Deanship of Graduate Studies
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Inhibitory Effect of *Moringa Oleifera* leaf Extract and *Olive* leaf Extract on Advanced Glycation End Product Formation and Tablet Formulation Development of *Moringa Oleifera* leaf Extract.

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Thesis Approval

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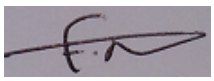
Internal Examiner: Dr. Fuad Rimawi, Signature: 

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Jerusalem- Palestine

2020\1441

Dedication

I dedicate this thesis, First, to my beloved mother and father, Wafa’ and Naser, I would not be the person I am today if it was not for them. Second, I would like to dedicate this work to my life partner, Mohammad, for his patience and unfaltering support. Third, dedicated to my sister Dema, my brothers Johar, Bara’ and Osama, to my friends, to all patients especially Diabetics who need such work, to those who sacrifice their lives for freedom, to the prisoners in the occupation prisons. Last but not least I dedicate this thesis to all merciful souls all over the world.

Iba’ N. A. Halabieh

Declaration

I certify that this thesis 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.

Signed: ……

Iba’ N. A. Halabieh

Date: 25\4\2020

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Abstract

In traditional Palestinian medicine, *Olive* tree is known for its therapeutic effectiveness against many diseases like diabetes. Where *Moringa oleifera* is known for the same effectiveness in India.

Under hyperglycemic conditions characteristic of type 2 diabetes mellitus, non-enzymatic formation of advanced glycation end products (AGEs) is accelerated and contributes to the development of many complications such as coronary heart disease, stroke, nephropathy, neuropathy, retinopathy and atherosclerosis. Therefore, inhibition of AGE formation represents a potential therapeutic target for the prevention and treatment of diabetic complications.

Therefore, the main aim of this study is to investigate the antiglycation effect of each *Moringa oleifera* and *olive* leaves extract, and investigating the antiglycation effect of the combination of them by (1:1) ratio. Also to investigate antioxidant activity, total phenolic and total flavonoids content for each of them, and analysing *Moringa oleifera* leaves extract using liquid chromatograghy HPLC, Moreover, to develop a food supplement from *Moringa oleifera* leaves extract to be used for diabetic complications.

In the present study, 99% ethanolic extracts of *Olive* leave and *Moringa oleifera* leave were extracted using Soxhlet apparatus. In vitro glucose-bovine serum albumin (BSA) assay was used to evaluate Anti-glycation formation of end products. Where scavenging effect of the both extracts and the combination of them by (1:1) were evaluated by DPPH assay. Total phenolic and total flavonoids content were estimating by Folin-Ciocalteau assay and colorimetric assay, respectively. HPLC analysis of *Moringa oleifera* leaves were characterized using Waters HPLC connected to UV-

PDA detector, while the extract of *Moringa oleifera* leaves was formulated in a tablet dosage form.

The obtained results show that Concentrations of 2.5-12.5 mg/mL of *Moringa oleifera* leaf extract and *Olive* leaf extract could inhibit AGE-formation by 13-47% and 0.1% to 36%, respectively. This superior inhibition due to the presence of bioactive compounds in both extracts which reflected in the results of total phenolic content which were 66.5 mg CEQ/g dry extract of *Moringa oleifera* leaves extrcact and 66.7 mg CEQ/g dry extract of Olive extract and the results of total flavonoids content assays which were 19.04 mg GAE/g dry extract of *Moringa oleifera* leaves extrcact and 45.2 mg GAE/g dry extract of Olive leaves extract. It was observed that the results for each extract alone were better than combined them together by (1:1) ratio. HPLC chromatogram confirms the presence of bioactive compounds in *Moringa oleifera* leaves extrcact with identification of Gallic Acid. Whereas tablet formulation indicated that the physical properties of *Moringa oleifera* leaf extract tablets could be highly enhanced when *olive* leaf extract added as a binder and active material to the formulation.

In the light of the findings of the study it recommends that *Moringa oleifera* leaf extract and *olive* leaf extract could be used as a treatment of diabetic complication and promoting over all body health.

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Abbreviations

AGEs : Anti-glycation End products

OLE: Olive leaf extract

MOE : Moringa oleifera leaf extract

TFC : Total Flavonoids Content

TPC: Total phenolic Content

DPPH : 1,1-diphenyl 2-picrylhydrazyl (DPPH•) radical

BSA : bovine serum albumin

CEQ: Catechin equivalent

GAE : Gallic Acid Equivalent

HPLC: High Performance Liquid chromatography

UV-PDA: Ultra violet photo diode Array

LOD : Loss on drying

Chapter One: Introduction

1.1 *Moringa Oleifera*

Moringa Oleifera Lam (syn. M. ptreygosperma Gaertn.) (Figure 1) is one of the best known and most widely distributed and naturalized species of a monogenetic family Moringaceae .The tree ranges in height from 5 to 10 m . It is found wild and cultivated throughout the plains, especially in hedges and in house yards, thrives best under the tropical insular climate, and is plentiful near the sandy beds of rivers and streams. It can grow well in the humid tropics or hot dry lands can survive destitute soils and is little affected by drought.

It tolerates a wide range of rainfall with minimum annual rainfall requirements estimated at 250 mm and maximum at over 3000 mm and a pH of 5.0–9.0. *Moringa oleifera*, native of the western and sub Himalayan tracts, India, Pakistan, Asia Minor, Africa and Arabia is now distributed in the Philippines, Cambodia, Central America, North and South America and the Caribbean Islands. In some parts of the world *Moringa Oleifera* is referred to as the ‘drumstick tree’ or the ‘horse radish tree’, whereas in others it is known as the kelor tree. While in the Nile valley, the name of the tree is ‘Shagara al Rauwaq’, which means ‘tree for purifying’. In Pakistan, *Moringa oleifera* is locally known as ‘Sohanjna’ and is grown and cultivated all over country. (Anwar, F., et al., 2007)

Figure 1.1 Moringa oleifera tree

1.2 Chemical and Phytochemical Screening of Moringa Oleifera

H. Mihammad et al. reported the chemical and Phytochemical screening in their review on promising phytochemical, nutritional and glycemic control studies on *Moringa oleifera* in tropical and sub-tropical regions as the following:

1.2.1 Chemical screening of *Moringa Oleifera*

The fresh *M. oleifera* leaves contained less nutrients as compared to dried ones, as the dried leaves enclosed 17 times Ca of the milk, ten times vitamin A of the carrots, 15 times K of the bananas and 25 times the iron of spinach, 27.2% (protein) or 0.58–0.73 g protein/g, 5.9% moisture, 17.1% fat, 38.6% carbohydrates, essential amino acids and carotenoids . The secondary metabolites were also observed, appeared to be involved in plant defense mechanisms and the trypsin inhibitor against serine proteinases.

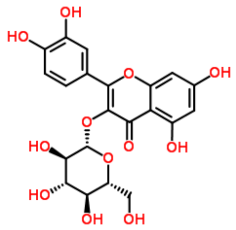
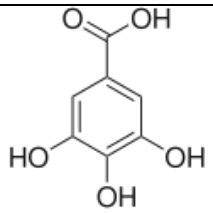
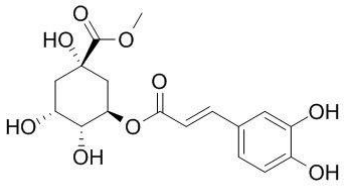
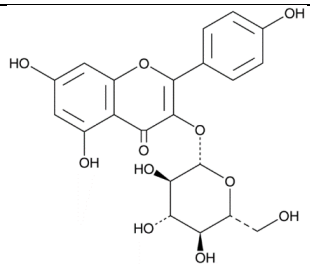
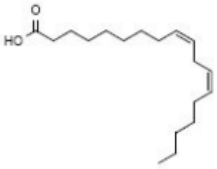
1.2.2 Phytochemical screening *Moringa Oleifera*

Flavonoids and phenolic acids are collectively referred as phenolic compounds. They are classified into several subgroups including: flavone, flavanone, flavonol, isoflavonoid, anthocyanidin, and chalcones.

The high performance liquid chromatography (HPLC) analysis also indicated the presence of phenolic acids (Gallic, ellagic, chlorogenic and ferulic acid) and flavonoids: kaempferol, quercetin, isoquercetin, astragalin and rutin. Quercetin and kaempferol were the predominant flavonols in *Moringa oleifera* leaves. The leaves as well enclosed niazirin, niazirinin, 4-[(4′-O-acetyl-Lrhamnosyloxy) benzyl] isothiocyanate, niaziminin A and B, quercetin-3-O-(6″-malonyl-glucoside), kaempferol-3-O-glucoside and kaempferol-3-O-(6″-malonyl-glucoside), 3-

caffeoylquinic and 5-caffeoylquinic acid. It was also reported that the leaves had enough amount of carotenoids, epicatechin and o-coumaric acid (Muhammad, H., et al., 2016). Another study also isolated and reported the same components. (Mishra, G. et al., 2011)

Table 1.1: The main phytochemical components in *Moringa oleifera* and their structure

#	Chemical structure	Component chemical name
1		quercetin-3-O-glucoside
2		Gallic Acid
3		3- caffeoylquinic acid
4		Kaempferol-3-O-D-glucoside
5		Linoleic acid

Previous studies reported the Phytochemicals profile of *Moringa oleifera* leaf extracts and fractions and illustrated in the table below:

Table 1.2: Phytochemicals profile of *Moringa oleifera* leaf extracts and fractions*

Test Extract	Alkaloids	Tannins	Flavanoids	Phenolic	Saponins	Steroids	Glycosides	Reduced suger
95%EtoH	+	+++	+++	+++	++	+	++	-
50% EtoH	-	+	++	+	++	+	+++	-
Water	-	-	+	+	+	-	++	++

* (Fahmy, T. et al. ,2015)

1.3 Nutritive Properties of *Moringa Oleifera*

The leaves of this plant provide a rich source bioactive compounds as described in the previous section such as carotenoids, vitamins, minerals, amino acids, alkaloids, and flavonoids and a rare combination of phenolic compounds, including zeatin, quercetin, kaempferol, apigenin, and many other phytoconstituents that offer essential and disease preventing nutrients to humans. (Karthivashan, G. et al. ,2015)

Every part of *Moringa oleifera* is a storehouse of important nutrients and antinutrients. Its leaves have a low calorific value and can be used in the diet of the obese. The pods are fibrous and are valuable to treat digestive problems and thwart colon cancer. (Fahmy, T. et al., 2015)

In another study, mineral analysis has been also shown that Leaves had high calcium, potassium, sulphur, magnesium, phosphorous and iron content whereas copper content was high in seeds. Calcium and iron content was high when compared with mushroom and leaves of cassava, amaranth, taro and pumpkin. Whereas the Vitamin analysis has been shown that Vitamin E (tocopherol) content was high in

leaves whereas seeds had high vitamin C (ascorbic acid) content. Vitamin B_2 (riboflavin) content of seeds was negligible and vitamin E was absent. Vitamin B_5 (pantothenic acid) and B_9 (folate) content were similar in leaves and seeds. (Chelliah, R. et al., 2017)

1.4 Medical use of *Moringa Oleifera*

So for centuries, *Moringa oleifera* has been used as a traditional medicinal source. Additionally, besides being edible, all the parts of the *Moringa* tree (e.g., pods, seeds, and leaves) have long been employed for the treatment of many diseases, and therefore, it was called a ‘‘miracle vegetable’’. (Alhakmani, F. et al. 2013)

A number of medicinal properties have been ascribed to various parts of this highly esteemed tree. Almost all the parts of this plant have been used for treatment of inflammation and infectious diseases along with cardiovascular, gastrointestinal, hematological and hepatorenal disorders. (Anwar, F., et al., 2007)

1.4.1 Antidiabetic activity

Several medicinal plants have been evaluated for their potential as therapeutic agent for diabetes. *Moringa Oleifera* is also an important component in this category. *Moringa Oleifera* leaves significantly decrease blood glucose concentration in Wistar rats and Goto-Kakizaki (GK) rats, modeled type2 diabetes. Another study indicated that the extract from *Moringa Oleifera* leaf is effective in lowering blood sugar levels within 3 h after ingestion. *Moringa Oleifera* leaves are potent source of polyphenols, including quercetin-3- glycoside, rutin, kaempferol glycosides, and other polyphenols. Thus, potential antidiabetic activity of *Moringa Oleifera* can be commercialized through the development of suitable technology with achieving anti-diabetic activity up to conventional drugs. (Fahmy, T. et al., 2015)

F. Anwar et al. evaluate the antidiabetic activity of *Moringa Oleifera* leaf extract and three of its active ingredients (moringinine, quercetin and chlorogenic acid). Alcoholic extracts of *Moringa oleifera* leaf, moringinine, quercetin and chlorogenic acid; were tested against diabetic rats induced by alloxan. And the result of this study suggests that alcoholic extract of *Moringa oleifera* leaf possess potent antidiabetic activity and also is a good source of natural antioxidants. (Anwar, F., et al., 2007)

1.4.2 Antioxidant activity of *Moringa oleifera*

Moringa oleifera is a rich source of antioxidants. It has been reported that aqueous extracts of leaf, fruit and seed of *Moringa oleifera* act as an antioxidant. During a study reporting antioxidant property of freeze dried Moringa leaves from different extraction procedures, it was found that methanol and ethanol extracts of Indian origin *Moringa oleifera* have the highest antioxidant activity with 65.1 and 66.8%, respectively. Another study comparing palm oil with *Moringa oleifera* seeds for their antioxidant potential and they found that *Moringa oleifera* seed are superiors for radical scavenging. (Anwar, F., et al., 2007)

1.4.3 Anti-bacterial properties of *Moringa Oleifera*

In a recent study, aqueous extracts of *Moringa oleifera* was found to be inhibitory against many pathogenic bacteria, including Staphylococcus aureus, Bacillus subtilis, Escherichia coli, and Pseudomonas aeruginosa in dose dependent manner. *Moringa oleifera* leaves extract was found to be effective in checking growth of fungi Basidiobolus haptosporus and Basidiobolus ranarums. Beside antibacterial activity of *Moringa oleifera* oils, it also possess anti-fungal activity. (Farooq, F. et al., 2012)

1.4.4 Anti-inflammatory activity of *Moringa Oleifera*

Moringa Oleifera plant parts have substantial anti-inflammatory activity. For instance, the root extracts exhibits significant anti-inflammatory activity in carrageenan induced rat paw oedema. The crude methanol extract of the root inhibits carrageenaninduced rat paw edema in a dose dependent manner after oral administration. Moreover, literatures show that extract of the seeds of *Moringa Oleifera* exhibit anti-inflammatory activity against ovalbumin-induced airway inflammation in guinea pigs. This is to conclude that amelioration of inflammation associated chronic diseases can be possible with the potent anti-inflammatory activity of *Moringa Oleifera* bioactive compounds. (Anwar, F., et al., 2007)

1.4.5 Cardiac and circulatory stimulant

Moringa Oleifera is reported with somewhat cardiac and circulatory stimulant activity. Root bark of Moringa contains alkaloid moringinine which acts as cardiac stimulant through its effect on sympathetic nervous system. The aforementioned effects can also result due to the prevention of hyperlipidemia. It has been demonstrated that *Moringa Oleifera* prevents hyperlipidemia in male Wister rat due to iron deficiency. It was found that *Moringa Oleifera* leaf extract cause significant changes in cardiovascular parameters. They reported in this study *Moringa Oleifera* leaf extract as hypolipidimic, lowering body weight, heart weight, serum triglyceride level and serum cholesterol level in experimental animals. (Anwar, F., et al., 2007)

1.4.6 Antihypertensive, diuretic and cholesterol lowering activities

Moringa Oleifera leaves contain bioactive compounds which have direct effect on blood pressure, and thus stabilizing it. Nitrile which is the one of *Moringa Oleifera* compounds leading to blood pressure lowering effect, In addition, mustard oil

glycosides and thiocarbamate glycosides present in *Moringa Oleifera* leaves diuretic activity of *Moringa Oleifera* exists in its roots, leaves, flowers, gum and the aqueous infusion of seeds. Moreover, *Moringa Oleifera* leaves also has effect on lowering cholesterol. (Anwar, F., et al., 2007)

1.4.7 Hepatoprotective activity

Moringa Oleifera has shown significant hepatoprotective activity in several studies. Ethanol extraction of the leaves showed significant protection against liver damage induced by antitubercular drugs in rats. Moreover, *Moringa Oleifera* leaves extracts also showed significant protection against CCl_4 induced liver damage in albino rats. Also *Moringa Oleifera* root and flowers effective for this manner. And this is due to flavonoid (Quercetin), which may be responsible for its potent hepatoprotective activity. (Anwar, F., et al., 2007)

1.4.8 Antitumor activity

Moringa Oleifera extract has been found as a potent anticancer plant and several bioactive compounds with significant antitumor activity have been discovered from *Moringa Oleifera*. Such as niazimicin that found in the leaves Furthermore, niazimicin also shows the inhibition of tumor promoter teleocidin B4-induced Epstein-Barr virus (EBV) activation. Another study involving 11 plants used in Bangladeshi folk medicine, *Moringa Oleifera* was considered as potential source of anticancer compounds. Beside leaves, seed extracts also have anticancer activity through its effects on hepatic carcinogen metabolizing enzymes, and antioxidant property. (Anwar, F., et al., 2007)

1.5 Antiglycation and Diabetes complications

AGEs are a diverse class of biomolecules resulting from non-enzymatic reactions between lipid, nucleic acid, or protein substrates and reducing sugars such as glucose and fructose. In conditions of hyperglycemia, glycation leads to cellular damage by impairing the function of intracellular and extracellular proteins. This often affects the half-life of the biomolecule and, in cases such as insulin, decreases the molecule's bioactivity .Some AGEs, such as Nε-(carboxymethyl)lysine (CML), interact with AGE receptor (RAGE), which elicits activation of NF-KB, participates in the initiation and progression of atherosclerosis, and contributes to inflammatory events that contribute to macro and micro vascular complications of diabetes and linked to chronic diseases and reducing the overall immune system efficiency. During the conjugation of reducing sugars to biomolecules, several oxidative transformations occur resulting in oxidative stress. Dietary antioxidants, such as flavonoids, therefore offer at least two means of protection, by inhibiting oxidative formation of AGEs and AGEs it selves, and by scavenging reactive oxygen species (ROS). Current strategies targeting AGE production have met with limited success, notably due to side-effects associated with synthetic inhibitors. A nutritional approach based on foods with high flavonoid content represents an alternative approach to AGE inhibition with a reduced risk of adverse effects. (Beaulieu, L. et al. ,2010)

1.6 Olive leaf extract as strong natural antioxidant and regulating blood sugar

Olive leaves have been used for centuries in folk medicine to treat diabetes. Recently, the medicinal properties of olive products have focused on its polyphenols

(particularly oleuropein and hydroxytyrosol). M. de Bock *et al.* in their clinical study assess the effects of supplementation with olive leaf polyphenols (51.1 mg oleuropein, 9.7 mg hydroxytyrosol per day) on insulin action and cardiovascular risk factors in middle-aged overweight men. They found that Supplementation with olive leaf polyphenols for 12 weeks significantly associated with a 15% improvement in insulin sensitivity compared to placebo. There was also a 28% improvement in pancreatic beta cell responsiveness in overweight middle-aged men at risk of developing the metabolic syndrome. Hence, compared to drugs that only improve insulin secretion, olive leaf extract improves both insulin sensitivity and pancreatic Beta cell secretory capacity. (De Bock, M. et al., 2013)

On other hand, K. Wojcikowski *et al.* studying the Antioxidant capacity of 55 medicinal herbs traditionally used to treat the urinary system and they found that the highest radical-scavenging activity was found in olive leaf. This because that olive leaves found by others to contain a mixture of phenolic compounds with significant antioxidant activity. (Wojcikowski, K. et al., 2007)

1.7 Problem statement

Recent global estimates suggest that the number of diabetic people which is over 180 million in 2000 will be doubled by 2030; the global trend is now to use natural sources to fight these diseases, their complications and promote overall body health. Due to the presence of biologically active compounds including natural antioxidants in the traditional plants such as *Moringa oleifera* and *Olive leaf* extract, and represent a culturally suitable adjuvant treatment that may assist in providing protection from the development of diabetic complications. Using *Moringa oleifera* and *Olive leaf* extract for diabetes and diabetes complications and promoting immunity become as a major concern especially in these days with the spread of Corona virus (COVID-19) and the need of diabetics to naturally increase their immunity and overall heath.

This study therefore aimed to investigate in-vitro antiglycation activity of *Moringa oleifera*, *Olive leaf* extract and the combination of the tow extracts by (1:1) ratio, and to develop a natural food supplement in tablet dosage form for *Moringa oleifera* leaf extract.

1.8 Objectives of this study

The main goals of this research are:

1- Studying in- vitro anitglycation assay, antioxidant activity, total phenolic and total flavonoids content for *Moringa oleifera* leaf extract and *Olive* leaf extract.

2- HPLC analysis of Moringa *oleifera* leaf extract and identification of its constituents.

3- Tablet formulation and process development of food supplement contains *Moringa oleifera* leaf extract as an active material for diabetic complications.

Chapter Two: Literature Review

On reviewing the available literature, it is evident that extensive research has been carried out to address various aspects of *Moringa oleifera*. With this in mind, in an attempt to simplify and delimit the subject to some extent, this chapter will focus primarily on Antiglycation Activity, Antioxidant, total phenolic and flavonoids content in *Moringa oleifera* and *Olive* leaf, tableting formulation, and HPLC analysis that has been carried out on Moringa oleifera.

P. Nunthanawanich *et al*, studying the effect of *Moringa oleifera* leaf extract on the level of fructosamine, *Moringa oleifera* leaf extract powder was obtained by extraction the dried leaves with water at 100˚C for 3 hours, then subjected to a spray dryer after filtration with Whatman filter paper. The Glycated BSA was performed by the following method: BSA (10 mg/mL) was incubated with glucose or fructose (0.5 M) in 0.1 M phosphate buffer (pH 7.4) containing 0.02 % sodium azide (NaN3) with or without (MOE) (0.5–2.0 mg/mL) and Aminoguanidine (1.0 mg/mL) as a Positive control at 37 ℃ for 4 weeks. The fluorescent AGEs were determined by a spectrofluorometer at excitation and emission wavelengths of 355 nm and 460 nm. Total phenolic content of *Moringa oleifera* leaf extract were also studied and it was determined by the Folin-Ciocalteu method. The results were reported that the percentage of inhibition of AGE formation by *Moringa oleifera* leaf extract (0.5–2.0 mg/mL) ranged from 14.52–40.65 % in glucoseglycated BSA and 45.82–65.43 % in fructose-glycated BSA. However, they compared the percentage of inhibition of *Moriega Oliefera* with aminoguanidine and they reported that *Moriega Oliefera* has less potent in the inhibition of AGE formation when compared with aminoguanidine at the same concentration (1 mg/mL). Whereas the result of total phenolic content was

38.56 ±1.50 mg Gallic acid equivalents/g dry extract. (Nunthanawanich, P. et al. ,2016)

In the same context, W. Sangkitikomol, *et al.* also reported the effect of *Moringa oleifera* on advanced glycation end-product formation and they studied antioxidant activity and Total flavonoid and polyphenol contents. *Moringa Oleifera* leaf extract were prepared from dried leaves and extracted in 1000 mL 80% methanol in water in an ultrasonic bath for 60 min at 40°C, it was centrifuged then it was concentrated using a vacuum rotary evaporator and freeze-dried. The formation of AGE was assessed using the fluorescence method, 20 μL of each four various concentrations of Moringa Oleifera leaf extract from (2.5 mg/mL – 10 mg/mL), phosphate buffer was added to the freshly prepared reaction mixture containing 400 μL of (1 mg/mL) bovine serum albumin (BSA) and 80 μL of (1.0 M) glucose in phosphate buffer, pH 7.4 and incubated at 60°C for 72 h. The amount of glycated BSA was measured based on fluorescence intensity with a spectrofluorometer. The excitation and emission wavelengths used were 370 nm and 440 nm, respectively. Total flavonoid contents were determined by colorimetric reaction with some modifications. Absorbance was determined at 510 nm against the reagent blank. An oxygen radical absorbance capacity (ORAC) assay were performed to assess the antioxidant levels, and a modified FCP assay was used to determine polyphenol contents in *Moringa Oleifera* leaf extract. The results were reported that *Moringa Oleifera* leaf extract could inhibit 10-45% AGE formation in a dose-dependent manner. Total flavonoid content is reported as Quercetin equivalents (QE) mM/kg dried mass and it is 286±12 QE mM/kg dry mass for *Moringa Oleifera* leaf extract. Whereas the result of Anti-oxidant and Total polyphenolics content assay were of

9307 ± 364 TE mM/kg dry mass, 218 ± 1 GE mM/kg dry mass, Respectively. (Sangkitikomol, W. *et al.*, 2014)

There are many studies that investigate the antioxidant activity of Olive leaves; one of them is P. Goldschmidt Lins *et al.* in their study "In vitro antioxidant activity of olive leaf extract (Olea europaea L.) And its protective effect on oxidative damage in human erythrocytes", they evaluated total phenolic, total flavonoid content, and anti-oxidant assay for olive leave extract. Dried olive leaves were subjected to removal of n-hexane-soluble compounds using a Soxhlet extractor then it were extracted in methanol/water (80:20, v/v), under agitation and it was centrifuged, filtered, and then methanol was removed under reduced pressure. The Folin-Ciocalteau colorimetric method was used for estimation of total polyphenol content of the extract. Free radical DPPH (2,2-diphenyl-1-picrylhydrazyl) is used to evaluate the antioxidant activity. The total flavonoid content was estimated according to pharmacopeia method (1989). The results shown that Total phenolic and flavonoid contents were 131.7 ±9.4 mg gallic acid equivalents/g dry weight (dw) and 19.4 ±1.3 mg quercetin equivalents/g dw, respectively. And for DPPH assay it was (EC50 13.8 ±0.8 µg/mL). (Goldschmid Lins, P. *et al.*, 2018)

Regarding inhibitory effect of AGEs formation V. Kontogianni, et al. studied in their work "Olive Leaf Extracts Are a Natural Source of Advanced Glycation End Product Inhibitors" In vitro effect of two different olive leaf extracts (an aqueous and a methanolic) on AGE formation. 50 g of fresh leaves of olive leaves were extracted separately both with 250 mL of distilled water boiled for 1 hour and with 250 mL of methanol macerated for 7 days in the dark at room temperature. The extract was separated by filtration and the solvent was evaporated under vacuum. In vitro

glycation of BSA were performed by the following: BSA (10 mg/mL, fatty acid-free) was modified in vitro at 37˚C by the reducing sugars, ribose or fructose (500 mM). All incubations were carried out in 0.1M phosphate buffer (pH 7.4) in the absence and presence of different concentration of extracts (1–100 lg/mL), pure compounds (1–100 lM). All solutions contained 3mM sodium azide to prevent bacterial contamination. After 3 and 21 days formation of pentosidine was monitored by measuring its characteristic fluorescence using the excitation and emission maxima of 370 and 440 nm, respectively. The results showed that the methanolic olive leaf extract inhibited fluorescent AGE formation in a bovine serum albumin (BSA)-ribose system by 43.07% at the concentration of 100μg/mL, whereas the aqueous extract had no effect in both BSA fructose and BSA-ribose systems. (Kontogianni, V. *et al.*, 2013)

The same works for testing the formation of Advanced Glycation End products were performed for different types of plants such as *Vaccinium vitis-idaea*, one of these works is L. Beaulieu *et al*. article which studying inhibitory effect of the Cree traditional medicine Wiishichimanaanh (*Vaccinium vitis-idaea*) on advanced glycation endproduct Formation. 80% ethanolic extract was prepared for AGEs test , incubation media containing BSA (1.0 mg/mL), glucose (100 mM), fructose (100 mM) and either *V.vitis-idaea* berry extract, or vehicle (80% Ethanol: 20% H_2O, v/v) in 100 mM sodium phosphate buffer were prepared. Ten different concentrations of the berry extract (0.39–200 g/mL) were prepared to determine concentration-dependent responses. The positive control was Quercetin (2.5 g/mL). The incubation media then incubated at 37ºC in darkness with shaking for 7 days. The formation of fluorescent AGEs was quantitatively assessed using fluorometer at excitation and emission wavelengths of 355 nm and 460 nm, respectively. The results reported that

Vaccinium vitis-idaea extract could inhibit 5-80 % of glycated end products formation in a dose- dependent manner. With IC_{50} 13.5 g/mL of the extract tested samples. (Beaulieu, L. et al., 2010)

In addition, other studies investigate the antiglycation assay for 17 medicinal plants. Like in this study "Inhibition of Advanced Glycation End Product Formation by Medicinal Plant Extracts Correlates with Phenolic Metabolites and Antioxidant Activity" The assessment of inhibitory effects on in vitro AGE formation of ethanolic extracts of 17 medicinal plants was reported. Each different dried medicinal plant were processed and extracted twice with 80% ethanol. The AGEs assay were conducted as the following: Bovine serum albumin (BSA) (1mg/mL) was incubated with 100mMglucose/100mMfructose in 100mM sodium phosphate monobasic monohydrate buffer (pH 7.4) with 80% ethanol vehicle (negative control), with extract or with quercetin (positive control). Multiple concentrations of each extract (0.39–200 μg/mL) were tested. The samples then incubated for 7 days at 37°C in darkness on a mechanical shaker. Fluorescence was measured using a micro plate reader at excitation and emission wavelengths of 355 nm and 460 nm, respectively. The results shown that most extracts inhibited fluorescent AGE formation with IC_{50} values ranging from 0.4 μg/mL to 38.6 μg/mL. (Harris, C. et al., 2011)

In regards of antioxidant, total phenolic and total flavonoids content Moringa oleifera: Water, aqueous methanol, and aqueous ethanol extracts of freeze-dried leaves of Moringa oleifera Lam. from different agroclimatic regions were examined by P.Siddhuraju and K. Becker for radical scavenging capacities and antioxidant activities. All leaf extracts were capable of scavenging peroxyl and superoxyl radicals. Similar scavenging activities for different solvent extracts of each collection

were found for the stable 1,1-diphenyl 2-picrylhydrazyl (DPPH•) radical. Among the three different *Moringa oleifera* samples, both methanol and ethanol extracts of Indian origins showed the highest antioxidant activities, 65.1 and 66.8%, respectively, in the â-carotene-linoleic acid system. Nonetheless, increasing concentration of all the extracts had significantly ($P < 0.05$) increased reducing power, which may in part be responsible for their antioxidant activity. The major bioactive compounds of phenolics were found to be flavonoid groups such as Quercetin and Kaempferol. On the basis of the results obtained, *Moringa oleifera* leaves are found to be a potential source of natural antioxidants due to their marked antioxidant activity. This is the first report on the antioxidant properties of the extracts from freeze-dried *Moringa oleifera* leaves. Overall, both methanol (80%) and ethanol (70%) were found to be the best solvents for the extraction of antioxidant compounds from *Moringa oleifera* leaves. (Siddhuraju , P. et al. ,2003)

M. Hamed *et al*. estimate the total phenolic contents (TPCs) and in vitro antioxidant of different solvent extracts of *Moringa oleifera* leaves. The antioxidant activity of different solvent extracts of *Moringa oleifera* leaves were estimated using three antioxidant assays and the total phenolic contents (TPCs) were also evaluated using Folin-Ciocalteu's assay. The results showed that the TPCs values of the tested extracts were varied from 309.52 to 43.28 mg gallic acid equivalent/g dry extract. The reducing power antioxidant activities (RPAA) were 0.434, 0.402, 0.395, 0.149, 0.143 and 0.124, while the total antioxidant capacity (TAC) values were 316.43, 203.35, 181.56, 86.70, 76.62 and 50.83 mg ascorbic acid equivalent/g dry extract; for n-Butanol, Ethyl acetate, 85% Methanol, H_2O, CH_2C_{l2}, and pet. Ether extracts, respectively. The study concludes that *Moringa oleifera* leaves showed promising antioxidant activities. (Hamed, M. et al. ,2017)

Same finding of antioxdidant activity have been noted in W.Fitriana et al. study of Antioxidant Activity of *Moringa oleifera* Extracts. *Moringa oleifera* leaves were extracted with methanol, ethyl acetate, dichloromethane and n-hexane. The antioxidant activity of extracts were evaluated by 1,1- diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging activity assay and an improved 2,2'-azino-bis-[3-ethylbenzothiazoline sulphonate] (ABTS) radical cation decolorization assay in vitro. Trolox was used as standard with IC_{50} 5.89 μg/mL in DPPH assay and 3.06 μg/mL in ABTS assay. The methanol extract showed the highest free radical scavenging activity with IC_{50} value of 49.30 μg/mL in DPPH assay and 11.73 μg/mL in ABTS assay. This study provided that *Moringa oleifera* leaves possess antioxidant (Fitriana, W. et al. ,2016)

L. Pari *et al.* reported the anti-oxidant activity and HPLC analysis of Phenolic acids of *Moringa Oliefera* leaf extract in their study "Antioxidant Activity of the Crude Extracts of Drumstick Tree (*Moringa Oleifera* Lam.) and Sweet Broomweed (Scoparia Dulcis L.) Leaves". Fresh leaves of *Moringa* leaves were chopped into small pieces and soaked overnight in methanol at solid material to solvent ratio of 1:3 (w/v), The solvent was evaporated using a rotary evaporator.The content of total phenolic compounds in extract was estimated using the Folin-Ciocalteu's phenol reagent and (+) Catechin was used as a standard. Scavenging of DPPH radical was done as the following, 0.1 mL of methanolic solution containing 0.04 to 0.20 mg of the extract was mixed with 2 mL of methanol and then 0.25 mL a methanolic solution of DPPH (1 mmol/L) was added. The mixture was vortexed for 1 min, and then left standing at room temperature for 20 min and the absorbance of this solution was subsequently read at 517 nm. For the HPLC finger print analysis of phenolic compounds present in extracts a Shimadzu system with photo-diode array

detector, C18 (4 × 250 mm, 5 μm, Phenomenex) column were used. A flow rate of 1 mL/min, and gradient elution of acetonitrile- water-acetic acid (5:93:2, v/v/v) [solvent A] and of acetonitrile-water acetic acid (40:58:2, v/v/v) [solvent B], 0– 50 min solvent B from 0 to 100%; and injection volume of 20 μL were applied; whereas the separation of compounds was monitored at 280 and 320 nm. The results shown that the content of total phenolics in extract of *Moringa oleifera* leaves was 118 mg/g, *and* scavanged 65% of DPPH used for the assay with value of Total Antioxidant Activity (μmol Trolox/mg) is 0.636 ± 0.024. The HPLC chromatogram o of *Moringa oleifera* leaves *extract* was characterized by six peaks recorded at wavelength of 280 nm , and 320 nm. Compounds giving peaks 1-6 on chromatogram from *Moringa oleifera* leaves extracts were characterized by maxima of UV, Compounds 1–4 did belong to phenolic acids and were derivatives of caffeic, *p*-coumaric or ferulic acid. (Pari, L. et al. ,2007)

Moreover, G. Oboh, et al. characterize the phenolic constituents in *Moringa oleifera* leaves extracts using (HPLC- DAD) analysis. *Moringa oleifera* leaves were purchased from local market, air dried at room temperature, and pulverized. Then powdered sample (5 g) was soaked in 100mL of distilled water for about 24h at 37˚C, the mixture then filtered and centrifuged. (HPLC- DAD) analyses were carried out under gradient conditions using C_{18} column. The extract was analyzed at a concentration of 20mg/mL, and the standard concentration ranges of Gallic acid were from 0.050 to 0.450mg/mL and it was detected at 254nm. The results shows that the extract contain may of phenolic compounds such as: gallic acid (105.67mg/g), catechin (20.19mg/g), chlorogenic acid (79.31mg/g), and ellagic acid (52.95mg/g) and flavonoids such as quercetin (137.81mg/g),quercitrin (74.9mg/g), isoquercitrin (75.65mg/g), kaempferol (106.75mg/g), and rutin (60.38mg/g). It was conclude that

Gallic acid, chlorogenic acid, quercetin, and kaempferol were the most abundant phenolic compounds identified in the leaf extract. (Oboh, G. et al. ,2015)

On the other hade, regarding tablet formulation**,** An attempt has been made to develop new herbal formulation by *(*Mahajan, S. *et al.* ,2013), illustrated in "Formulation And Evaluation Of Herbal Tablets Of *Moringa Oliefera* leaves Extract "study the herbal tablet formulation was developed from the Ethanolic Extract of *Moringa Oliefera* leaves by direct compression process. *Moringa* was compressed into tablet using various excipients, micro crystalline cellulose, sodium starch glycolate, ethyl cellulose, magnesium stearate, lactose anhydrous and evaluated for physical parameters such as Thickness, friability, hardness and disintegration time. The optimized formulated herbal tablet with 22.5 mg of Ethyl cellulose and 79.5 mg of Lactose anhydrous, was kept for Stability studies were carried out at 40˚C/75 % RH for 30 days. The results were showed no significant changes on evaluation parameters of tablet.

M. Denis (Denis, M., 2012) in his study "formulation development of *Moringa oleifera* film coated tablets" also formulated four batches of basic formulations of *Moringa oleifera* leaf powder. Moringa leaf powder (72.3%) and a disintegrant (corn starch BP 7.3 % or 9.1 % w/w with respect to the total tablet weight), Gelatin, PVP (K-30), Lactose(filler), SLS , Talc and Magnesium stearate were used as inactives in the four formulations. Physical appearance, weight uniformity, hardness and friability and Disintegration test was done as a quality control tests for the four formulations. It was concluded that the formula granulated with 0.7 % PVP as a binder and 9.1 % corn starch as disintegrant is the best formula for film coating tablet.

Moreover, J. Muazu *et al.* (Muazu1, J. et al., 2014) aimed to formulate a standard dose of aqueous extract of Moringa oleifera leaves into tablets and to determine a suitable binder for the formulation. Aqueous extract of *Moringa oleifera* leaves was extracted and formulated using different binders which included Maize Starch, Gelatin and Micro-crystalline Cellulose (MCC) to find out which one produce better tablets of aqueous extract of *Moringa oleifera* leaves. Formulations were characterized using various parameters such as physicochemical properties (bulk density, tapped density, moisture content, Hausner's ratio, Carr's index, ash value), strength (friability and crushing strength) and release properties (disintegration and dissolution times tests). The result showed that tablets formulated with Gelatin as a binder has lowest friability and disintegration time compared to those formulated with either MCC or maize starch. The crushing strengths were all within the acceptable limit (3 – 6 KgF) except maize starch which was higher. And it was recommended that Gelatin is preferable in the formulation of *Moringa oleifera* tablets.

Same in "Optimization of formulation and processing of *Moringa oleifera* and *spirulina* complex tablets " studied by Y. Zheng *et al.* (Zheng, Y. *et al.* ,2017), aimed to prepare a more comprehensive nutrition, more balanced proportion of natural nutritional supplement tablets with *Moringa oleifera* leaves and *spirulina* the two nutrients which have complementary natural food ingredients. On the basis of research *Moringa oleifera* leaves with *spirulina* nutrient composition was determined on M. oleifera leaves and spirulina ratio of raw materials, and the choice of microcrystalline cellulose, sodium salt of caboxy methyl cellulose (CMC),magnesium stearate excipient, through single factor and orthogonal experiment, selecting the best formula tablets prepared by powder direct compression technology, for preparation of *Moringa oleifera* and *spirulina* complex tablets. The results were shown that the

best ratio of raw material for the *Moringa oleifera* leaves powder: *spirulina* powder was 7:3, the best raw materials for the tablet formulation was 88.5%, 8.0% microcrystalline cellulose, CMC 2.0%, stearin magnesium 1.5%, the optimum parameters for the raw material crushing 200–300 mesh particle size, moisture content of 7%, tableting pressure 40 kN.

Chapter Three: Experimental Part

3.1. Materials and Reagents:

The leaves of *Moringa Oleifera* were collected from Jenin city, Palestine and Dried Olive Leaf extract were collected from Palolea Company with internal lot Number of (OL 01\0917).

Ethanol 99% which used in the extraction was purchased from Al-madi store, Ramallah. And the chemical reagents which used for Anti-glycation test, antioxidant assay, Total phenolic and total flavonoid content were obtained from the collage of Chemistry store, Al-Quds University. Bovine Serum Albumin (BSA), Monopotassium phosphate (KH_2PO_4), Disodium phosphate (Na_2HPO_4), Sodium chloride (NaCl), distilled water, Fructose, Glucose, DPPH, methanol, Folin- Ciocalteu reagent, sodium bicarbonate, sodium nitrite, aluminum chloride, sodium hydroxide. .

Quercetin standard CAS No. (117-39-5) Q4951 and Gallic Acid standard CAS No. (149-91-7) G7384 were purchased from sigma Aldrich.

Natural Plant mixture was obtained from local market Hizma, it is marketed as a folk natural mixture to reduce blood sugar. It is contain: Cinnamon 8%, mustard 48%, marjocam 20%, rashad 8%, radishes 8%, kizha 8%.

HPLC grade Acetonitrile (439134), Methanol (34860), and glacial Acetic Acid (ARK2183) were purchased from techno-line, Ramallah.

3.2. Sample preparation of *Moringa* Oleifera *leaf extract* and *Olive leaf* extract:

The leaves of *Moringa Oleifera* were dried in shade and dry place at the ambient temperature. And then freed from foreign matter and grounded into coarse powder.

50 g of *Moringa Oleifera* powder were extracted through Soxhlet using 500 ml of 99% Ethanol for 4 hours until the colour of recycled ethanol changed from green to light yellow.

The same extraction procedures and conditions of *Moringa Oleifera extraction* were performed to the *Olive leaves*. Dried *Olive leaves* were obtained from Palolea Company, and freed from foreign matter and grounded into coarse powder. 50 g of *Olive leaves* powder were extracted through Soxhlet using 500 ml of 99% Ethanol for 4 hours until the colour of recycled ethanol changed from green to light yellow.

The solvent of the both extracts were evaporated using vacuum rotary evaporator (chemistry lab- Al-Quds University) under reduced pressure, both were stored at 4˚C until needed.

3.3. Loss on Drying Test :

2g of each extracts of *Moringa Oleifera* and *olive Leaf* extract were distributed well in the aluminium dish of moisture analyser OHAUS (MB 35) operated at 105C^{o}.

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

The method was performed as previously described (Harris, C. et al., 2009) with some modifications as follows:

3.4.1. Preparation of Incubation media :

1- (100mM) sodium phosphate monobasic monohydrate buffer (pH 7.4) was prepared by weigh 0.544g of (KH_2PO_4) , 2.2g of (Na_2HPO_4), and 6.7g of (NaCl) in 1 litter of distilled water..

2- Stock solution of (1mg/ml) Bovine Serum Albumin (BSA) was prepared in (100mM) sodium phosphate monobasic monohydrate buffer (pH 7.4).

3-Stock solution of (100mM glucose/100mM fructose) mixture was prepared in (100mM) sodium phosphate monobasic monohydrate buffer (pH 7.4).

3.4.2. Preparation of extract samples:

Five different concentrations of *Moringa Oleifera* leaf extract, *Olive leaf* extract, and 1:1 *Moringa Oleifera* leaf extract to *Olive leaf* extract samples were prepared with ethanol 99% from 2.5 mg/ml to 12.5 mg/ml for each extracts to determine concentration-dependent responses. The weights of the extracts were weighted equivalent to dry base.

3.4.3. Test Samples:

The tests samples of both extracts and controls were prepared in Eppendorf with sample volume of (1000µL), each sample repeated in duplicate or triplicate.

3.4.3.1. 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 *Moringa Oleifera* leaf extract , *Olive leaf* extract ,and 1:1 *Moringa Oleifera* leaf extract to *Olive leaf* extract samples were prepared in Eppendorf and incubated in incubator shaker (biochemistry lab, Al-Quds University) at 37°C for 7 days

3.4.3.2. Positive control:

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 prepared in Eppendrof with five different concentrations from 2.5 mg/ml to 12.5 mg/ml, and incubated in incubator shaker at 37°C for 7 days.

3.4.3.3. *Negative control:*

100 µl of BSA ,100 µl of sugar solution ,300 µl of phosphate buffer(pH 7.4), and 500 99% Ethanol were prepared in Eppendrof and incubated in incubator shaker at 37°C for 7 days.

3.4.4. *Fluorescence*-based assay of the inhibition of AGE formation:

After 7 days of incubation, the formation of fluorescent antiglycation End products (AGEs) in each sample were quantitatively assessed using fluorometer (Fluoroskan Ascent & FL, Ascent software) (Nutrition and Health Research institute, Al-Quds University) at excitation and emission wavelengths of 375nm and 455nm, respectively. The fluorescence readings for the experimental treatment (containing BSA, sugar and either extract or pure standard) and the negative control were blanked against BSA, phosphate buffer, and the appropriate extract blanks to exclude baseline fluorescence. The corrected fluorescence readings (F) for the negative control ($F_{\text{negative control}}$) and for the experimental treatments ($F_{\text{experimental corrected}}$) were used to determine the percentage of inhibition of AGE formation by the following formula:

$$\%\ \text{inhibition} = \frac{(\text{F negative control} - \text{F experimentalcorrected})}{\text{F negative control}} * 100\ \%$$ [xviii]

3.5. Free radical scavenging activity using DPPH (DPPH)

DPPH assay is based in the measurement of scavenging ability of antioxidants towards the stable DPPH radical and it was determined spectrophotometrically by (EMC-61PC-UV Spectrophotometer) using the method of (Brand-Williams, W. *et al*, 1995).

The test procedures were performed as follows: 100 µL of each different concentrations of Torlox as positive control, *Moringa Oleifera* leaf extract, *Olive leaf*

extract, and 1:1 *Moringa Oleifera* leaf extract to *Olive leaf* extract as tested samples were add to 3.9mL aliquot of 0.0634 mM of DPPH solution in methanol (95%). Absorbance of the DPPH radical without an antioxidant, i.e., negative control, was also measured. The mixtures were vortexes for 5-10 sec. the change in the absorbance of the samples were measured at 515 nm after 30 min. Standard curve was prepared using different concentrations of trolox. The results expressed as a percentage of inhibition the control.

The percentage inhibition of DPPH of the test sample and known solutions of Trolox were calculated by the following formula:

$$\%\ of\ inhibition = \frac{A^{\circ} - A}{A^{\circ}} * 100\ ,$$

Where A˚ where is the absorbance of a solution of 100 µL methanol 95% and 3.9 mL of DPPH at 515 nm and A is the absorbance of the sample extract at 515 nm.

3.6. Total phenolic content (Folin-Ciocalteau assay)

Total phenolic were determine using Folin-Ciocalteau method and reagents (Singleton,V. et al. ,1965) (40 µL) of *Moringa Oleifera* leaf extract, *Olive leaf* extract, and 1:1 *Moringa Oleifera* leaf extract to *Olive leaf* extracts or Gallic acid standard were mixed with 1.8 mL of Folin- Ciocalteu reagent (pre-diluted 10 fold with distilled water) and allowed to stand at room temp for 5 min, then 1.2 mL of sodium bicarbonate (7.5 %, w\v) was added to the mixture. After standing for 60 min at room temperature, absorbance was measured at 765nm. Aqueous solutions of known Gallic acid concentrations in the range of 10-500 mg\L were used for calibration. Results were expressed as mg Gallic acid equivalents (GAE)\g sample.

3.7. Total flavonoid content (colorimetric assay)

The determination of total flavonoids was performed according to the colorimetric assay of (Kim, D. *et al.* ,2003). The test procedures were performed as follows: 4 mL Distilled water was added to 1 mL of the *Moringa Oleifera* leaf extract, *Olive leaf* extract, and 1:1 *Moringa Oleifera* leaf extract to *Olive leaf* extracts in a test tube. Then, 0.3 mL of 5 % sodium nitrite solution was added, followed by 0.3 mL 0f 10% aluminum chloride solution. Test tubes were incubated at ambient temperature for 5 min , and then 2mL of 1M sodium hydroxide were added to the mixtures. Immediately, the volume of the reaction mixtures was made to 10 mL with distilled water. The mixtures were thoroughly mixed using test tube shaker and the absorbance of the pink color developed was determine at 510 nm. Aqueous solution of known Catechin concentrations in the range of 50 – 100 mg \L were used for calibration and the results were expressed as mg Catechin equivalents (CEQ)\g sample.

3.8. HPLC Analysis

3.8.1. HPLC conditions

HPLC was performed on a Waters system (e 2695) connected with photo-diode array detector (PDA). The separation was conducted on C18 (4.6 × 150 mm, 3.5 µm,XBridge). The elution was performed on a gradient solvent system using 0.5% glacial acetic acid (solvent A) and Acetonitrile (solvent B) as mobile phase. The rations were as follows:

Table 3.1: Gradient flow rate of the HPLC method

Time (min)	Solvent A %	Solvent B %
0	90	10
25	0	100
30	0	100
32	90	10
37	90	10

The flow rate was 1.0 mL/min at room temperature. The UV-PDA detector was monitored at 254 nm at 2D-channel, 3D-channel was also monitored range from 210-400 nm at resolution 1.2 nm, and the injection Volume for all samples and standards was 20 µL. The quantitative HPLC analysis of Gallic Acid compound was calculated according to its peak area.

3.8.2 Standard preparation

20µg\mL of Gallic acid standard was prepared in methanol, sonicate for 15min, and then it was filtered through 0.45µm filter.

3.8.3 Sample preparation

2mg\mL of *Moringa Oleifera* leaf extract was prepared in methanol, sonicate for 15min, and then it was filtered through 0.45µm filter.

3.9. Tablet formulation of Moringa *Oleifera* leaf extract

Moringa Oleifera leaf extract tablets were prepared using two formulas as illustrated in the tables below:

Table 3.2 : *Moringa oleifera* leaf extract tablet formula 1

Materials	Percentage	Amount per tablet (mg)	Function
Moringa Oleifera leaf extract" dry base"	50.42%	180	Active ingredient
Avicel	27.05%	96.6	Diluent
Corn starch	18.03%	64.4	Binder
Ac-di-sol	4 %	14.2	Disintegrate
Magnesium Stearate	0.5 %	1.8	Lubricant
Total	100 %	357	

Table 3.3: Moringa oleifera leaf extract tablet formula 2

Materials	Percentage	Amount per tablet (mg)	Function
Moringa Oleifera leaf extract "dry base"	33.61%	120	Active ingredient
Olive leaf extract" dry base"	16.8%	60	Active Material and binder
Avicel	26.75%	95.49	Diluent
Corn starch	17.84 %	63.66	Binder
Ac-di-sol	4 %	14.28	Disintegrate
Aerosil	0.5 %	1.785	Flowing agent
Magnesium Stearate	0.5 %	1.785	Lubricant
Total	100 %	357	

The tablets of two formulas were prepared by drying the extracts of *Moringa* leaf and\or Olive leaf extract and mixed them with the rest inactives. Then the powder were pressed using a tablet pressing machine.

Quality control testing was performed to the tablets to ensure their quality against BP2013 and home method standards as the following:

Table 3.4: Moringa Oliefera tablets specification

#	**Test**	**Limits**	**Ref.**
1	Physical Appearance	Green to dark Green	Home method
2	Shape	rounded tablets	Home method
3	Average Weight	357 mg ± 5%	BP 2013
4	Weight Uniformity	Non deviated by 10% , NMT 2Tab by 5% from Avg.	BP 2013
5	Disintegration Test	Max 30 Min	BP 2013
6	Hardness	NLT 40 N	BP 2013
7	Friability	NMT 1%	BP 2013
8	Length	7-9 mm	Home method
9	Thickness	5-7 mm	Home method

Weight and weight uniformity of the tablets were performed by Analytical Balance (Ohaus) for 20 tablets and the results expressed as Average weight±SD. Hardness testing were performed for 10 tablets and expressed as average hardness±SD using hardness tester (pharma tester). While disintegration was performed for 6 immediate release tablets using disintegration tester (pharma tester), and the results expressed in the same manner. However, Friability testing was performed using friability tester (pharma tester) as the following: a sample of whole

tablets corresponding to approximately 6.5 g is taken (w1) the sample then placed in friability tester and rotated for 100 round, the sample then de-dusted and (w2) was accurately weight.

% friability calculated using the following formula:

$$\text{Friability} = \frac{w_1 - w_2}{w_1} \times 100\%$$

While the tablets diminutions measured using a manual micrometer.

3.10. Statistical analysis

Antiglycation Assay, Antioxidant, total phenolic and flavonoid content samples were performed in duplicate or in triplicate of each concentration or samples of *Moringa Oleifera* leaf extract, *Olive leaf* extract, and 1:1 *Moringa Oleifera* leaf extract to *Olive leaf* extracts and concentrations were independently analyzed. In HPLC analysis 2 injections of Gallic acid standard and *Moringa Oleifera* leaf extract sample were performed. The results are expressed as means ± standard deviation.

Chapter four: Results and Discussion

4.1. Plants Extraction

The weight of wet extracts obtained after evaporation of the solvent of *Moringa Oleifera* leaf extract and *Olive leaf* extract were (38g for MOE and 33g for OLE) respectively.

4.2. Loss on drying of the extracts

Loss on drying of *Moringa Oleifera* leaf extract was: 88.27 % and for *Olive leaf* extract was: 66.51%. Which mean that the weight of dried extract and yield percentage of *Moringa Oleifera* leaf extract and *Olive leaf* extract were (4.4 g, 11g) and (8.8% , 22%) respectively.

4.3. Antiglycation End Products (AGEs) Assay

Glycation was monitored at concentration 12.5mg\mL for each sample, the percentage of inhibition was found that: *Moringa Oleifera* leaf extract (47%), *Olive* leaf extract(36.1 %), *Moringa Oleifera* leaf extract and *Olive leaf* extract(1:1)(30.4%) and Natural Plants mixture(88.3%), The positive control Quercetin inhibited formation of fluorescent AGEs by 96.2% as shown in figure(4.1).

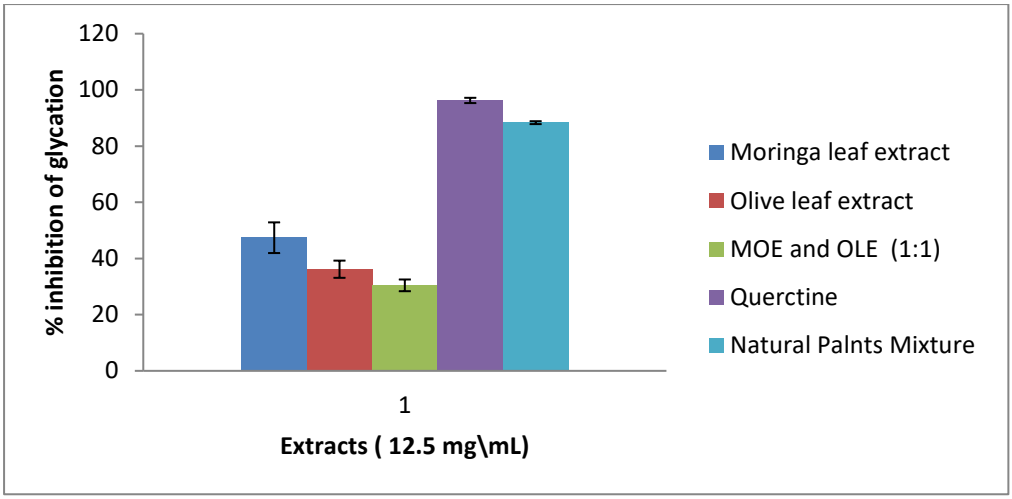


Figure 4.1: Anti-glycation activity of the extracts

Regarding the effect of different concentrations of positive control and extracts on fluorescent AGE formation, five concentration (2.5 mg\mL- 12.5 mg\mL) of positive control, extracts of MOE, (1: 1) MOE to OLE, and natural plants mixture were found to inhibit the formation of fluorescent AGEs as shown in figure(4.2-4.4 and 4.6). The correlation coefficient for Moringa Oleifera leaf extract r= 0.975, for Moringa Oleifera leaf extract and Olive leaf extract (1:1) r= 0.9247. Whereas, positive control figure(4.2), Olive leaf extract, Table (4.1), and Natural Plants mixture, figure (4.6) give a weak linear response in the tested concentrations.

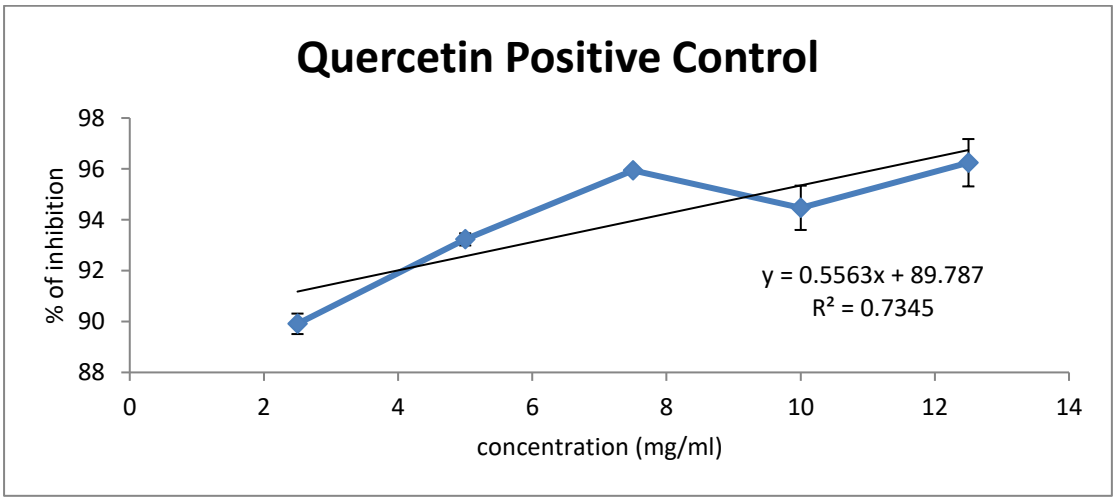


Figure 4.2: Concentration dependent effects of Quercetin positive control on in vitro formation of fluorescent AGEs.

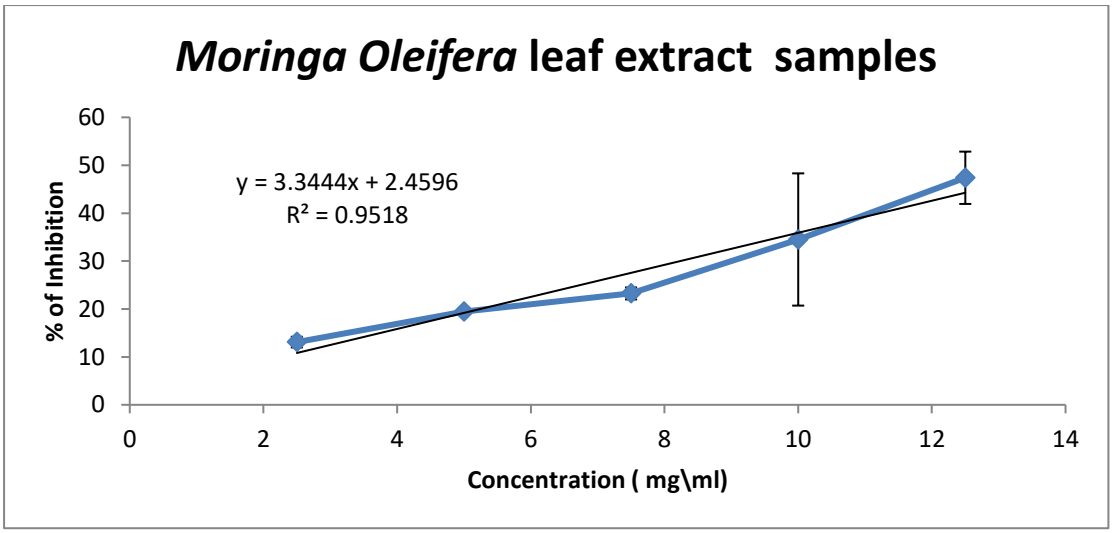


Figure 4.3: Concentration dependent effects of Moringa Oleifera leaf extract on in vitro formation of fluorescent AGEs.

Table 4.1: Concentration dependent effects of Olive leaf extract on in vitro formation of fluorescent AGEs.

Concentration (mg\mL)	% of inhibition	SD value
2.5	0.11%	2.27
5	0.3 %	1.72
7.5	32%	2.09
10	35.4%	2.15
12.5	36.2 %	3.06

Expected superior inhibitions were observed of Quercetin a known AGE inhibitor and served as a positive control, about 90% inhibition of AGEs formation was detected at lower concentration used in this assay, and 96.2% for higher concentration (12.5 mg\mL), Figure (4.2) although the correlation coefficient of the positive standard were indicate weak linear response due to work on high concentrations of tested samples. However, It was observed from the figure (4.3) that the percentage of inhibition of AGE started from 13% for 2.5mg\mL of *Moringa Oleifera* leaf extract

and linearly increased with increasing concentration to 47.3% for 12.5mg\mL. Same finding was reported by (Sangkitikomol, W. et al. ,2014) who state that *Moringa Oleifera* leaf extract could inhibit 10-45% AGE formation in a dose-dependent manner.

Almost the same linear trend was observed for *Olive* leaf extract samples with concentrations 7.5 mg\mL, 10 mg\mL and 12.5 mg\mL, Table (4.1), the correlation coefficient for these three concentrations were r=0.93 with excluding the lower concentrations (2.5 and 5 mg\mL) of OLE. It is also obvious that each extracts alone show better inhibition of AGE formation rather than combined them by1:1 ratio, figure (4.4) and figure (4.5).

On the contrary, the combination of different natural plants mixture which obtained from the local market and combined with 6 plants material (Cinnamon 8%, mustard 48%, marjocam 20%, rashad 8%, radishes 8%, kizha 8%) shows a superior percentage of inhibition of AGE at higher concentrations where the % of inhibition was 88% for 12.5mg\mL of the sample, figure (4.6). This indicates that combining of some plant materials may give additive and\or synergistic effect on the formation of AGEs.

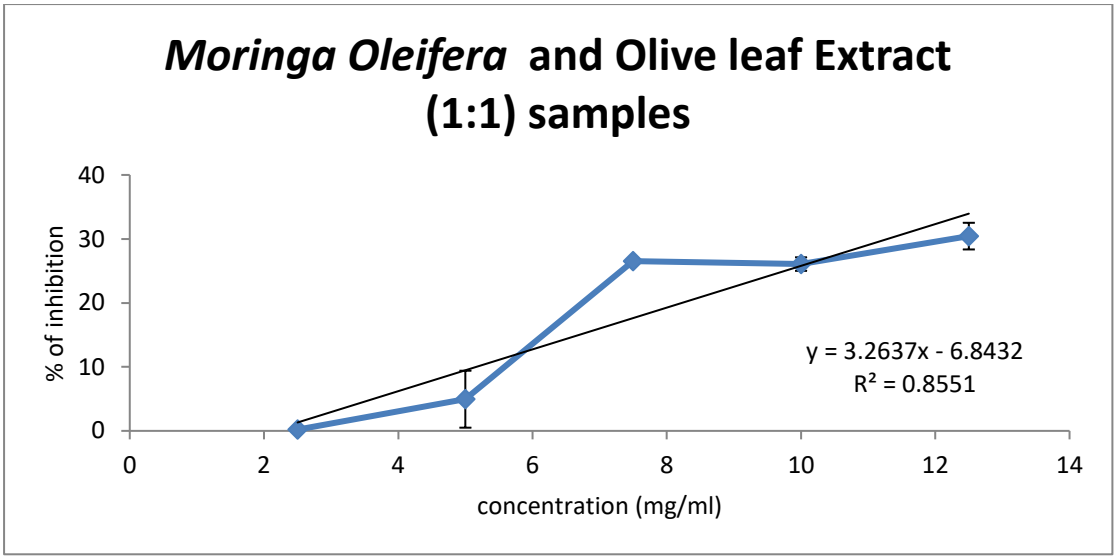


Figure 4.4: Concentration dependent effects of Moringa Oleifera and Olive leaf Extract (1:1) on in vitro formation of fluorescent AGEs.

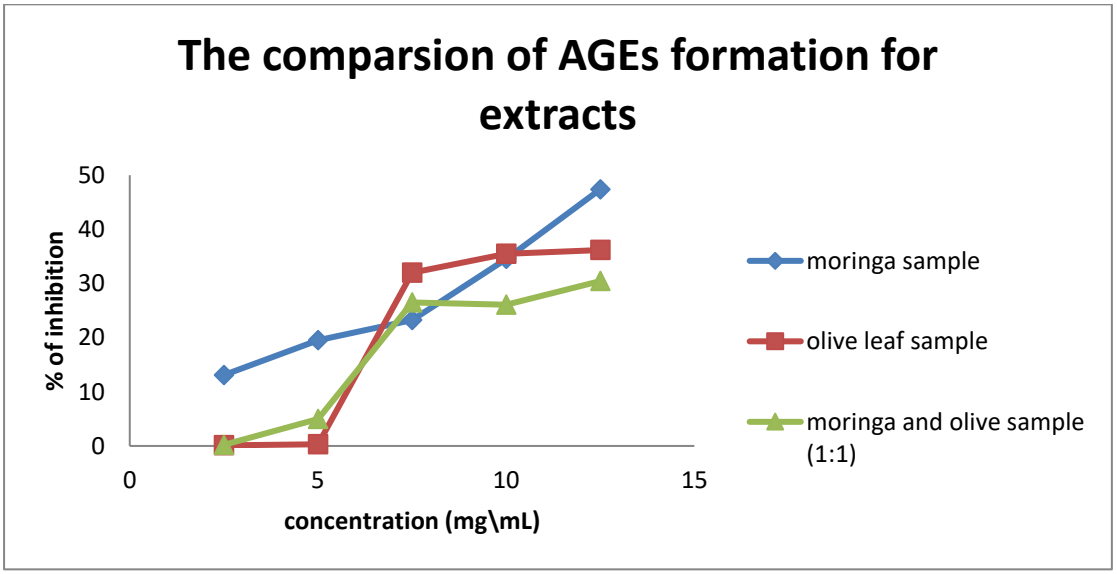


Figure 4.5: The comparison of concentration dependent effects of Moringa Oleifera, Olive leaf and (1:1) of each extract samples on in vitro formation of fluorescent AGEs.

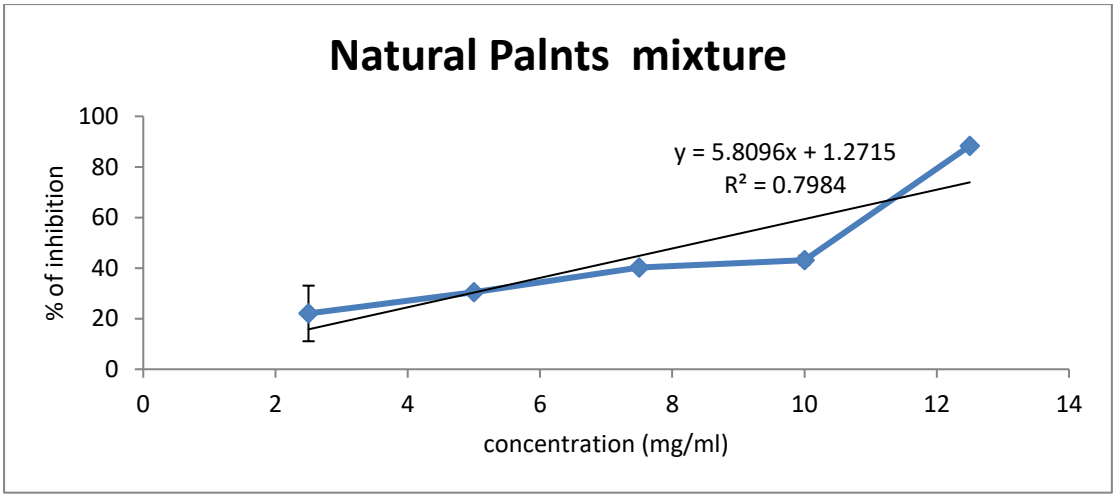


Figure 4.6: Concentration dependent effects of Natural Plants mixture on in vitro formation of fluorescent AGEs.

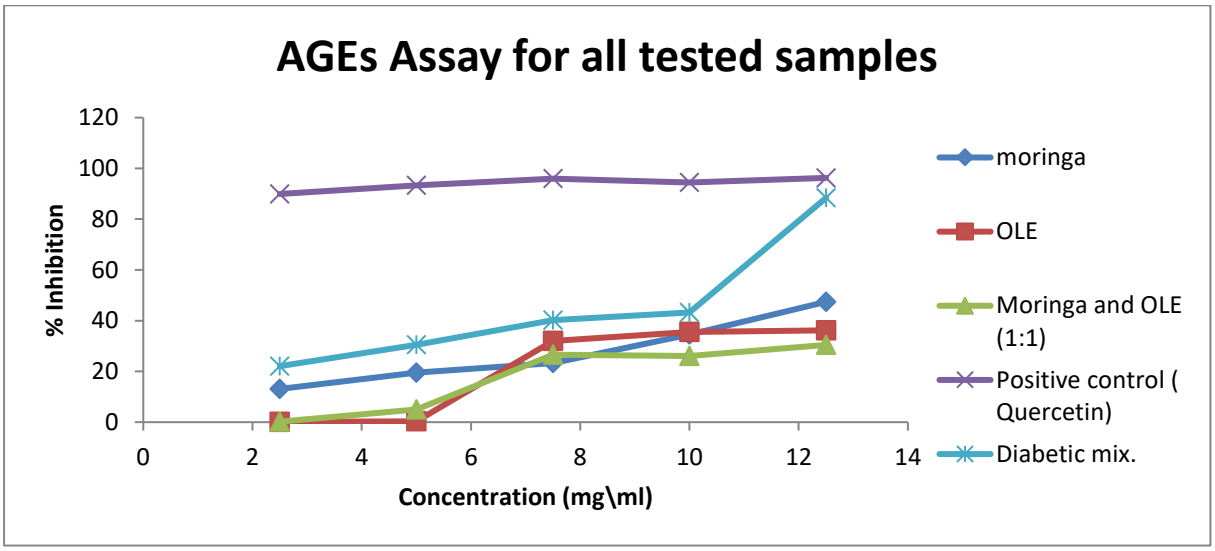


Figure 4.7: The comparison of concentration dependent effects of all tested samples on in vitro formation of fluorescent AGEs.

Form the figure (4.7), it was observed a close slope values, around 4, for *Moringa Oleifera* leaf extract, *Olive leaf* extract, and (1:1) *Moringa Oleifera to olive leaf extracts* curves which indicated the concentration dependant relationship of the inhibitory effect of glycation. On the other hand, very far value for the slope of the positive standard curve, around 0.5, which is too close to zero slope.

4.4. DPPH-radical-scavenging effect

Free radical scavenging capacity (Absorbance) of *Moringa Oleifera leaf* extract and *olive* leaf extract was detected by PDDH Assay and expressed as % of scavenging effect.

The scavenging effect of *Moringa Oleifera* leaf extract, *Olive leaf* extract, *Moringa Oleifera to olive leaf extracts by* (1:1) ratio , and Trolox positive standard at (12.5 mg\ml) were 35.3 %, 83.8 %, 36.2% and 92.1% respectively, figure(4.8).

Similar studies (Pari, L. et al., 2007) reported stronger DPPH scavenging effect which was (65%) for the methanolic extract of *Moringa Oleifera* leaf extracted. Whereas in this study 99% ethanol was used in the extraction.

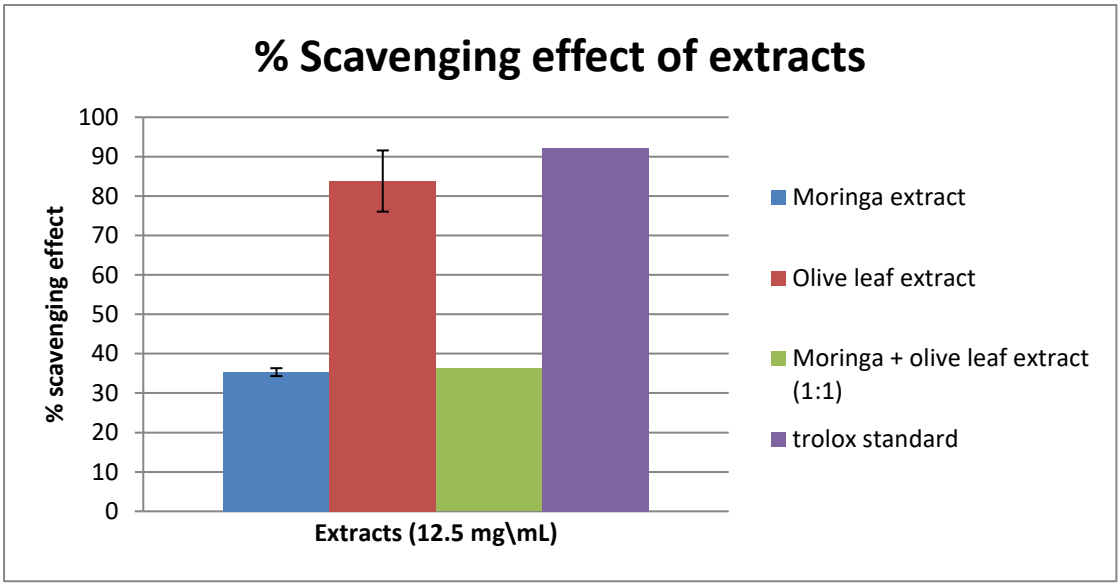


Figure 4.8: % Scavenging effect activity of the extracts

In the manner of dose-dependent of scavenging effect, the correlation coefficient for Trolox positive standard were r=0.4942 and this is due to working on high concentrations of standard, whereas the correlation coefficient for *Moringa Oleifera* leaf extract r= 0.863 and *Olive* leaf extract r= 0.985. Where five concentrations (2.5

mg\mL- 12.5 mg\mL) of *Moringa Oleifera* leaf extract and *Olive leaf* extracts were tested. The percentage of inhibition of DPPH free radicals for *Olive* leaf extract graphs increased as the amount of extracts increased, figure (4.11). It was observed that 12.5 mg\mL of *Olive* leaf extract gives a superior antioxidant activity 83.8% which is very close to the inhibition of positive control at the same concentration. However, The percentage of inhibition of 7.5 mg\mL, 10 mg\ml and 12.5mg\mL of *Moringa Oleifera* leaf extract were increased by increasing the concentration, while 2.5 mg\mL and 5 mg\ml of the same extract gives a very weak response, figure(4.10). No response was found for the concentrations from 2.5 mg\mL to 10 mg\mL of the combination of *Moringa Oleifera* leaf extract and *Olive leaf* extracts (1:1) samples. But the percentage of inhibition of the rest concentration (12.5 mg\mL) was 36.2 %, figure (4.12). Which indicate that this combination may give additive or synergistic effect on higher concentrations.

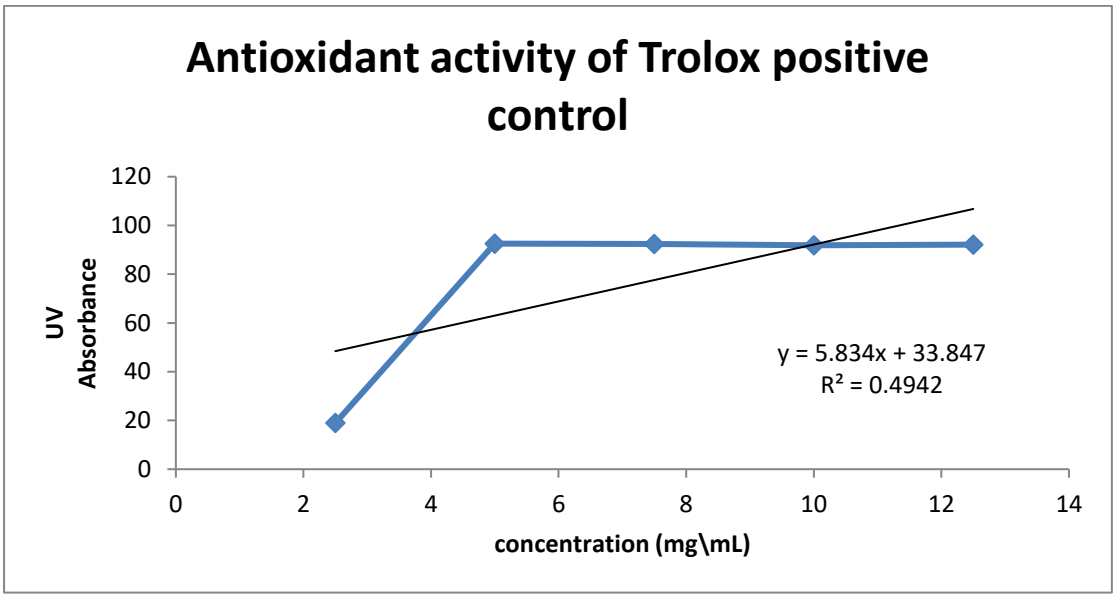


Figure 4.9: DPPH Scavenging activity of Trolox positive control.

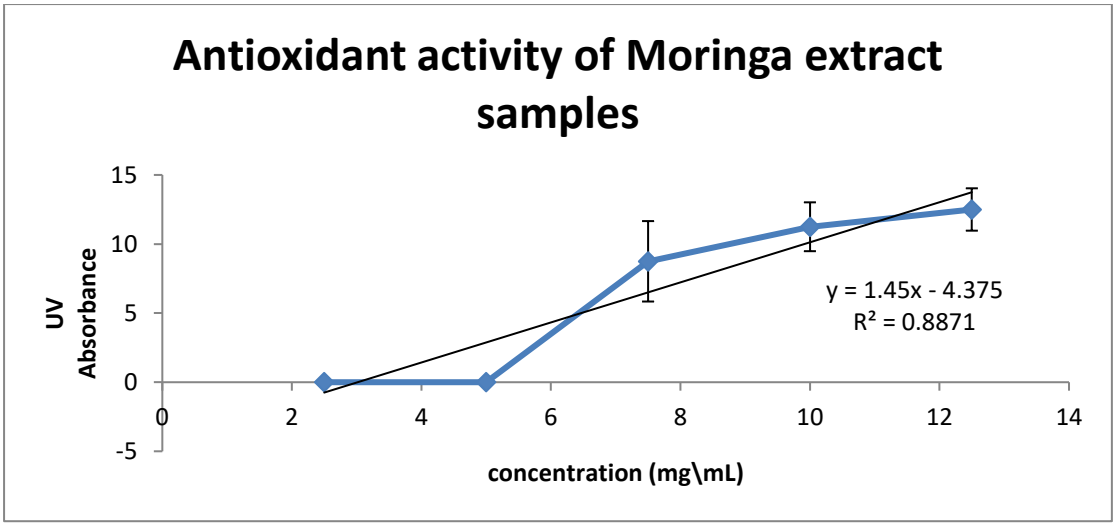


Figure 4.10: DPPH Scavenging activity of Moringa Oliefera leaf extract.

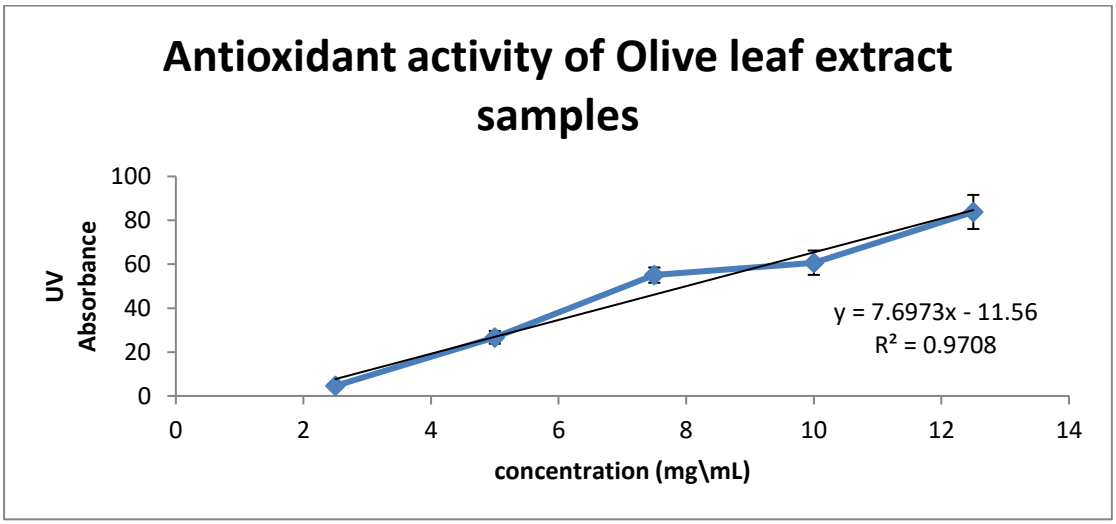


Figure 4.11: DPPH Scavenging activity of Olive leaf extract.

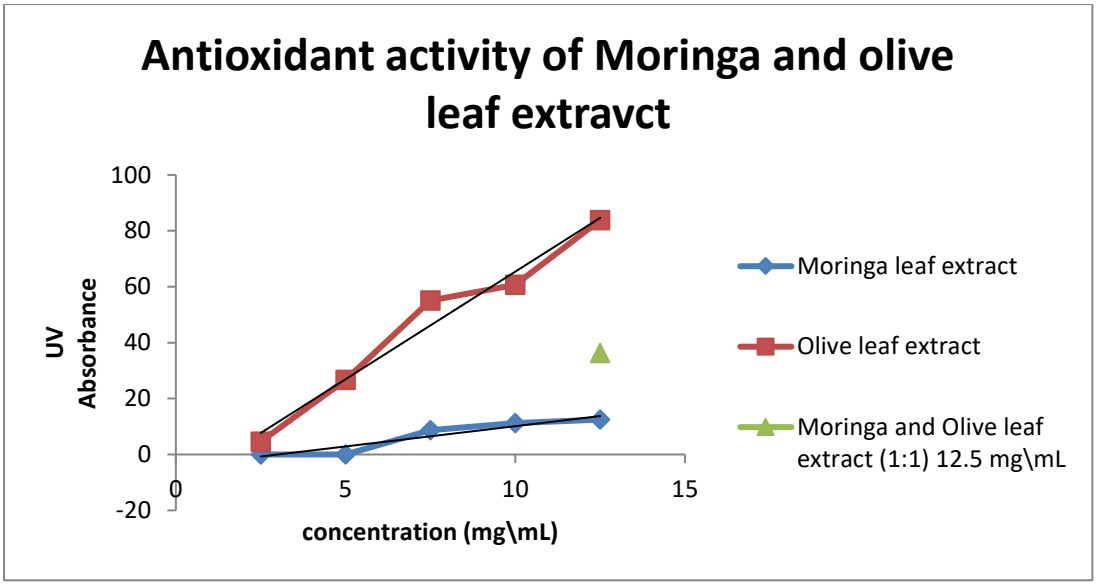


Figure 4.12: DPPH Scavenging activity of Moringa leaf extract and Olive leaf extract.

4.5. Total flavonoids and total phenolic content

The content of total Flavonoids in the extract of *Moringa oleifera* leaves was 66.5 mg CEQ\ g dry extract, 66.7 mg CEQ\ g dry extract for the extract of olive leaves, and 46 mg CEQ\ g dry extract of the combination of *Moringa oleifera and Olive* leaf Extracts by (1:1), Figure(4.13).

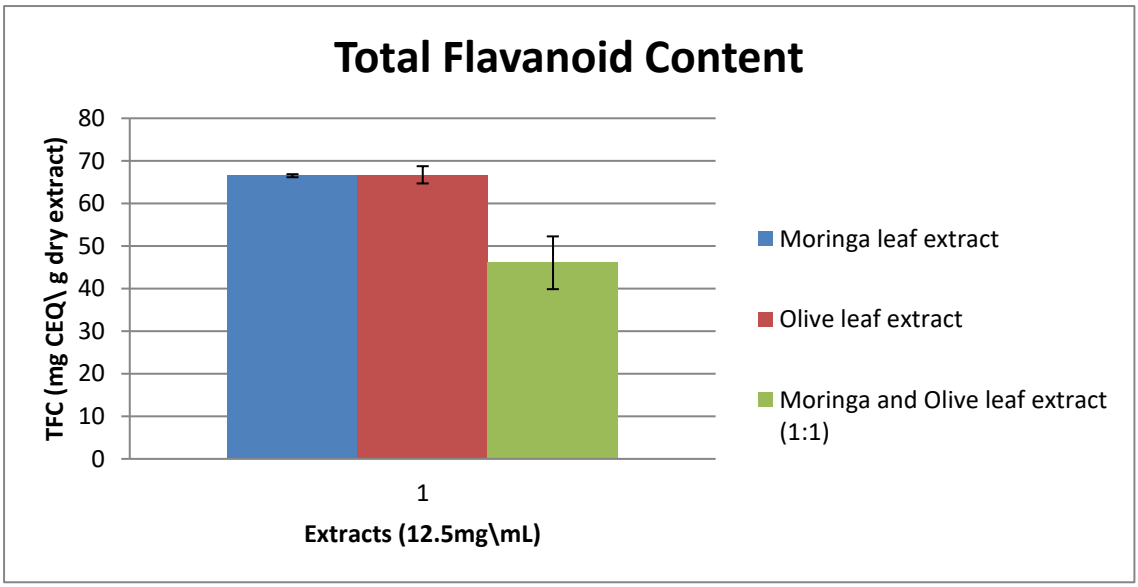


Figure 4.13: Total Flavonoid Content of extracts (Colorimetric Assay).

Whereas the total phenolic content in the extracts of *Moringa oleifera* leaves, olive leaves, and the combination of *Moringa oleifera and Olive* leaf were 19.04 mg GAE\ g dry extract , 45.2 mg GAE \ g dry extract , and 24.5 mg GAE \ g dry extract respectively, Figure(4.14).

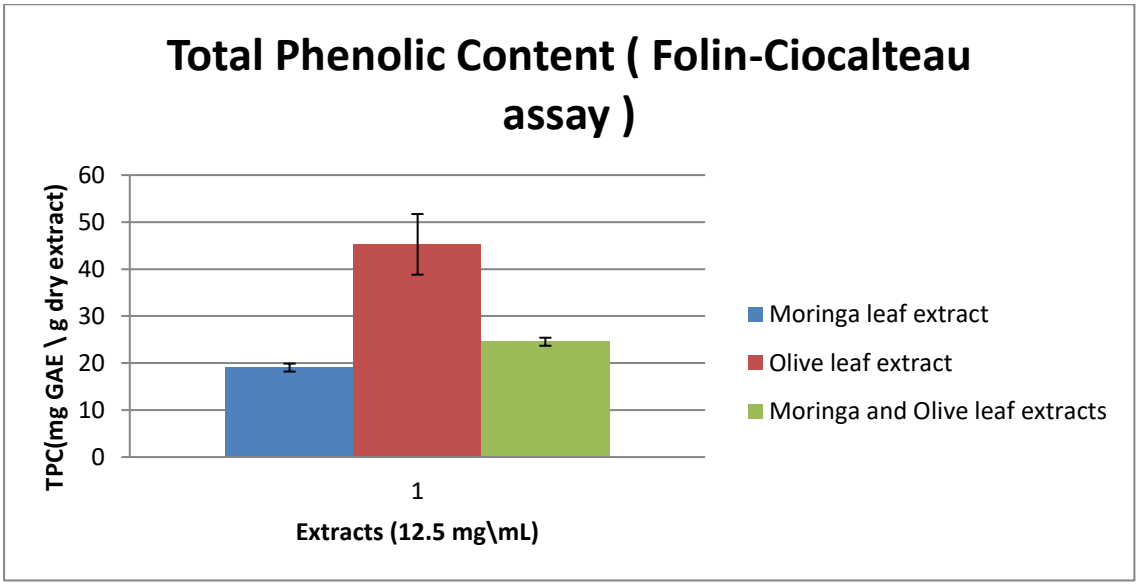


Figure 4.14: Total Phenolic Content of extracts (Folin-Ciocalteau assay).

4.6. HPLC analysis

The HPLC chromatogram of Moringa *oleifera* leaves was characterized at wavelength of 254 nm as shown in the below figure (4.15).

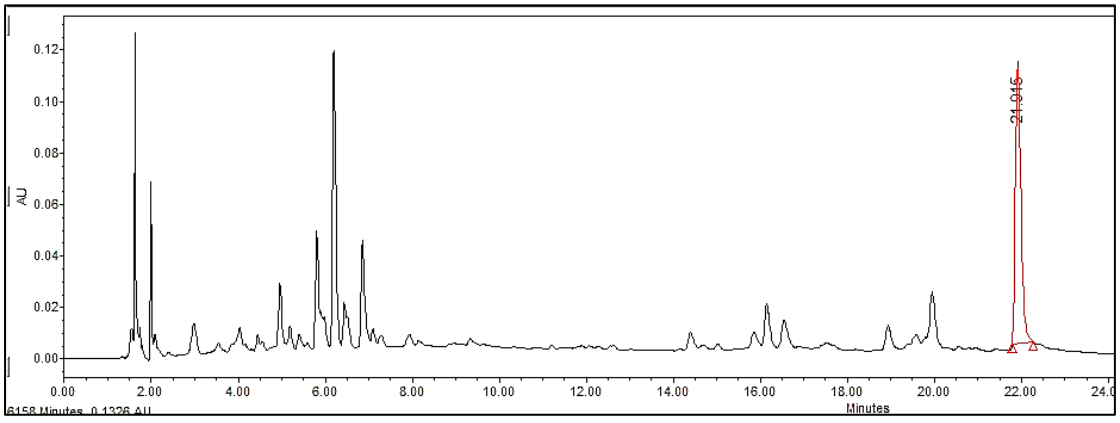


Figure 4.15: HPLC chromatogram for Moringa oleifera leaves extract at 254nm.

This HPLC chromatogram at wavelength 254 nm was characterized by the peak recorded with retention time 21.9 min by maxima of UV spectra at 255.7 nm, figure (4.16).

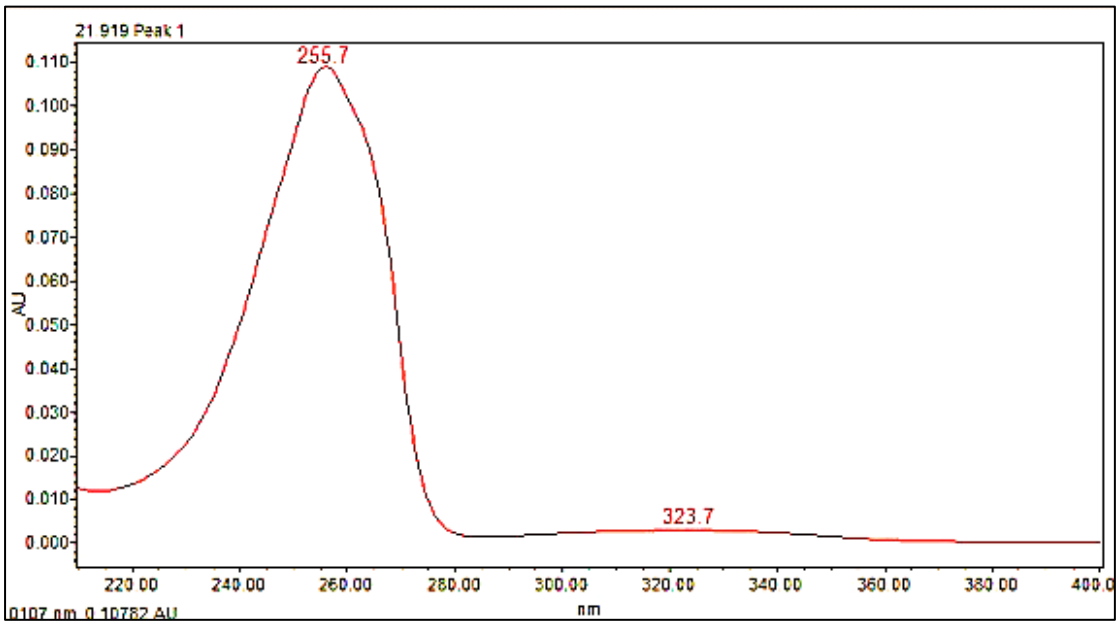


Figure 4.16: UV-PDA spectra of the peak separated from Moringa oleifera leaves extract with retention time 21.9 min using HPLC method.

It was found same retention time (21.9 min), Figure (4.17), and same maxima of UV spectra (255.7 nm) of the pack of Gallic Acid standard that characterized using same HPLC method, Figure (4.18). So this peak was identified as Gallic Acid in the extract.

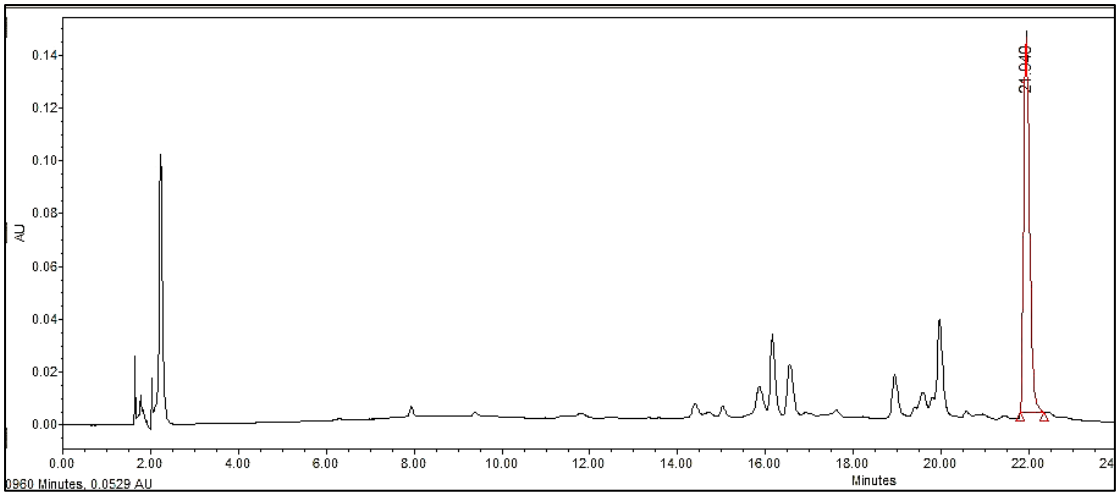


Figure 4.17: HPLC chromatogram for Gallic Acid standard at 254nm.

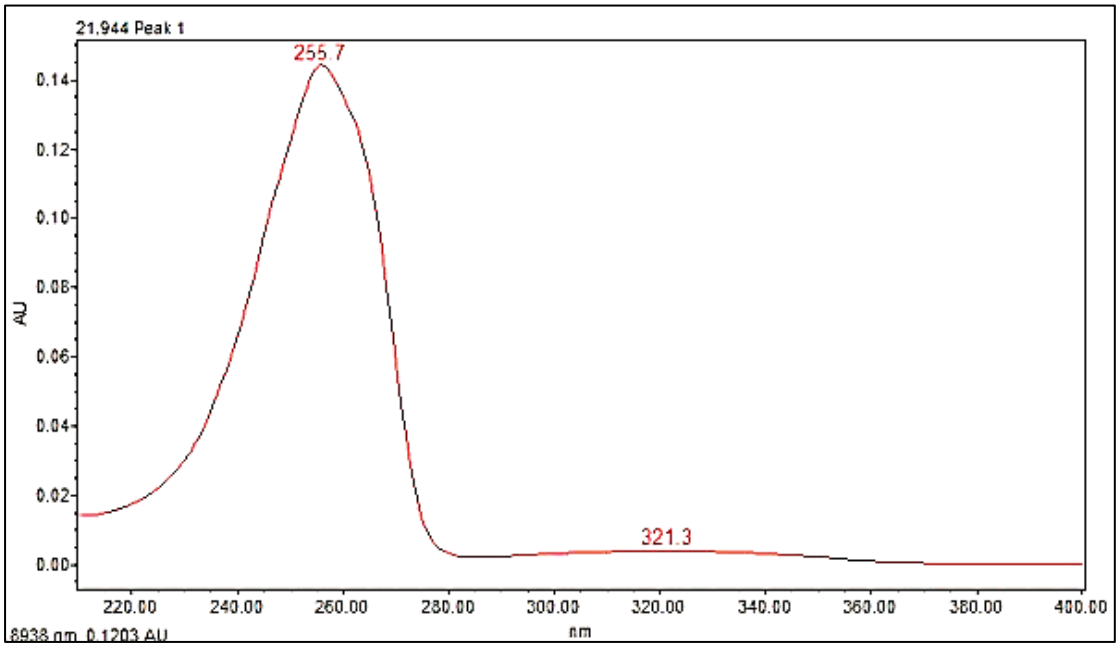


Figure 4.18: UV-PDA spectra of the peak separated from Gallic Acid standard with retention time 21.9 min using HPLC method.

For further identification, separately, a little bet around 0.3 mL of Gallic Acid standard was added to Moringa *oleifera* leaves extract sample (standard addition), and each of the three samples were re-analysed using the same HPLC method in the same run, Figure (4.19-4.21), it was found that the peak area of Moringa *oleifera* leaves extract sample increased from (1080840 µV*s) to (1276535 µV*s) Table (4.2). And

the Assay of Gallic acid increased from 1.38% in Moringa *oleifera* leaves extract sample to 1.63% in standard addition of Moringa *oleifera* leaves extract sample. Meanwhile, the HPLC chromatogram for these samples at wavelength 254 nm was characterized by the peak recorded with retention time 21.9 min by maxima of UV spectra all at 255.7 nm, Figure (4.22). This obtained results were confirmed the identification of Gallic acid. Previous study of (Alam, P. *et al.*, 2016) identifies Gallic acid in *Moringa oleifera* leaves extract. And (Pari, L. *et al.*, 2007) reported that Phenolic acids are the dominant phenolic constituents of the *Moringa olifera* leaves extract.

Table 4.2: chromatogram HPLC results of Gallic acid standard addition to Moringa oleifera leaves extract sample.

	Retention time (min)	AUC(µV*s)	Height (µV)
Gallic Acid standard	21.893	782503	88891
Moringa *oleifera* leaves extract	21.916	1080840	127207
Standard addition of Moringa *oleifera* leaves extract	21.932	1276535	147057

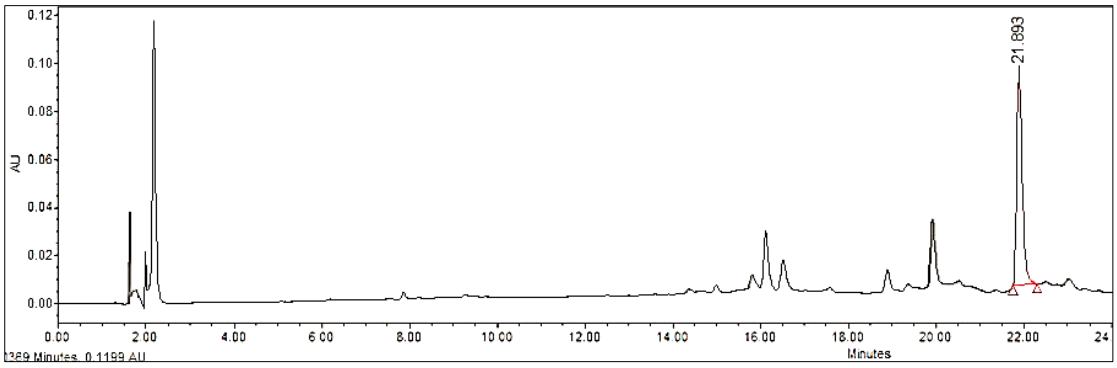


Figure 4.19: HPLC chromatogram for Gallic Acid standard at 254nm.

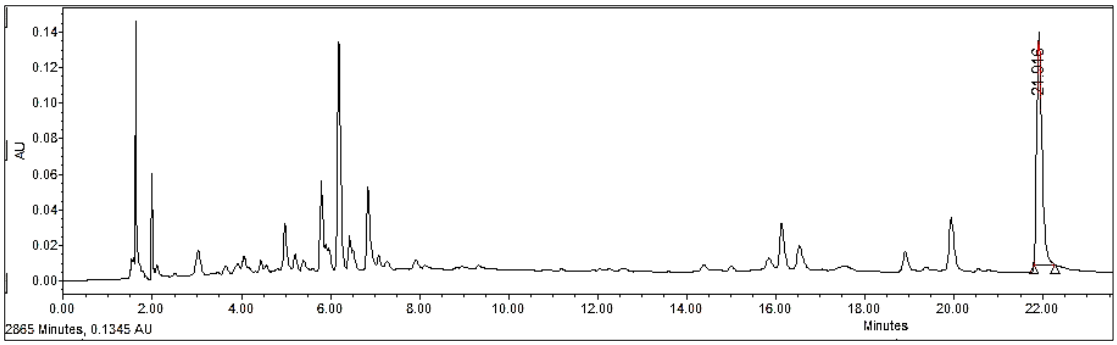


Figure 4.20: HPLC chromatogram for *Moringa oleifera* leaves extract at 254nm.

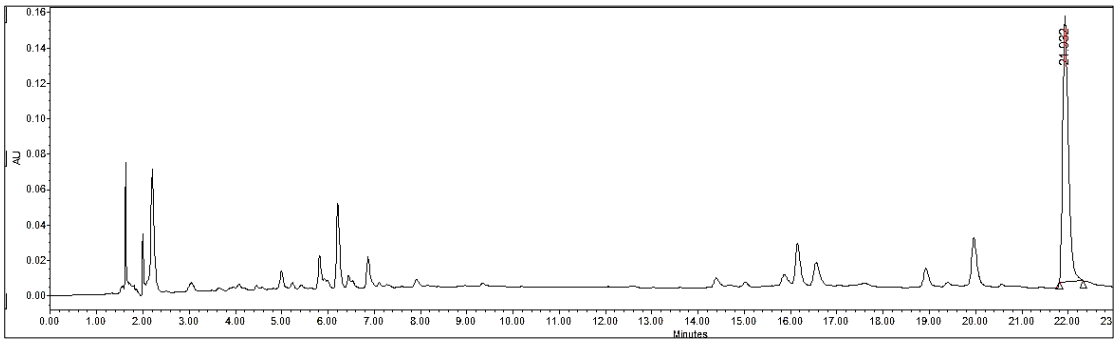


Figure 4.21: HPLC chromatogram for standard addition of *Moringa oleifera* leaves extract at 254nm.

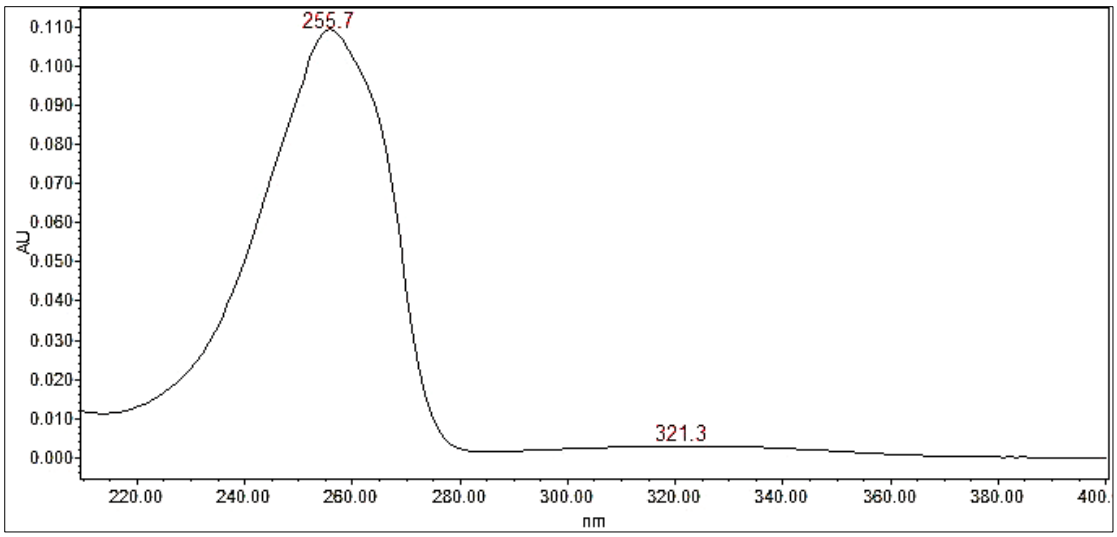


Figure 4.22: UV-PDA spectra of the peak separated from standard addition of Gallic Acid standard for *Moringa oleifera* leaves extract with retention time 21.9 min using HPLC method.

Discussion

Besides the identification of Gallic Acid in *Moringa oleifera* leaves extract using HPLC, and the presence of another bioactive compound that also reflected in anti-oxidant, total phenolic content, total flavonoids content assays of the tow extracts in this study, previous studies reported the presence of bioactive compounds such as Gallic Acid and kaempferol (Sangkitikomol, W. et al. ,2014) in *Moringa oleifera* leaves extract and Oleuropein, Rutin, Verbascoside and hydroxytyrosole in *Olive* leaves extract (Lubada, M., 2013).

Therefore, the therapeutic effects of *Moringa oleifera* leaf and Olive leaf extracts may be due to these combinations of various bioactive compounds. With respect to achieving good glycemic control, a diet combination that regulate the glucose level in the blood and reduces AGE consumption should be the target for management of diabetic patients. And this study was found that MOE and OLE inhibit AGE formation in a dose-dependent manner.

However, Glycation is the non-enzymatic formation of adducts between amino groups and the carbonyl groups of reducing sugars. A sugar reacts with the free amino group of a biological amine to form an unstable compound in the early stage, a Schiff base, which then undergoes rearrangement to form more stable Amadori products (early glycation products). These Amadori products degrade to form a variety of reactive dicarbonyl compounds, such as glyoxal, methyl glyoxal and deoxyglucosones, which are much more reactive than the initial sugars. In the late stage of glycation, due to dehydration, oxidation and cyclization reactions, irreversible compounds, called advanced glycation end products (AGEs), are formed.(Karolak I. *et al*., 2016)

Moreover, Previous studies indicate that high blood glucose can auto-oxidize to form hydrogen peroxide and ketoaldehyde compounds in the presence of transition metal ions, which then accelerates the formation of AGE (Sangkitikomol, W. et al. ,2014). Indicated also that AGE could modify LDL cholesterol levels, which becomes easy to oxidize, is deposited in vessel walls, and finally causes plaque formation. These particles are easy to oxidize and are potent initiators of atherogenesis and its associated vascular damage. *Moringa oleifera* leaves were found to possess hypolipidemic and a tiatherosclerotic activities, and have therapeutic potential for the prevention of cardiovascular diseases (Sangkitikomol W. *et al.*, 2014). In the same context, the European Food Safety Authority (EFSA) has approved polyphenol's ability to protect LDL particles from oxidation. In addition, Olive leaves extract supplementation was associated with a 15% improvement in insulin sensitivity compared to placebo. There was also a 28% improvement in pancreatic β-cell responsiveness. (EFSA Journal, 2011)

The inhibitory mechanism of flavonoids against glycation may be due to their antioxidant properties. A reduction of more than 50% AGE intake could reduce approximately 30% of the circulating AGE within 1 month (W. Sangkitikomol et al. ,2014) [16]. *Moringa oleifera* leaf and *Olive* leaf is efficient enough to inhibit AGE formation. The inhibitory effect of them was approximately around 50% (Figure 2). *Moringa oleifera* leaf and *Olive* leaf is a natural source that has been long consumed as food or herbs. Therefore, a presentation of *Moringa oleifera* leaf and *Olive* leaf in tablets food supplement provide a safe, beneficial, and easy to handle source of alternative medicine.

4.7. Tablet formulation of *Moringa oleifera* leaves extract

Due to that each extracts alone behave better than a combination of them in 1:1 ratio, *Moringa oleifera* leaf extract were formulated with less than half ratio of olive leaf extract in a form of tablets.

The results obtained for *Moringa oleifera* leaf extract tablets using formula 1 were showed that friability and hardness of tested tablets needs to be modified to reach the satisfied limits. Using starch as a binder was not sufficient to bind *Moringa oleifera* leaf extract and other inactives in the formula 1. Thus, using a little percentage of olive leaf extract in formula 2 enhanced very well friability and hardness of tested tablets as shown in the table below:

Table 4.3: Quality control results for obtained *Moringa oleifera* leaf extract tables using formula 2

#	**Test**	**Limits**	**Results**
1	Physical Appearance	Green to dark Green	Confirm
2	Shape	rounded tablets	Confirm
3	Average Weight	357 mg ± 5%	352.705±6.4 mg
4	Weight Uniformity	Non deviated by 10% , NMT 2Tab by 5% from Avg.	Confirm
5	Disintegration Test	Max 30 Min	25 ± 1.1 min
6	Hardness	NLT 40 N	47.09 ± 3 N
7	Friability	NMT 1%	0.1%
8	Length	7-9 mm	8.08 mm
9	Thickness	5-7 mm	6.14 mm

Figure 4.23: *Moringa oleifera* leaf extract tables using formula 2.

Chapter 5 : Conclusion and future work

Moringa oleifera leaf extract and *Olive* leaf extract were found to inhibit the formation of advanced glycation End product formation , it was also found that each extract contain high amount of phenols and flavonoids which behave strong scavenging effect.

HPLC Analysis of *Moringa oleifera* leaf extract confirming the presences of bioactive compounds in the extract, and Gallic Acid was identified as one of phenolic acids presents in *Moringa oleifera* leaf extract. This finding suggested that *Moringa oleifera* leaf extract and *Olive* leaf extract might use as food supplement to prevent glycation formation and therefor diabetic complications. Thus, *Moringa oleifera* leaf extract and less than half ratio of *olive* leaves were formulated in a cheap, easy to handle and swallow tablets food supplement to achieve this purpose.

Future work

1- Investigate the best ration of *Moringa oleifera* leaf extract and Olive leaf extract which gives synergistic effect on advanced glycation end product formation.

2- Modify the HPLC method of Analysis and validate it.

3- Investigate the best harvesting time of *Moringa oleifera* leaves.

4- Perform clinical Study and\or in vivo study for estimate the effect for *Moringa oleifera* leaf extract on regulating glucose level in the blood.

5- Clinically estimate the effect of *Moringa oleifera* leaf extract and Olive leaf extract on diabetic complications.

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inflammatory properties" (ID 1882), "contributes to the upper respiratory tract health" (ID 3468), "can help to maintain a normal function of gastrointestinal tract" (3779), and "contributes to body defences against external agents" (ID 3467) pursuant to Article 13(1) of Regulation (EC) No 1924/2006.

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Appendices

Appendix (7.1 a): Anti- glycation results for all tested samples

Negative Control Samples	
Fluorescence Response	Average Fluorescence Response
6.460	6.421
6.258	
6.545	

Appendix (7.1 b): Anti- glycation results for all tested samples

Moringa Oleifera samples				
concentration (mg/ml)	Fluorescence Response	% of inhibition	Average % of inhibition	Standard Deviation
2.5	5.538	13.75175206	13.08726574	1.11
	5.663	11.8050148		
	5.541	13.70503037		
5	5.207	18.90671235	19.49332918	0.512
	5.155	19.71655505		
	5.146	19.85672014		
7.5	4.898	23.71904688	23.24144733	1.25
	4.868	24.18626382		
	5.02	21.8190313		
10	4.96	22.75346519	34.50656699	13.8
	3.227	49.74303068		
	4.429	31.02320511		
12.5	3.579	44.26101853	47.38618076	5.46
	3.583	44.19872294		
	2.973	53.69880081		

Appendix (7.1 c): Anti- glycation results for all tested samples

Olive Leave extract samples				
concentration (mg/ml)	Fluorescence Response	% of inhibition	Average % of inhibition	Standard Deviation
2.5	6.328	1.448372528	0.109017287	2.279488859
	6.331	1.401650833		
	6.583	-2.5229715		
5	6.331	1.401650833	0.295904065	1.729824773
	6.345	1.183616259		
	6.53	-1.697554898		
7.5	4.297	33.07895966	31.97840419	2.097875956
	4.523	29.55925868		
	4.283	33.29699424		
10	4.277	33.39043763	35.48772258	2.151078027
	4.149	35.38389659		
	4.001	37.68883352		
12.5	4.165	35.13471422	36.1729741	3.062849038
	3.877	39.61999689		
	4.253	33.76421118		

Appendix (7.1 d): Anti- glycation results for all tested samples

Olive Leave extract and *Moringa Oliefera Extract (1:1)* samples				
concentration (mg/ml)	Fluorescence Response	% of inhibition	Average % of inhibition	Standard Deviation
2.5	6.4	0.327051861	0.202461	0.176199
	6.416	0.077869491		
5	5.901	8.098427036	4.9525	4.449013
	6.305	1.806572185		
7.5	4.709	26.66251363	26.50677	0.220248
	4.729	26.35103566		
10	4.699	26.81825261	26.07071	1.057191
	4.795	25.32315839		
12.5	4.561	28.96745055	30.43918	2.081345
	4.372	31.9109173		

Appendix (7.1 e): Anti- glycation results for all tested samples

Natural Plants Mixture samples				
concentration (mg/ml)	Fluorescence Response	% of inhibition	Average % of inhibition	Standard Deviation
2.5	5.503	14.29684	22.06821367	10.99038
	4.505	29.83959		
5	4.426	31.06993	30.47033172	0.847955
	4.503	29.87074		
7.5	3.866	39.79131	40.17287027	0.539608
	3.817	40.55443		
10	3.571	44.38561	43.16305871	1.728948
	3.728	41.94051		
12.5	0.7719	87.97851	88.34137985	0.513178
	0.7253	88.70425		

Appendix (7.1 f): Anti- glycation results for all tested samples

Querctine Positive Control samples				
concentration (mg/ml)	Fluorescence Response	% of inhibition	Average % of inhibition	Standard Deviation
2.5	0.6661	89.62622644	89.91045009	0.401952928
	0.6296	90.19467373		
5	0.424	93.39666719	93.22846909	0.237868034
	0.4456	93.06027099		
7.5	0.2635	95.89627784	95.94144214	0.063871972
	0.2577	95.98660645		
10	0.3946	93.85453979	94.47048746	0.871081551
	0.3155	95.08643513		
12.5	0.199	96.90079427	96.24279707	0.93054856
	0.2835	95.58479988		

Appendix (7.2 a): Antioxidant effect of all tested samples

• Absorbance of 100µL of methanol 95% and 3.9mL of DPPH =4.3133		
Trolox Standard		
concentration (mg/ml)	scavenging capacity (Absorbance)	Scavenging effect %
2.5	3.497	18.92518
5	0.3212	92.55327
7.5	0.3261	92.43966
10	0.3478	91.93657
12.5	0.3382	92.15914

Appendix (7.2 b): Antioxidant effect of all tested samples

Moringa Oliefera leaf Extract samples				
concentration (mg/ml)	scavenging capacity (Absorbance)	Scavenging effect %	Average Scavenging effect %	Standard Deviation
12.5	2.7432	36.40136	35.31751	1.532805
	2.8367	34.23365		
10	3.8406	10.95913	11.21183	0.357381
	3.8188	11.46454		
7.5	4.1142	4.615955	2.558366	2.909871
	4.2917	0.500777		
5	4.3133	0	0	0
	4.3133	0		
2.5	4.3133	0	0	0
	4.3133	0		
Olive Leave extract samples				
concentration (mg/ml)	scavenging capacity (Absorbance)	Scavenging effect %	Average Scavenging effect %	Standard Deviation
12.5	0.4612	89.30749	83.81286	7.770584
	0.9352	78.31823		
10	1.5264	64.61178	60.69715	5.536132
	1.8641	56.78251		
7.5	2.045	52.58851	55.04022	3.467254
	1.8335	57.49194		
5	3.2499	24.65398	26.70693	2.903313
	3.0728	28.75988		
2.5	4.069	5.663877	4.591612	1.516412
	4.1615	3.519347		

Appendix (7.2 c): Antioxidant effect of all tested samples

Olive Leave extract and *Moringa Oliefera Extract (1:1)* samples		
concentration (mg/ml)	scavenging capacity (Absorbance)	Scavenging effect %
12.5	2.7498	36.24835

Appendix (7.3): Total phenolic Content results for all samples

The standard curve of the Gallic Acid gives the following liner equation: Y = 0.0027 x – 0.1172				
Moringa sample 12.5mg\ml	Absorbance	mg GAE \ g dry extract	Average mg GAE \ g dry extract	Standard deviation
Sample 1	0.5456	19.63852	19.04	0.846433
Sample 2	0.5052	18.44148		
Olive leaf extract sample 12.5mg\ml	Absorbance	mg GAE \ g dry extract	Average mg GAE \ g dry extract	Standard deviation
Sample 1	1.5644	49.82519	45.26519	6.448814
Sample 2	1.2566	40.70519		
Olive leaf extract sample and Moringa sample (1:1) 12.5mg\ml	Absorbance	mg GAE \ g dry extract	Average mg GAE \ g dry extract	Standard deviation
Sample 1	0.6907	23.93778	24.54222	0.854814
Sample 2	0.7315	25.14667		

Appendix (7.4): Total Flavonoids content results for all tested samples

The standard curve of the Catechin gives the following liner equation: y = 0.0048x + 0.0034				
Moringa sample 12.5mg\ml	Absorbance	mg CEQ \ g dry extract	Average mg CEQ\ g dry extract	Standard deviation
Sample 1	4.009	66.76	66.50833	0.35591
Sample 2	3.9788	66.25667		
Olive leaf extract sample 12.5mg\ml	Absorbance	mg CEQ \ g dry extract Average mg CEQ\ g dry extract		Standard deviation
Sample 1	4.0927	68.155	66.7225	2.025861
Sample 2	3.9208	65.29		
Olive leaf extract sample and Moringa sample (1:1) 12.5mg\ml	Absorbance	mg CEQ\ g dry extract	Average mg CEQ\ g dry extract	Standard deviation
Sample 1	3.0299	50.44167	46.06083	6.195434
Sample 2	2.5042	41.68		

Appendix (7.5): HPLC results for Moringa leaf extract sample

	Injection	Retention time	AUC(µV*s)	Height (µV)
Gallic Acid standard	1	21.955	1356805	152424
	2	21.940	1319830	142074
	Injection	Retention time	AUC(µV*s)	Height (µV)
Moringa *oleifera* leaves extract	1	21.915	980134	106793
	2	21.925	974441	105868

Appendix (7.6): Quality control results of Moringa leaf extract tablets using formula 2

Weight and weight uniformity					
Weight of tablets (mg)				Average weight (mg)	SD
357.3	343.3	347.4	349.7	352.7	6.42
340	362.9	351.2	356.6		
358.6	361.4	355.7	351.2		
346	354	361.1	347.9		
352.2	360.6	352.8	344.2		
Hardness Values (N)		Average Hardness (N)			SD
50.3	43.5	47.09			3.04
46.1	42.6				
48.2	43.2				
49	46.4				
50.5	51.1				
Disintegration time	Average Disintegration time (min)				SD
27	25.83				1.16
26					
26					
27					
25					
24					

تأثير الخاصية المثبطة لمستخلص أوراق المورينجا و مستخلص أوراق شجرة الزيتون في تكون المنتجات النهائية لتفاعل السكر مع البروتين المتقدمة و تطوير صياغة لحبوب مستخلص أوراق المورينجا

اعداد : إباء ناصر الدين علي حلبية

اشراف: الدكتور ابراهيم كيالي

ملخص :

تُعرَف شجرة الزيتون في الطب التقليدي الفلسطيني لفوائدها العديدة، حيث تساعد في علاج العديد من الأمراض ، والتي من أهمها مرض السكري، فيما أن أوراق المورينجا معروفة بذات الفعالية العلاجية في الهند.

تعمل زيادة نسبة السكر في الدم لدى مرضى السكري من النوع الثاني إلى تسريع التكوين غير الإنزيمي للمنتجات النهائية لعملية ارتباط السكر بالبروتين المتقدمة (AGEs) حيث تلعب هذه المنتجات دوراً رئيسياً في العديد من المضاعفات مثل : أمراض القلب التاجية والسكتة الدماغية واعتلال الكلية والاعتلال العصبي ، وتصلب الشرايين . لذلك يمثل تثبيط تكوين المنتجات النهائية لعملية ارتباط السكر بالبروتين المتقدمة هدفاً علاجياً محتملاً للوقاية والعلاج من مضاعفات مرض السكري.

لذلك، من الأهداف الرئيسية في هذه الدراسة هي فحص أثر مستخلص أوراق المورينجا و أوراق الزيتون و الخليط بينهما بنسبة (1:1) في مكافحة تكون المنتجات النهائية لعملية ارتباط السكر بالبروتين المتقدمة ، و أيضاً يهدف هذا البحث لدراسة نسبة مضادات التأكسد و تحديد مجموع الفينولات و الفلافونويدات و تحليل مستخلص اوراق المورينجا باستخدام جهاز الكروماتوجرافيا السائلة، و أيضاً تطوير مكمل غذائي من مستخلص المورينجا ليساعد في الوقاية من مضاعفات مرض السكري.

في هذه الدراسة ، تم استخلاص أوراق المورينجا و أوراق الزيتون باستخدام كحول الإيثانول (99%) باستخدام طريقة الاستخلاص الساخن المتكرر (Soxhlet)، ومن ثم تم تقييم المنتجات النهائية لعملية ارتباط السكر بالبروتين المتقدمة في المختبر بواسطة فحص ألبومين المصل البقري مع الجلوكوز ، بينما تحددت نسبة مضادات التأكسد للمستخلصين و الخليط بينهما بنسبة (1:1) باستخدام طريقة الجدور الحرة (DPPH). وقد تم تحديد مجموع الفينولات باستخدام طريقة فولين سيوكالتيو ، و فحص الفلافونويد باستخدام طريقة المقاييس اللونية واستخدم جهاز الكروماتوجرافيا السائلة الموصول مع جهاز كاشف الأشعة فوق البنفسجية، لتحليل مستخلص المورينجا، بينما تم تشكيل مستخلص المورينجا إلى أقراص ليتم أخذه كمكمل غذائي.

توصل البحث إلى أن التركيز من (12.5 – 2.5 ملغم\ملم) من مستخلص أوراق المورينجا و أوراق الزيتون قادرة على تثبيط إنتاج المنتجات النهائية لعملية ارتباط السكر بالبروتين المتقدمة بشكل فعال، حيث أن مستخلص أوراق المورينجا قادر على تثبيط ما نسبته (13-47%) بينما ورق الزيتون قادر على التثبيط بنسبة (0.1%-36%). حيث تعود نتيجة هذه النسب العالية إلى وجود المركبات النشطة حيوياً في كلا المستخلصين، وهذا أظهرته نتائج فحص مجموع الفينولات والفلافونويدات، حيث كان مجموع الفلافونويدات لمستخلص المورينجا و مستخلص ورق الزيتون بالترتيب كالتالي : (66.5 و 66.7 ميليغرام مكافئة للكاتشين لكل غرام من المستخلص الجاف). بينما كانت نتيجة الفينولات لمستخلص المورينجا و مستخلص ورق الزيتون بالترتيب: (19.04 و 45.2 ميليغرام مكافئة للجاليك أسيد لكل غرام من المستخلص الجاف).

تم الاستنتاج من خلال النتائج التي توصل إليها البحث أن تصرف مستخلص ورق الزيتون و مستخلص المورينجا كل على حدا أفضل من إضافة كلا المستخلصين معاً بنسبة (1:1) ، و من خلال فحص الكروماتوجرافيا السائلة تم تأكيد وجود مركبات حيوية في مستخلص المورينجا، وقد تم تعريف مركب الجاليك أسيد في المستخلص ذاته. بينما تم تشكيل مستخلص المورينجا إلى مكمل غذائي على شكل أقراص، حيث وُجِد أن اضافة مستخلص ورق الزيتون بنسبة قليلة إلى الصياغة يعمل على تحسين الخصائص الفيزيائية للأقراص بشكل ملحوظ جداً.

و بهذا الشكل يمكن الاستفادة من الفوائد الكبيرة الموجودة في مستخلص المورينجا و مستخلص ورق الزيتون و خاصة المرتبطة بالوقاية من مضاعفات مرض السكري عن طريق حبوب مكمل غذائي طبيعي سهل الاستخدام و البلع.