, chapter two is going to focus on antiglycation activity, antioxidants, antibacterial activity, total phenolic and flavonoids content in flaxseed oil, Flaxseed oil supplementation reduces skin irritation and enhances skin barrier function and health. Flaxseed oil extract as a therapy for hypercholesterolemia: HMG-COA reductase inhibitory activity and HPLC analysis of flaxseed oil .

2. Medical effect of flaxseed oil

2.1 Antioxidant effect

The aim of this study was to find out the phytochemical components of flaxseed solvent extract as well as its free radical scavenging abilities. To undertake phytochemical analysis of extracts, exploratory screening procedures of phytoconstituents in various solvents were applied. Furthermore, methanolic and butanolic extracts provided larger quantities of total phenolic components (47.015.40 and 43.332.77 g gallic acid equivalents/g of extract, respectively) and flavonoids (30.890.09 and 29.550.15 g Quercetin equivalents/g of extract, respectively). The antioxidant activity of petroleum ether, benzene, ethyl acetate, methanolic, and butanolic extracts of flaxseed was investigated in vitro. Although the hydrogen peroxide scavenging activity of petroleum ether and ethyl acetate extracts was considerable, butanolic extract had a higher DPPH radical scavenging activity (Alachaher, et al .,2018).

Same finding of antioxidant activity has been noted in Amin, et al.(2014) study of antioxidant activity of flaxseed extracts. The proximate composition, phytochemical screening and antioxidant activity of ethanol and chloroform extracts of flaxseed oil, were investigated in this work. Free radical scavenging activity was calculated using the DPPH and hydrogen peroxide methods to calculated the antioxidant activity of extracts. Using the DPPH and hydrogen peroxide methods, the median inhibitory concentration (IC_{50}) was determined. The IC_{50} value of ethanol extract of flaxseeds according to the DPPH technique is 256.313 g/ml, while according to the hydrogen peroxide method, it is 33.718 g/ml.

In the same context, Flaxseed is high in phenolic compounds like lignans, phenolic acids, flavonoids, phenylpropanoids, and tannins. The proximate composition, phytochemical screening, total phenolic, total flavonoids, and antioxidant activity of acetone 70%, methanol 70%, ethanol 70%, and water extracts of flaxseed .were investigated in this work. The free radical scavenging activity of extracts was measured using the DPPH technique. The DPPH technique was used to calculate the (IC50). The IC50 value of flaxseed acetone extract is 90.76, while methanol extract is 55.74, ethanol extract is 65.21, and water extract is 97.40. Methanol extracts outperformed ethanol, acetone, and water extracts (Hanaa MH., et al.2017).

2.2 Antibacterial effects:

The antibacterial activities of flaxseed fixed oil studied by Katihwas., et al. (2011) The therapeutic effectiveness of flaxseed fixed oil in bovine mastitis was examined in vitro and in vivo. Method The disc diffusion method and minimum inhibitory concentration (MIC) determination were used to assess the in vitro antibacterial activity of flaxseed fixed oil against a variety of microorganisms. The oil's in vivo efficacy was assessed in nine mastitis-affected cows divided into three groups (three in each group) after a once-daily intramammary infusion of oil, cefoperazone, or an oil-cefoperazone combination for seven days, with the California mastitis test score, somatic cell count, and microbial count in milk samples monitored. Results the oil has antibacterial efficacy against Staphylococcus aureus (S.aureus), Streptococcus agalactiae, and Escherichia coli (E.coli) that were comparable to cefoperazone in vitro.

The test same to the aforesaid were done and the same results were obtained by Jabbar., et al.(2016) The goal of this investigation was to examine if flaxseed oil had antibacterial and antibiofilm efficacy against some locally isolated bacterial pathogens. Neither Escherichia coli (E.coli) nor Enterococcus faecalis had any inhibitory effect. Methicillin resistant Staphylococcus aureus, (MRSA), Methicillin-Sensitive Staphylococcus aureus (MSSA), Klebsiellapneuminae, and Staphylococcus epidermidis.On the other hand, The flaxseed had antibiofilm activity against all of the bacteria examined (MSSA, MRSA, S. epidermidis, and K. pneumoniae) and inhibited them to varying degrees. Flaxseed oil was used to heal wounds in the laboratory. Finally, flaxseed oil is a fantastic alternative drug that may be used to treat bacterial wound infections.

In addition, Hady., et al.(2017) investigated the antimicrobial activity of flaxseed oil. The data revealed the existence of 52 bacterial isolates, 37 (71%) of which were gram–positive bacteria, categorized as Staphylococcus aureus (S. aureus) (20%), Streptococcus pyogenes (S. pyogenes) (9%), and Streptococcus pneumoniae (S. pneumoniae) (1%). 15% of the total Only 15 (29%) of the bacteria were gram-negative, with E. coli (73%) being the most prevalent, followed by P.aeruginosa (4%), and Protuse mirabilis (4%). The antibiotic susceptibility of S. aureus and E.coli bacteria, which are the most common G+ve and G-ve nosocomial pathogens in Najaf hospitals, was tested using antibiotic susceptibility pattrons in order to choose one isolate from S. aureus and one isolate from E.coli that had the highest resistance to most antibiotics for the remaining steps of study.

2.3. Diabetes type 2 and Glycation of Hemoglobin in blood

The goal of the study was to see how omega-3 fatty acid supplementation affected antioxidant capacity in people with type 2 diabetes. For that purpose, 114 people with type 2 diabetes were at random assigned to one of three groups: fish oil (n=42), flax seed oil (n=35), or maize oil (n=37) for 180 days. In the fish oil group, the level of glycosylated hemoglobin in the blood was considerably lower. After 180 days, the concentrations of malondialdehyde in the fish oil, flax seed oil, and maize oil groups increased considerably, while superoxide dismutase declined dramatically, but no important differences were seen when any two of the three groups were compared, with high-density lipoprotein cholesterol levels (Wang .,et al.2021).

In contrast, Type two diabetes is characterized by elevated fasting blood serum glucose and insulin concentrations, as well as the percentage of hemoglobin expressed as Hemoglobin A1c(HbA1c), are some of the signs and symptoms of type 2 diabetes. Flaxseed oil supplementation was thought to improve blood glucose, insulin, and HbA1c profiles. Patients were seen twice before therapy, three months apart, on two separate dates (visits 1 and 2). Both groups consumed less than 10 grams of oil each day. The involvement group consumed 5.5 g of Alpha Linolenic Acid (ALA) each day. Fasting blood serum glucose, insulin, and HbA1c levels were unaffected by flaxseed oil. High dosages of flaxseed oil had no impact on glycemic management in type 2 diabetics, according to the findings (Barre ., et al. 2008).

2.4. HMO-CoA reductase inhibitory activity of flaxseed

One of the biggest hazardous factors for coronary heart disease and atherosclerosis is hypercholesterolemia. The impact of flaxseed oil supplementation on blood lipid profile, apoprotein A (apo A), apoprotein B (apo B), Lipoprotein a Lp (a), homocysteine, and endotheline-1 (ET-1) in rats with high cholesterol diet-induced hypercholesterolemia was investigated in this study. A total of 60 male rats were used in this experiment. These findings imply that flaxseed oil might be useful in lowering cholesterol levels and treating dyslipidemia, as well as reducing the risk of cardiovascular problems associated with hypercholesterolemia(Hussein., et al . 2014).

In a similar context, Patade., et al.(2008)also mentioned the effect of flaxseed oil on hypercholesterolemia. The goal of the study was to evaluate how much a daily dose of 30 grams of flaxseed, a rich source of lignans, omega-3 fatty acids, and fiber, added to the diet of Native American postmenopausal women for three months improved their lipid profiles. Fifty-five Native

American postmenopausal women with mild to moderate hypercholesterolemia (5.1 to 9.8 mmol/L) were randomly allocated to one of three groups. To examine lipid parameters, overnight fasting venous blood was obtained at base and at the conclusion of the therapy period. The findings of this study show that regular flaxseed eating reducing the risk of cardiovascular disease in Native American postmenopausal women, as seen by decreased total cholesterol levels.

Furthermore, cardiovascular diseases (CVDs) are one of most important causes for mortality worldwide.The major and rate-limiting enzyme in the cholesterol production pathway is reductase (HMGCoA). Therefore, the main goal of this study was to look at how nutraceuticals and medicinal herbs affect HMGCoA reductase . The results show that nutraceuticals such as natural foods, isolated nutrients, herbal items, and dietary supplements reduce HMGCoA reductase expression and activity (Hussein., et al . 2014).

2.5 Supplementing with flaxseed oil improves skin health

Flaxseed or borage oil were given to two groups of women for 12 weeks in this study. Skin reddening was reduced in both groups, With flaxseed or borage oil, skin hydration was considerably enhanced after 12 weeks of therapy comparison to week 0 ($P<0.05$). After 12 weeks, the flaxseed oil group showed a further drop. When comparing week 0 and week 12 ($P<0.05$), surface examination of live skin demonstrated that flaxseed and borage oil significantly reduced skin roughness and scaling. The placebo group had no effect on any of the indicators except hydration. The findings show that dietary lipids can be used to influence skin characteristics (De Sprit.,et al. 2009).

In the same context, skin sensitivity is a prevalent condition in the Western population, and it is linked to changes in skin properties such as skin barrier function, moisture, and physiology. The goal of this study was to investigate how healthy participants with sensitive skin responded to regular flaxseed and safflower seed oil intake. The 12-week intervention was designed as a randomized, double-blind research with two women therapy groups (n = 13). Only a substantial roughness of the skin improves and moisture was detected with safflower seed oil; however, the benefits were less dramatic and observed next period than with flaxseed oil, and the plasma n-6/n-3 FA (fatty acid) ratio increased. The findings show that consuming flaxseed oil on a daily basis improves skin health (Neukam .,et al. 2010).

Also, Cosmetics has been around for a long time. Humans use cosmetics to seem nice and beautiful, and natural beauty is a blessing. Herbal cosmetics, including as cleanser, moisturizer, toner, lotions, and creams, are used on a regular basis. Essential oils are concentrated liquids made up of a complex variety of volatile components that may be collected from certain plant organs. Flaxseed is used to make hair oil and hair gel, both of which serve to moisturize and nourish the hair. Flaxseeds are also utilized as a nutritional supplement and in the production of various foods (Fale., et al 2022).

Chapter Three
Materials and Methods

3.1 Chemical material

Used Materials: Absolute Ethanol (EtOH), Ethanol 99%,petroleum ether, Methanol,Ethyl acetate, Dimethylsulfoxide (DMSO), Folin – ciocalteau reagent (10 time diluted) .Sodium Carbonate solution (Na_2Co_3), Gallic acid, Aluminum chloride (AlCl3)2%, sodium acetate Methanol, Quercetin (reagent), DDPH (2,2-Diphenyl-1-picrylhydrazyl propane hydrochloride), Ascorbic acid, Bacterial cultures (plate count agar), Antibiotic control (Penicillin, Gentamicin), Bovine Serum Albumin (BSA), Monopotassium phosphate (KH_2PO_4), distilled water(DW), Disodium phosphate (Na_2HPO_4), Sodium chloride (NaCl), distilled water, Fructose and Glucose, Cetylstearyl alcohol, Isopropyl Myristate, GlycerilMonostearate, Vaseline, Glycerin, triethanolamine, sorbitol, methyl paraben, perfume and water.

Materials were purchased from Sigma Aldrich.

3.2 Equipment and Apparatus

Soxhlet apparatus, Rotary evaporator, spectrophotometer (UV2550), spectrophotometer with Temperature control, Graduated cylinder, Evaporating dish, Beaker, Test tubes Micro pipettes, Spatula, Thermometer, Funnel were used in the Chemistry, Biology, laboratories for environmental sciences at Al-Quds University.

3.3 Methods

3.3.1 Collection and Identification of Flaxseed:

The flaxseed under study was bought in summer 2020 from a spice shop in Ramallah, Palestine. It was identified by Dr. Khalid Sawalha (Associate Professor of Plant Biotechnology) from the Biology Department, Sciences Faculty - Al-Quds University. Flaxseed has a Binominal name, which is Linum usitatissium, and a family name is Linaceae.

3.3.2 Plant Sample:

Flaxseeds were manually cleaned to remove husks and extraneous materials, then homogenously ground using a mechanical grinder till got a fine powder.

3.3.3 Oil extraction

3.3.3.1 Preparation of flaxseed oil ethanol extract:

Ethanol extract of the flaxseeds was made through soxhlet apparatus (model FA-46) extraction process using 15gm of flaxseed which needed 300 ml of ethanol solvent, and this process took 2 hours to get the chemical components contained in flaxseeds.

Once the process has finished, the ethanol evaporated by Rotary evaporator under decreased pressure at 25C° and then it was switched to 40C° (Khattab et al., 2013).

Figure 3.3 soxhlet apparatus (model FA-46)

3.3.4 Total phenolic content (Folin–Ciocalteu assay)

The total phenolic content was calculated using the Folin-Ciocalteu assay. Samples containing polyphones are reduced by Folin-cioacalteu regent there by producing colored complex. Flaxseed oil extracts (0.50 mg) were dissolved in ethanol (99%) (20 mL), 1ml of sample's or gallic acid (1000ppm) were mixed with 5 mL of Folin-Ciocalteu reagent (diluted 10-fold) and 5 mL (75 g/L) sodium carbonate. After incubation at 25°C for 30 min, the quantitative phenolic estimation was performed at 765 nm by UV spectrophotometer (EMC-61PC-UV Spectrophotometer). The phenolic concentration of extracts was evaluated from a gallic acid calibration curve of 20 µg/ ml, 30µg/ml, 40 µg/ml, 50µg/ml, 60µg /ml, 70 µg/ml, and 80µg/ml. µg/mL ethanoic Gallic acid by putting the value of absorbance vs. concentration.

3.3.5 Total Flavonoid Content (TFC)

A percentage of 5% of quercetin was used to make the standard calibration curve. The standard solutions of quercetin were prepared by serial dilutions using distilled water (10 - 50- 100-200 µg / ml).1 ml of diluted standard quercetin solutions or extracts was separately mixed with 3 ml of methanol, 200µl of 2% aluminum chloride solution, 200µl of 1M sodium acetate solution, and 5.6 ml of distilled water. The solution was incubated for 30 min at room temperature, The absorbance of the reaction mixtures was measured against blank at 420 nm wavelength with (EMC-61PC-UV Spectrophotometer).

3.6. RP-HPLC analysis of flavonoids

The high performance liquid chromatography (HPLC) technique is frequently used to examine the flavonoids concentration in natural extracts, for both separation, and identification of these chemicals. For quercetin analysis, a C18 column (15 cm with a UV detector) was utilized in HPLC using UV a detector (4 micrometer particle size) and a water mobile phase at wavelength of 254 nm: (50:50) Methanol standard of 0.01 g per 10ml (v/v) at flow rate of 1.0 ml per minute (Baskaran et al., 2015).

3.7. Fluorescence-based assay of the inhibition of AGE formation

The approach was carried out as formerly reported (Wang et al., 2009), with the following amendment:

a. Preparation of Incubation media

1- A sodium phosphate monobasic monohydrate buffer (pH 7.4) (100mM) was produced.

2- In (100mM) sodium phosphate monobasic monohydrate buffer, a stock solution of (1 mg/ml) (BSA) was produced (pH 7.4).

3- In (100mM) sodium phosphate monobasic monohydrate buffer, a stock solution of (100mM glucose/100mM fructose) combination was produced (pH 7.4).

b. Preparation of extract samples

Five various concentrations of flaxseed oil, which were prepared using ethanol 99%, and these concentrations began from 2.5 mg/ml to 50 mg/ml for each extracts to determine concentration-dependent responses.

c. Test Samples

The test samples of both extracts and controls were prepared in test tube with screw cap with sample volume of (1000µL), every sample was repeated in duplicate.

Extract samples

100 µl of BSA, 100 µl of sugar solution, 300 µl of phosphate buffer (pH 7.4), and 500 µl of each five concentration of flaxseed oil, were prepared in Test tube with screw cap and incubated in incubator shaker (Environmental Sciences Laboratory lab) at 37°C for 7 days.

Positive control

In test tubes, 100 µl of BSA, 100 µl of sugar solution, 300 µl of phosphate buffer (pH 7.4), and 500 µl of Quercetin standard (Q4951) were made with five various doses ranging from 2.5 mg/ml to 12.5 mg/ml, and incubated in an incubator shaker at 37°C for 7 days.

Negative control

In a test tube with screw cover, 100 µl of BSA, 100 µl of sugar solution, 300 µl of phosphate buffer (pH 7.4), and 500 µl of Ethanol 99 percent were made and incubated at 37°C for 7 days in an incubator shaker.

d. Fluorescence-based assay of the inhibition of AGE formation:

The development of fluorescent antiglycation End products (AGEs) in each sample was measured using a fluorometer (Albaraj lab, Al-Quds University) at excitation and emission wavelengths of 455 nm and 375 nm, respectively, after 7 days of incubation. To eliminate baseline fluorescence, the experimental treatment (including BSA, sugar, and either extract or pure standard) and the negative control had their fluorescence values blanked against BSA, phosphate buffer, and the relevant extract blanks. The percentage of inhibition of AGE production was calculated using the corrected fluorescence readings (F) for the negative control (F negative control) and experimental treatments (F experimental corrected).

3.8 Preparation of cream

Hand cream was made according the following researcher-developed formula:

Part one: selected materials and determined their weights, and these materials are listed as follows: Cetylstearyl alcohol, Isopropyl Myristate, GlycerilMonostearate, flaxseed oil and Vaseline.

Part two: selected materials and determined their weights, and these materials are listed as follows: Glycerin, triethanolamine, sorbitol, methyl paraben and water.

Part three: selected a proper perfume.

Procedure: materials mentioned in part one and part two were heated to 75 C° and then materials of part two were added to materials of part one. And then at the temperature of 40C°the selected perfume was added to the mixture.

3.9 Antibacterial activity

The antibacterial test by using agar disc diffusion (Kathwas et al., 1999). Negative controls were created utilization the same solvents (90% ethanol) as were used to dissolve the samples. Standard antibiotics Gentamicin (10 micrograms/disc) and Penicillin served as positive controls for the microorganisms studied (10 unit). Antibacterial effectiveness was measured by the diameter of the zones of inhibition surrounding the disc against the microorganisms tested.

3.9.1 Microorganism

The University of Al-Quds' College of Health Professions Department submitted clinical isolates of Escherichia coli (E.coil), Staphylococcus aureus(S.aureus), Methicillin-resistant Staphylococcus aureus, (MRSA), and Pseudomonas aeruginosa(p. aeruginosa).

3.9.2 Preparation of medium

Bacteria needed highly particular environmental conditions to grow, including adequate dietary energy, the proper temperature, and the right humidity. We must utilize culture medium to generate these conditions in order to develop these cells in the lab. Muller Hinton agar is used as solid culture medium in Petri plates. After the bacterial inoculum was created in broth, Mueller Hinton Agar (MHA) was made and sterilized by autoclaving for around 30 minutes.The medium was placed in sterile petri plates. For 10-15 minutes, they were allowed to harden. Plates were placed in a plastic bag and flipped upside down to prevent moisture from gathering on the medium's surface.

3.9.3 Bacterial culture

Bacteria were injected into nutritional broth and cultured at 37°C for 24 hours before being diluted to a concentration equivalent to MacFarland nephlometer tube no. (108 cfu/ml) using a spectrophotometer set to 625 nm (optical density 0.08 to 0.1). Using a cotton swab, the inoculum was uniformly spread on the surface of MHA after it had hardened.

3.9.4 Antibacterial

The disc diffusion technique was utilized to test the antibacterial activity of four distinct bacteria strains. To disinfect the disc (5mm diameter), it was autoclaved for around 30 minutes. The germs were then disseminated across Muller Hinton agar plates. Reference antibiotic disc was put on the surface of MHA as a positive control. Later, with 50 µ l in each disc, sterile discs were

impregnated with plant extracts (at varied concentrations) and negative controls (solvent). The plates were then incubated at 37°C for 24 hours.

3.10 Antioxidant assay

(DPPH) Assay described by Amin and other (Amin et al 2014) 3 ml of 4% DPPH methanolic solution added to 1 ml of sample and 6ml methanol .The solution was incubated for 30 min at room temperature. The absorbance of the reaction mixtures was measured against blank at 517 nm wavelength with ((EMC-61PC-UV Spectrophotometer). The percentage of DPPH scavenging effect (%) was calculated by following equation:

DPPH scavenging effect (%) =A0 -A1 /A0 × 100

Where A0 = absorbance of control.

A1 = absorbance of standard.

3.11 Enzyme assay

The enzyme and the substrate are less stable when added to the assay buffer. Hence, it is very important to add the different components according to the order mentioned in this procedure in order to obtain best results.

The HMG-CoA reductase stock solution had a concentration of 0.5–0.75 mg/mL. Each crude extract (50 µ g) was combined with a reaction mixture containing nicotinamide adenine dinucleotide phosphate (400µ M), HMG-CoA substrate (400µ M), and potassium phosphate buffer (100 mM, pH 7.4) containing potassium chloride (120 mM), ethyl enediaminetetraacetic acid (1 mM), and dithiothreitol (5 mM), as well as HMG-CoA (Baskarn et al ., 2015).

Before beginning, set the spectrophotometer at 37 °C and 340 nm, with a kinetic program:

• 1 ml sample: read every 20 seconds for up to

5 minutes.

The HMG-Co A reductase inhibition (%) was evaluated using the formula below:

$$\text{Inhibition}\,\% = \left(\frac{\Delta\ \text{Absorbance control} - \Delta\ \text{Absorbance test}}{\Delta\ \text{Absorbance control}} \right) \times 100 \quad (1)$$

Chapter Four
Results and Discussion

4. Phytochemical screening

4.1. Total phenolic contents (TPC)

TPC of flaxseed oil extracts for two way, flaxseed oil was extracted using ethanol 99% and petroleum ether as solvents, and the results are shown in **Table 4.1**

The result of the (TPC) was extracted using ethanol resulted in (231.0mg /g)which is higher than what was extracted using petroleum ether (53.5 mg /g).

With respect to TPC, Several previous researches showed lower percentage as they came up with a result of (47.01±5.40 mg\g), and that was attributed to using different solvent (Methanol) (Alachaher et al ,.2018).

Table 4.1: (TPC as mg Gallic acid/g DW).

Solvent	**TPC (Mg/g)**	**TFC**
Ethanol 99.9	230.81 ±0.02mg /g	223.72 ±0.04mg/g
petroleum ether	53.531 ±0.007mg/g	-

***Abbreviation:** DW, distilled water

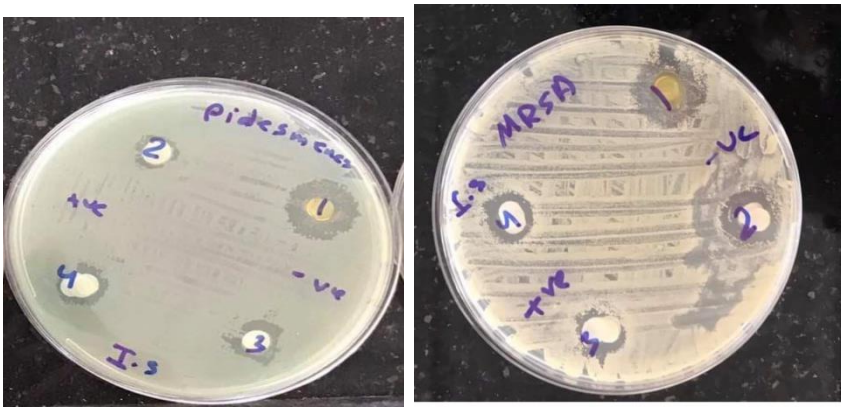
4.2 Total flavonoids content (TFC):

The results of the Aluminum chloride test for determined (TFC) were presented in Table 4.1, as the results showed that the flaxseed oil addressed in this study the Total phenolic content has a higher value comparing to their correspondent total flavonoid content.

With respect to TFC, Several previous researches showed lower percentage as they came up with a result of (30.89±0.09 mg\g), and that was attributed to using different solvent (Methanol) (Alachaher et al ,.2018).

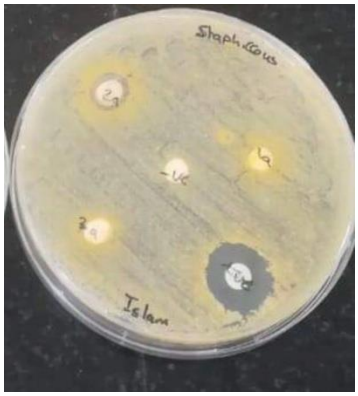
4.3 Antibacterial activity

The extracts from the flaxseed oil (extracted using petroleum ether) showed antibacterial activities against MRSA, Pseudomonas, Staphylococcus, was extracted using ethanol 99 % showed antibacterial activities against Staphylococcus only. The existence of positive control (Gentamicin (10 micrograms/disc) and Penicillin 10 unit) by using the disc diffusion method. The zones of inhibition (Fig.4.3 and fig 4.4) were calculated and the average results of zones of inhibition were presented in Table 4.2.



Flaxseed oil antimicrobia activity Against p.aeruginose	Flaxseed oil antimicrobial against MRSA

Figure 4.3 Zone inhibition of flaxseed oil (extracted using petroleum ether)



Flaxseed oil antimicrobial activity against S.aureus	Flaxseed oil antimicrobial activity against S. aureus

Figure 4.4 Zone inhibition of flaxseed oil (extracted using ethanol)

Table 4.2 The inhibition zone (mm) of extract is shown as follows:

Flaxseed oil	**Bacteria type**	**Average zone of inhibition (mm)**
Flaxseed oil (extracted by petroleum ether)	MRSA	12 mm
Flaxseed oil (extracted by ethanol 99%	MRSA	ND
Flaxseed oil (extracted by ethanol 99%	E-Coli	ND
Flaxseed oil (extracted by petroleum ether)	E-Coli	ND
Flaxseed oil (extracted by ethanol 99%	P.aeruginosa	ND
Flaxseed oil (extracted by petroleum ether)	P. aeruginosa	11.25 mm
Flaxseed oil (extracted by ethanol 99%	S.aureus	9mm
Flaxseed oil (extracted by petroleum ether	S.aureus	12mm
* ND : : Not detected.		

In other previous researches, results showed inhibition of E-Coli due to using solvent n-hexane (Jabber et al ,. 2015).

4. 4 Antioxidant assay

Table 4.3 The result of scavenging activity for flaxseed was extracted using ethanol 99.9% and petroleum ether. The DPPH test was used to determine antioxidant activity.

Name of solvent	**AS (Absorbance standard) Average**	**Ac (control)**	**AE (Absorbance sample) Average**	**% scavenging activity**
Ethanol 99 %	0.47	1.29	1.71	4.02±0.02
Petroleum ether	0.47	1.29	1.74	1.7±0.2

*Abbreviation: AS: absorbance standard; AC: Absorbance control; AE: absorbance sample

Table 4.3 shows the antioxidant activity of flaxseed extracted by ethanol 99% and petroleum ether analyzed in this research. As can be seen from the data, the extraction with ethanol contains4.019mg/g. where comparative to the antioxidant activity of flaxseed extracted with petroleum ether 1.741 mg/g, flaxseed extracted by ethanol in this research, had a higher antioxidant activity than that extracted using petroleum ether.

Respecting antioxidant activity of flaxseed, multiple researches concluded that it has a result of (7.35 mg\g) as they used the ferric reducing antioxidant power (FRAP) (Alachaher et al ,.2018).

4.5 Enzyme assay

HMG-CoA reductase inhibitory activity of the flaxseed oil examined based on Spectrophotometric measurements.

HMG-CoA reductase catalyzes the rat-limiting step in synthesis of cholesterol. In the study, the inhibition of the anzyme may reflect the potential of flaxseed oil in cholesterol reduction.

Table 4.4 Anti -HMG-CoA reductase activity of flaxseed oil extracted

Scientific name	Family name	Inhibition %
Linumusitatissimum	Linaceae	56.3%

*Abbreviation:HMG-CoA,hydroxy-3-methyl -glutaryl-coenzyme A

The inhibition flaxseed oil (extracted using petroleum ether) was presented in Table 4.4. The positive control applied in this study, simvastatin, showed enzyme inhibition of 85.1% (Baskaran et al ., 2015).

4.6. Preparation of cream

When used topically, the formula is moisturizing, smoothing, has a pleasant scent, absorbs rapidly (without leaving a film), does not irritate the skin (ph= 5.5-6), has an excellent color, is easily distributed on skin.

Figure 4.5 Cream of flaxseed

The samples stored at 8°C and 25°C showed no signs of liquefaction. The sample remained stable in the evaporimeter at 40° ever the 17th day. At different temperatures, the formulation remained stable. And this is an expected result as flaxseed oil has antibacterial potential.

Measurement: The percentage of flaxseed extract in cream = 2.01/99.8*100% = 2 %

Figure 4.6 Evaporimeter (left) creams in 40°C (middle)

4.7 Anti-glycation End Products (AGEs) Assay

Anti-glycation activity of the oil concerning the impacts of various concentration of positive control and extraction fluorescent AGE formation, five concentration (2.5 mg\mL- 12.5 mg\mL)for sample, and the results shown that the percentage of inhibition of flaxseed oil as follows: concentration of 12.5 mg /ml gave a higher inhibition concentration than 10 mg/ml , the inhibition were presented in Table 4.5.

Also, after reviewing previous researches with respect to Anti-glycation activity, the results were better and promising due to using clinical experiment (THAKUR et al ., 2009).

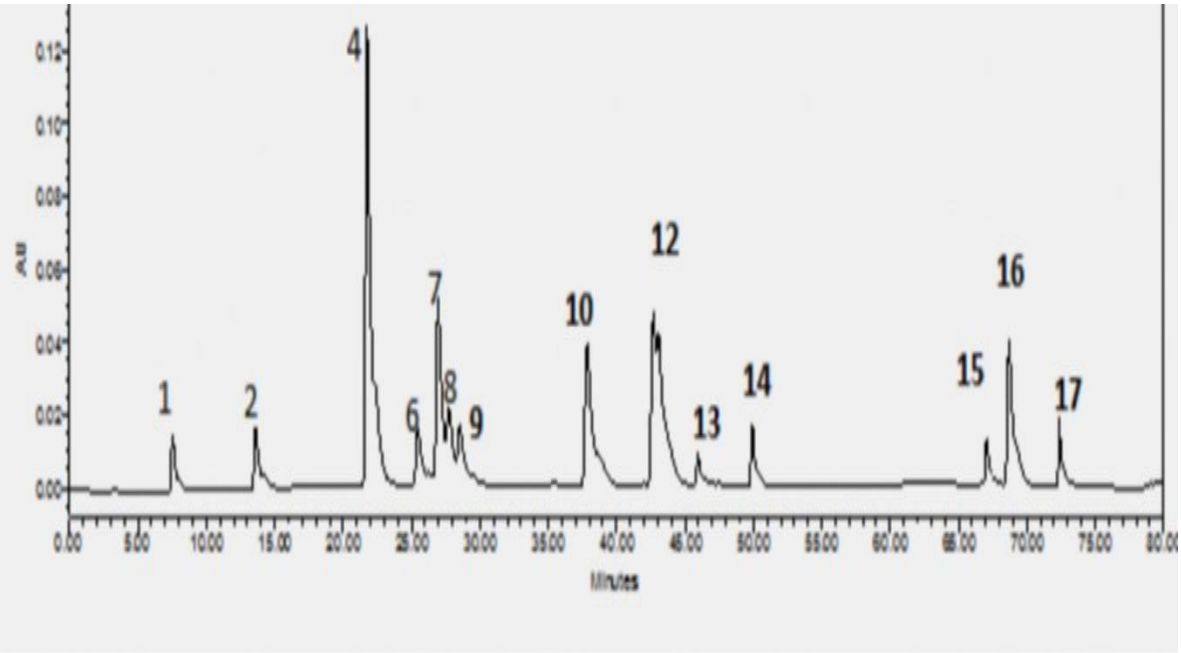
Table 4.5 Antiglycation results all tested samples

Concentration(mg/ml)	Fluorescence Response	% Inhibition
2.5	10.07	N
5	9.023	N
7.5	8.694	N
10	6.998	15.7
12.5	5.814	29.9

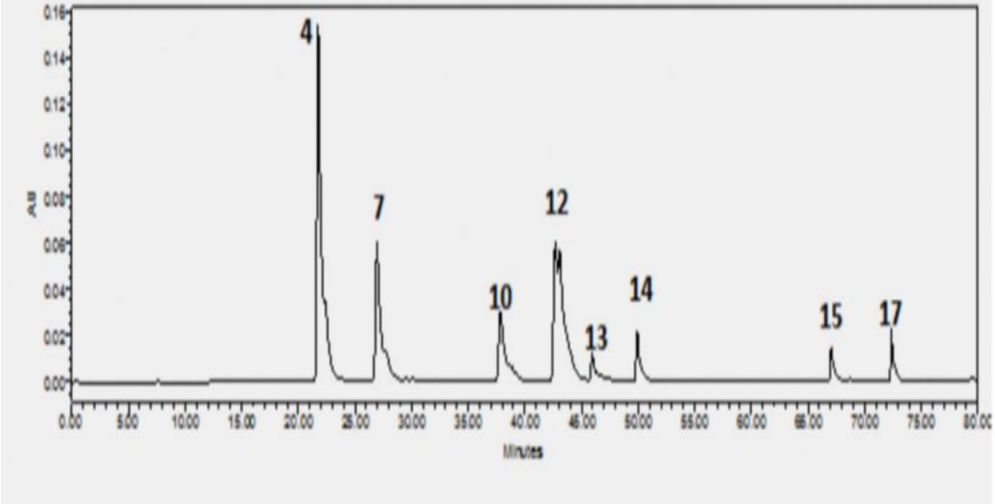
* N: negtive results

4.8. HPLC analysis of the standards of polyphenolic compounds and flavonoids

The mixture of 17 standards were injected (20 µL) into the HPLC chromatograph and analysedusing the RP-phase method described above. Different wavelengths using the photodiode array detector were used as each compound has its own wavelength of maximum absorption (Table 4.7). Figure 4.8 shows the chromatograms of the standards mixture at different wavelengths (300 nm (a), 323 nm(b), 270 nm (c), and 290 nm (d)). As it is obvious from Figure4.8 (a-d), the 17 compounds were separated when different wavelengths were used. Table 4.7summarizes the retention times of the standards with maximum wavelength of absorption for each standard.



(a)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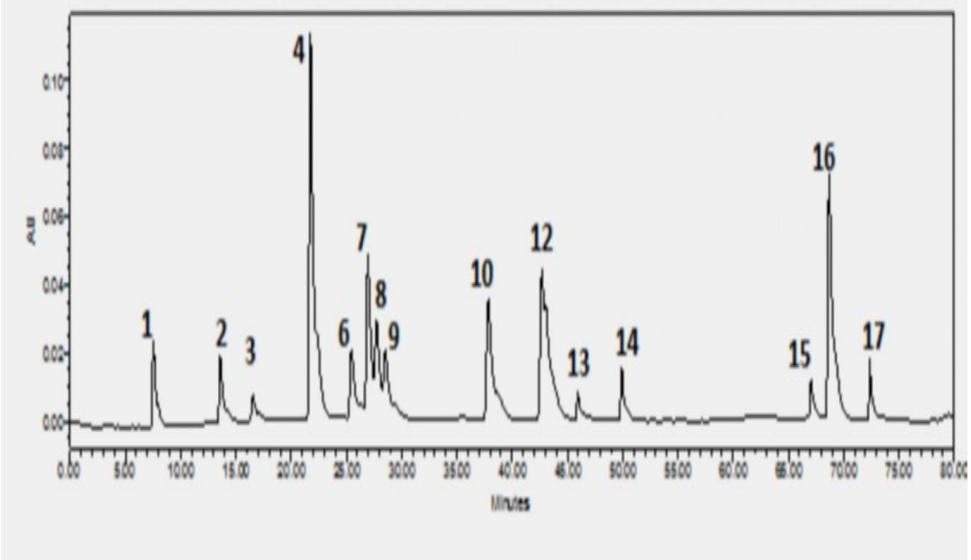
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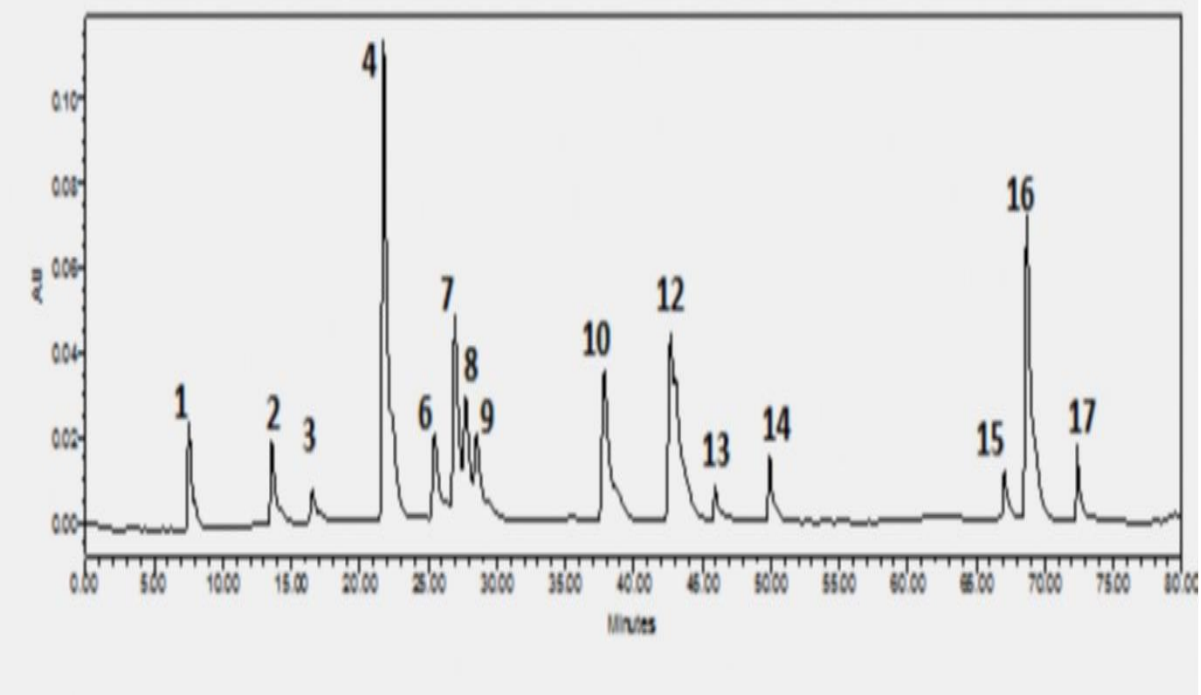

(d)

Figure 4.8 HPLC chromatogram of polyphenolic and flavonoid standards analysed using RP-HPLC method at 300 nm (a), 323 nm (b), 270 nm (c), and 290 nm (d).

Table 4.6 List of Standard compounds analyzed using RP-HPLC method with their retention times and maximum wavelength of absorption

Standard #	Standard name	Retention time (min)	Wavelength (nm)
1	Gallic acid	8.26	271
2	3,4-Dihydroxybenzoic acid	13.87	259
3	3,4Dihydroxyphenylacetic acid	16.57	280
4	Chlorogenic acid	21.64	323
5	4-hydroxyphenylacetic acid	24.55	274
6	Vanallic acid	25.42	260
7	Caffeic acid	26.92	322
8	Syringic acid	27.73	274
9	Isovanallic acid	28.55	259
10	Ferrulic acid	37.82	309
11	Sinapic acid	42.68	322
12	Rutin	43.1	323
13	Verbascoside	45.99	255
14	Trans-cinnamic acid	49.98	329
15	Quercetin	67.04	364
16	Trans-cinnamic acid	68.69	275
17	Kaempferol`	72.37	265

4.8.2 HPLC analysis of flaxseed extracts

The flaxseed extracts (excreted using ethanol) were analysed using the method developed for the standards. Figure4.9 shows the chromatogram extract at 280 nm). The following polyphenolic compounds were detected and identified by comparing the retention times of the peaks in the sample chromaogram of the extract with that of the standard:

1. Chlorogenic acid

2. Rutin

3. Trans cinnamic acid

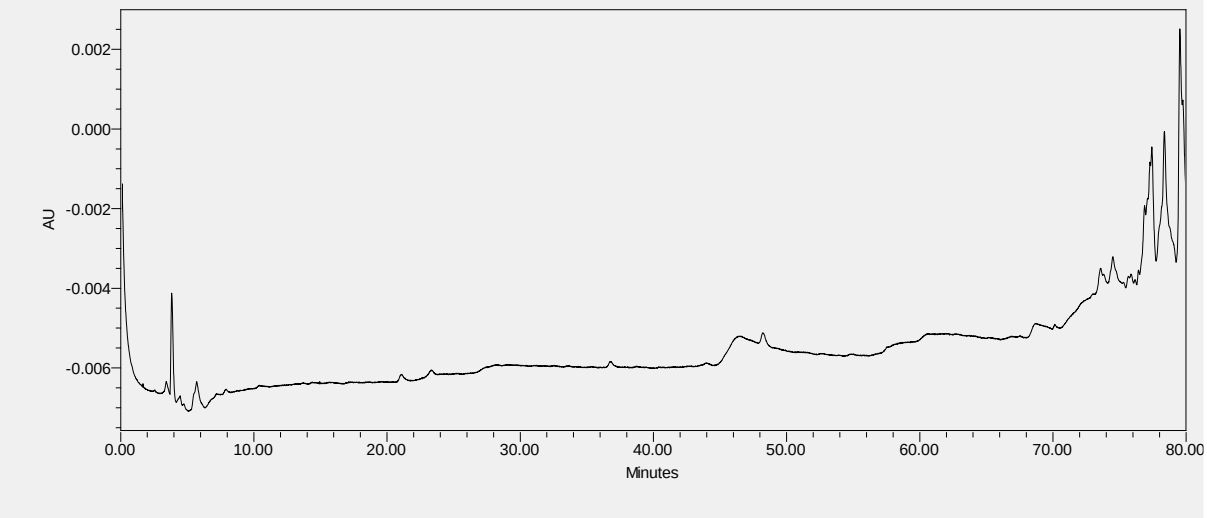

Figure 4.9 The HPLC chromatogram extract for flaxseed oil at (280 nm).

5. Conclusions and future work

5.1 Conclusions:

This research has concluded that flaxseed oil extract is a key player in decreasing cholesterol levels by inhibiting HGM-COA reductase activity, and that may be effective in treating hypercholesterolemia and associated disorders.

It was also discovered that each extract has a large number of phenols and flavonoids, both have strong antioxidant impact.

The HPLC approach was used to identify the polyphenolic components of the flaxseed oil extract and was found to be exact, accurate, and trustworthy. This research suggests that flaxseed oil extract might be utilized as a dietary supplement to enhance human health by preventing glycation and other associated diabetes consequences.

The findings of antibacterial activity in these experiments indicated the potential use of flaxseed oil extract with petroleum ether against resistant Pseudomonas, Staphylococcus, MRSA and flaxseed extract with ethanol against resistant Staphylococcus.

5.2 Future work:

1. We may then undertake further research and employ new approaches to achieve the greatest possible findings, which we can subsequently use to treat a variety of diseases such as reduce high blood pressure in vitro.

2. Clinically estimate the effect of flaxseed oil extract on cholesterol in blood.

3. Studying clinical and\or in vivo effect of flaxseed oilextract on regulating glucose level in the blood.

6. References:

1. Abdul Jabbar, O. et al. The effect of flaxseed extract on skin elasticity of the healing wound in rabbits. IIUM Med. J. Malaysia 18, 5–12 (2019).

2. Ahmad, M. A., Anjum, R., Haq, M. E. & Khan, N. M. Hepatoprotective effect of flaxseed oil on hypercholesterolemia induced hepatotoxicity. Pakistan J. Med. Heal. Sci. 11, 1158–1162 (2017).

3. Alachaher, F. Z., Dali, S., Dida, N. &Krouf, D. Comparison of phytochemical and antioxidant properties of extracts from flaxseed (Linumusitatissimum) using different solvents. International Food Research Journal vol. 25 (2018).

4. Amin, T. & Thakur, M. Original Research Article A Comparative Study on Proximate Composition , Phytochemical Screening , Antioxidant and Antimicrobial Activities of Linum usitatisimum L . (flaxseeds). 3, 465–481 (2014).

5. Andronie, L. et al. Characterization of flax and hemp using spectrometric methods. Appl. Sci. 11, (2021).

6. Asif, M. Chemical characteristics and nutritional potentials of unsaturated fatty acids. Chem. Int. 1, 118–133 (2015).

7. Baskaran, G. et al. HMG-CoA reductase inhibitory activity and phytocomponent investigation of Basellaalba leaf extract as a treatment for hypercholesterolemia. Drug Des.Devel.Ther.9, 509–517 (2015).

8. Bernacchia, R., Preti, R. & Vinci, G. Chemical Composition and Health Benefits of Flaxseed. Austin J. Nutr. Food Sci. 2, 1–9 (2014).

9. Barre, D. E., Mizier-Barre, K. A., Griscti, O. & Hafez, K. High dose flaxseed oil supplementation may affect fasting blood serum glucose management in human type 2 diabetics. J. Oleo Sci. 57, 269–273 (2008).

10. De Silva, S. F. & Alcorn, J. Flaxseed lignans as important dietary polyphenols for cancer prevention and treatment: Chemistry, pharmacokinetics, and molecular targets. P1.

11. De Spirt, S. et al. Intervention with flaxseed and borage oil supplements modulates skin condition in women. Br. J. Nutr. 101, 440–445 (2009).

12.Draganescu, D., Dodi, G., Stoica, I. &Popa, M. I. Hydrolysis studies of flaxseed extract by high performance liquid chromatography. Cellul. Chem. Technol. 51, 803–811 (2017).harmaceuticals 12, 21–38 (2019).

13. Dzuvor, C. K. O., Taylor, J. T., Acquah, C., Pan, S. &Agyei, D. Bioprocessing of functional ingredients from flaxseed. Molecules 23, 1–18 (2018).

14. Fale, S. K. &Bhoyar, S. K. A ComprehensiveStudy Of HerbalCosmetics Prepared From Flaxseed . 4, 106–112 (2022).

15. Wang, F., Zeng, Q., Gao, X., Zhao, P. & Li, C. Differential Modulation of Markers of Oxidative Stress and Glycation of Hemoglobin in Patients with Type 2 Diabetes Mellitus by Edible Oils Rich in Eicosapentaenoic Acid and Docosahexaenoic Acid. Curr. Top. Nutraceutical Res. 19, 84–89 (2020).

16. Gaafar, A .A. In Vitro antioxidant antimicrobial activities of Lingnan Flaxseed Extract linumsitissimumL.(2016).

17. Hady, A. A., Darweesh, M. F. &Motar, A. A. The Antibacterial of Essential Fatty Acid Semicarbazide Extracted from Flaxseed Oil Against Some Nosocomial Infection Bacteria in Iraq. Int. J. Curr. Pharm. Rev. Res. 8, 31–39 (2017).

18. Hanaa, M., Ismail, H., Mahmoud, M. & Ibrahim, H. Antioxidant activity and phytochemical analysis of flaxseeds (Linum Usitatisimum L). Minia J. Agric. Res. Dev. 37, 129–140 (2017).

19. Hussein, S. A., El-Senosi, Y. A., Ragab, M. R. &Hammad, M. M. F. Beneficial effect of flaxseed oil on lipid metabolism in high cholesterol diet fed rats. http://www.bvmj.bu.edu.eg.

20. Jabbar, H., Al-Mathkhury, F., Al-Dhamin, A. S. & Al-Taie, K. L. Antibacterial and Antibiofilm Activity of Flaxseed Oil. Iraqi Journal of Science vol. 57 (2016).

21. Jangale, N. M. et al. Dietary flaxseed oil and fish oil modulates expression of antioxidant and inflammatory genes with alleviation of protein glycation status and inflammation in liver of streptozotocin-nicotinamide induced diabetic rats. Food Chem. 141, 187–195 (2013).

22. Kaithwas, G., Mukerjee, A., Kumar, P. & Majumdar, D. K. Linum usitatissimum (linseed/flaxseed) fixed oil: Antimicrobial activity and efficacy in bovine mastitis. Inflammopharmacology 19, 45–52 (2011).

23. Katare, C., Saxena, S., Agrawal, S. & Prasad, G. Flax Seed: A Potential Medicinal Food. J. Nutr. Food Sci. 02, (2012).

24. Kasote, D. M. Flaxseed phenolics as natural antioxidants. Int. Food Res. J. 20, 27–34 (2013).

25. Khattab, R. Y. & Zeitoun, M. A. Quality evaluation of flaxseed oil obtained by different extraction techniques. LWT - Food Sci. Technol. 53, 338–345 (2013).

26. Lerman, R. H. Essential fatty acids. Altern.Ther.Health Med. 12, 20–31 (2006).

27. Mishra, K., Ojha, H. &Chaudhury, N. K. Estimation of antiradical properties of antioxidants using DPPH- assay: A critical review and results. Food Chem. 130, 1036–1043 (2012).

28. Mukker, J. K., Kotlyarova, V., Singh, R. S. P. & Alcorn, J. HPLC method with fluorescence detection for the quantitative determination of flaxseed lignans. J. Chromatogr. B Anal. Technol. Biomed.Life Sci. 878, 3076–3082 (2010).

29. Neukam, K. et al. Supplementation of flaxseed oil diminishes skin sensitivity and improves skin barrier function and condition. Skin Pharmacol. Physiol. 24, 67–74 (2011).

30. Oomah, B. D., Kenaschuk, E. O. &Mazza, G. Phenolic Acids in Flaxseed. J. Agric. Food Chem. 43, 2016–2019 (1995).

31. Oomah, B. D. Flaxseed as a functional food source. J. Sci. Food Agric. 81, 889–894 (2001).

32. Pag, A. I., Radu, D. G., Drăgănescu, D., Popa, M. I. &Sîrghie, C. Flaxseed Cake-a Sustainable Source of Antioxidant and Antibacterial Extracts Flaxseed Cake-A Sourceof Antioxidentand Antibacterial Extracts. Cellulose Chem. Technol vol. 48 (2014).

33. Patade, A. et al. Flaxseed reduces total and LDL cholesterol concentrations in Native American postmenopausal women. J. Women's Heal. 17, 355–366 (2008).

34. Pant, G., Simaria, C., Varsi, R. A. H., Bhan, P. &Sibi, G. In vitro anti-cholesterol and antioxidant activity of methanolic extracts from flax seeds (Linumusitatissimum L.). Res. J. Med. Plant 9, 300–306 (2015).

35. Patade, A. et al. Flaxseed reduces total and LDL cholesterol concentrations in Native American postmenopausal women. J. Women's Heal. 17, 355–366 (2008).

36. Pellizzon, M. A., Billheimer, J. T., Bloedon, L. A. T., Szapary, P. O. & Rader, D. J. Flaxseed reduces plasma cholesterol levels in hypercholesterolemic mouse models. J. Am. Coll. Nutr. 26, 66–75 (2007).

37. Satarupa Ghosh, DK Bhattacharyya, D. K. and M. G. Studies on Screening of Phyto Chemicals of Flax Seed Oil and its Defatted Flaxseed Meal and Oxidative Stability of the Oil Extracted by Polar and Non Polar Solvent System. Acta Sci. Nutr. Heal. 3, 168–172 (2019).

38. Savio, N., Street, B. & Francisco, S. The Context of Mobile Interaction. Int. J. Mob. Mark. 2, 5–7 (2007).

39. Sciences, B. &Elarab, N. B. Antioxidant, Antimicrobial and Anti-HCV Activities of Lignan Extracted from Flaxseed. Res. J. Agric. Biol. Sci. 6, 247–256 (2010).

40. Styrczewska, M., Kulma, A., Kostyn, K., Hasiewicz-Derkacz, K. & Szopa, J. Flax Terpenoid Pathway as a Source of Health Promoting Compounds. Mini-Reviews Med. Chem. 13, 353–364 (2013).

41. THAKUR, G., MITRA, A., PAL, K. & ROUSSEAU, D. Effect of Flaxseed Gum on Reduction of Blood Glucose Levels in Type 2 Diabetes.Int. J. Food Sci. Nutr.60, 126–136 (2009).

42.Wang, F., Zeng, Q., Gao, X., Zhao, P. & Li, C. Differential Modulation of Markers of Oxidative Stress and Glycation of Hemoglobin in Patients with Type 2 Diabetes Mellitus by Edible Oils Rich in Eicosapentaenoic Acid and Docosahexaenoic Acid. Curr.Top.Nutraceutical Res. 19, 84–89 (2020).

43. Zhang, Z. S., Wang, L. J., Li, D., Li, S. J. &Ozkan, N. Characteristics of flaxseed oil from two different flax plants. Int. J. Food Prop. 14, 1286–1296 (2011).

44. Zhu, L. et al. Dietary flaxseed oil rich in omega-3 suppresses severity of type 2 diabetes mellitus via anti-inflammation and modulating gut microbiota in rats. Lipids Health Dis. 19, 1–16 (2020).

Appendices

Appendix (a): (TPC as mg Gallic acid/g DW).

Name of solvent	TPC (Mg/g)	TFC
Ethanol 99.9	**230.81 ±0.02mg /g**	**223.72 ±0.04mg/g**
petroleum ether	**53.531 ±0.007mg/g**	**-**

***Abbreviation :**DW, distilled water

Appendix (b) The result of scavenging activity for flaxseed was extracted using ethanol 99.9% and petroleum ether

Name of solvent	AS (Absorbance standard) Average	Ac (control)	AE (Absorbance sample) Average	% scavenging activity
Ethanol 99 %	**0.47**	**1.29**	**1.71**	**4.02±0.02**
Petroleum ether	**0.47**	**1.29**	**1.74**	**1.7±0.2**

***Abbreviation: AS: absorbance standard; AC: Absorbance control; AE: absorbance sample**

Appendix (c): Anti-glycation results for all tested Samples

Concentration(mg/ml)	Fluorescence Response	% Inhibition
2.5	**10.07**	**N**
5	**9.023**	**N**
7.5	**8.694**	**N**
10	**6.998**	**15.7**
12.5	**5.814**	**29.9**

*** N: negtive results**

Appendix (d): Anti -HMG-CoA reductase activity of flaxseed oil extracted

Scientific name	Family name	Inhibition %
Linumusitatissimum	**Linaceae**	**56.3%**

Appendix (e): HPLC results for flaxseed extract sample

Name of Polyphenloic compounds	**Retention time (min)**	**Wevelength (nm)**
Chlorogenic acid	**21.64**	**323**
Rutin	**43.1**	**323**
Trans cinnamic acid	**49.98**	**329**

Appendix (f) The inhibition zone (mm) of extract is shown as follows:

Flaxseed oil	Bacteria type	Average zone of inhibition (mm)
Flaxseed oil (extracted by petroleum ether)	MRSA	12 mm
Flaxseed oil (extracted by ethanol 99%	MRSA	ND
Flaxseed oil (extracted by ethanol 99%	E-Coli	ND
Flaxseed oil (extracted by petroleum ether)	E-Coli	ND
Flaxseed oil (extracted by ethanol 99%	P.aeruginosa	ND
Flaxseed oil (extracted by petroleum ether)	P. aeruginosa	11.25 mm
Flaxseed oil (extracted by ethanol 99%	S.aureus	9mm
Flaxseed oil (extracted by petroleum ether	S.aureus	12mm
* ND: Not detected.		

Flaxseed oil extract and its medicinal benefits

مستخلص زيت بذور الكتان وفوائده الطبية

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المشرف الرئيسي: الدكتور إبراهيم كيالي

الملخص:

تعتبر بذور الكتان من "الأطعمة الصحية ذات القيمة الغذائية العالية"، فهي تحتوي على نسبة عالية من الأحماض الدهنية و منها أوميجا 3 وتحتوي على العناصر الضرورية للصحة الجيدة. و في عصرنا الحالي تُبذل جهود حثيثة لإنتاج أدوية ذات مصدر طبيعي والبحث عن مصادر نباتية بديلة المنشأ لتقليل الآثار الجانبية المرتبطة بالأدوية و تداعياتها على الجسم البشري. حيث تساهم المركبات و المنتجات الطبيعية في إيجاد و تطوير مستحضرات دوائية جديدة و مبتكرة من شأنها علاج العديد من الأمراض الشائعة بكفاءة و فعالية.

و في هذه الدراسة تم استخلاص زيت بذرة الكتان عن طريق جهاز Soxhlet حيث يعتبر هذا الجهاز أداةً فعالة لاستخلاص جميع المركبات النشطة تقريباً من بذرة الكتان.

كذلك تم تحديد مدى فعالية بذرة الكتان و نشاطها كمضاد للأكسدة باستخدام طريقة DPPH حيث تبين أن مستخلص بذرة الكتان بواسطة مذيب الإيثانول جاءت نسبته (4.019±0.02 mg\g) في حين أن مستخلص بذرة الكتان بواسطة مذيب أثير البترول أظهر نسبة (1.741±0.2 mg\g).

أيضاً تم تحليل محتوى الفينول لبذرة الكتان بالاعتماد على طريقة Folin-Ciocalteu و استخدم أيضاً جهاز UV-Visible spectrophotometer حيث تبين أن مستخلص بذرة الكتان بواسطة مذيب الإيثانول جاءت نسبته (230.8±0.02 mg\g) و هي بذلك أعلى مما جرى استخلاصه بواسطة مذيب أثير البترول و هي (53.5±0.07 mg\g). و كانت نسبة مركبات الفلافونيد (223.7±0.07 mg\g).

وكذلك تم تحديد نسبة تأثير مستخلص بذور الكتان لتقليل مستوى الكوليسترول في الدم، وتم ذلك باستخدام إنزيم 3-هيدروكسي -3مثيل جلوتاريل (HMG-CoA)، وهو انزيم مهم في مسار ميفالونات انتاج الكوليسترول حيث يودي تثبيط إنزيم HMG-CoA)الى خفض مستوى الكوليسترول لدى البشر والحيوان بشكل فعال. وكانت نسبة تأثير زيت بذور الكتان (56.3 %).

و أظهر تحليل بذرة الكتان باستخدام جهاز HPLC للمعايير و المركبات قيد البحث، و بعد مقارنة وقت الاستبقاء (Retention time) لذروة مستخلص بذرة الكتان مع المعيار المحدد. و أظهرت نتائج فحص مستخلص بذرة الكتان النتائج التالية:

1. حمض الكلوروجينيك

2. روتين

3. حمض السينامك المتحول

و حُدد نشاط مستخلص بذور الكتان المضاد للبكتيريا في المختبر باستخدام طريقة agar disc diffusion. حيث أظهرت المستخلصات من زيت بذور الكتان نشاطاً مضادًا للبكتيريا ضد كل من MRSA و Pseudomonas و Staphylococcus ،وأظهرت بذور الكتان المستخرجة باستخدام الإيثانول نشاطًا مضادًا للبكتيريا ضد المكورات العنقودية فقط.

وأخيراً توصلت الدراسة إلى أن زيت بذرة الكتان قادر على تثبيط إنتاج المنتجات النهائية للسكر، وذلك من خلال استخدام فحص مصل البروتين البقري مع الجلوكوز(BSA) في المختبر.