Journal of Current Medical Research and Opinion

CMRO 09 (03) 4731-4738 (2026)
DOI: https://doi.org/10.52845/CMRO/2026/9-3-7
www.cmro.in

CMRO
ISSN (O) 2589-8779
(P) 2589-8760

Original Research

Anti-Diabetic and Anti-Inflammatory Effects of Oral Administration of a Leaf Extract of *Origanum majorana* in Rats and Investigate Its Natural Components

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Abstract

The ancient methods of healing in the past were based on natural remedies and herbs. The last few decades have seen an upsurge in the interest of drug discovery researchers in herbs, as they may cause fewer problems and adverse effects. Medicinal and pharmacological research has been on the increase all over the world due to the increase in demand. In case of inappropriate use of glucose by the body a disease occurs, and the disease is termed as diabetes mellitus. It is a chronic disease that is accompanied by a disproportionate increase in the level of blood glucose, and is caused by either inadequate production of insulin or lack of enough sensitivity of the cells to its activity. An abrupt rise in the level of glucose in the blood, excessive urination, excessive thirst, and inexplicable weight loss are some of the symptoms of this condition. The main objective of this research was to examine the active components of an *Origanum majorana* leaf extract and the anti-inflammatory and anti-diabetic properties of this extract on mice. Twenty bioactive compounds were identified in the present study: Bis(2,4 dimethylpentadienyl)ruthenium(II), Pentacosanoic acid, methyl ester, 10-Octadecenoic acid, trans, Triacontanoic acid, octadecyl ester, Docosanedioic acid, dimethyl ester, 1,1'-Bicyclopentyl, 2-hexadecyl, Phenol, 2,6-bis(1,1-dimethylethyl), 6,10,14-Trimethylpentadecan-2-ol, n-Hexadecanoic acid methyl ester, 9-Octadecenoic acid (9Z)-, methyl ester, L-Proline, 1-acetyl, Oleoyl chloride, Dimethyl 2-methylisophthalate, Butanoic acid, 3-hexenyl ester, (E)-, 2,4,7-trimethyl-1H-indene, tau-Cadinol, 2-Octanol, 2,6-dimethyl, 7-Pentadecanone, 7-Pentadecanone, Phytol, and 2,2,3,5-Tetramethylhexane. The impacts of *Origanum majorana* Leaf methanolic crude extract were observed on the serum enzymes SGPT, SGOT and ALP in rats. The means of the extract given orally to the rats are (79.00±3.95, 89.02±4.67 and 26.00±1.89) in the *Origanum majorana* Leaf methanolic crude extract group, The values of the inhibitory power against Alpha-amylase were obtained as (76.00±4.90, 58.52±3.86, 31.00±2.18 and 27.06±1.15) respectively, depending on the type of extract (methanol crude extract, ethanol fraction, ethyl acetate fraction and Acarb. It was measured at (48.05±3.61, 35.00±2.70, 22.09±1.03 and 17.00±0.94) respectively. Most likely, the source of this anti-inflammatory potential is the presence of polyphenolic chemicals with anti-inflammatory effects and which may be used in the treatment of disorders related to diabetes.

Keywords: Oral Administration, *Origanum majorana*, Natural Components, Anti-Diabetic, Anti-Inflammatory

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Received: 06.01.2026 | **Revised:** 24.03.2026 | **Accepted:** 28.03.2026 | **Published:** 31.03.2026

Introduction

Germs may grow in hostile environments and may have positive as well as negative impacts on human health. They do not only accelerate the spoilage of food, they also make people sick. Food spoilage is a very undesirable process that is a great challenge to human beings. Plant antimicrobial peptides (AMPs) have been investigated in terms of their bioactivities towards various human, plant, and food infections [1, 2], among other reasons that accelerate food spoilage. Some of the numerous phenolic substances that possess antioxidant activity include the stilbenes, lignans, flavonoid and phenolic acids. Plants take advantage of properties of the above compounds to safeguard themselves against the destructive environmental factors such as ultraviolet light, temperature changes and mechanical stress. Their physiological impact on insects also offers a significant chemical barrier to herbivores due to the latter [3]. *Origanum majorana* is one of the species of Lamiaceae family that has undergone a vast body of research into its pharmacological activity. It has outstanding composites in its leaves such as rosmarinic acid, thymol, tannins and triterpenes and the essential oil fraction of the plant also comprises a rich chemical profile [4-6]. A significant amount of research has been conducted regarding the biological activities of the essential oil of the plant produced by its aerial part. Marjoram is a wonderful anti-bacterial and antiviral agent in the case of many common ailments such as food poisoning, tetanous infection in wounds, influenza, mumps, etc. Marjoram as well enhances the circulatory and cardiovascular systems, i.e., it reduces blood pressure, cholesterol formation, and the risk of hypertension significantly. (Asthma, sinus headaches, fever) are the symptoms of inflammation which improve the inflammation. Apply to aches and pains such as the back pain and muscle tension. Two of the examples of the emotional and neurological benefits include reduction of stress and lowering emotional reactivity [7, 8]. The principal phytoconstituents of the herb are linalool, terpenes, and tannins. This study was aimed at studying the antidiabetic and anti-inflammatory properties of a methanol extract of leaves of the plant *Origanum majorana*.

Materals and Methods

Origanum majorana is a leaf that was obtained at Medicinal Herbs Center in Hilla, Iraq. Also, they received Babylonian University College of Science recognition. The leaves were first rinsed by the process of shade drying and then ground in a fine powder with sterile distilled water to take off any dirt or other impurities on the leaf. These powdered samples (100g/500 ml) were subjected to the usage of methanol over 48 hours at 45 o C. The phytochemical components are extracted with the help of the soxhlet equipment. The extract was concentrated at 50 C ^{o} through soaking and pressure evaporation after which the residue was stored at 4 C^{o}. Organic maceration in ethanol, methanol and ethyle acetate produced some chemical compounds. Each type of solvent (100 mL) was added to the 25 grams of powdered plant material in a 500 mL conical flask, and then the mixture was covered by the mouth of the flask and placed in a shaker incubator at six hours, after which the mixture was cooled, filtered, and stored to be used in the future. This was followed by the use of vacuum pressure pump and the extracts were filtered using Whatman No.1 filter paper. A Rotary Vacuum Evaporator RE52 fitted with a water reservoir heated to 50 C ^{o} was used to evaporate the solvent present in the extract. Finally, the residues were gathered and stored at 4 C ^{o} to test their biologic activity.

The mass spectrometry-gas chromatography (GC-MS) analysis was done through Agilent 789OA gas chromatography. Helium was used as the carrier gas. The specifications of the capillary lumen were Aligent 19091S-433:1548.52849HP-5MS and 5% phenyl methyl silox 30 m and 250 m×0.25 m HP-5MS. The temperature was fluctuating between 80s and 300s. A sample that was diluted with hexane (1:100 v/v) was injected (2 ml).

α-Amylase inhibitory activity

The extract and fractions were analyzed concerning their α-amylase inhibitory activity

with the help of the standard procedures with some minor modifications. Fractions containing various concentrations of extracts and 0.5 milligrams per milliliter were mixed with fifty liters of phosphate buffer of pH 6.8 and 100 millimolar concentration. Ten liters of alpha amylase were added as well. Thereafter, the mixture was pre-incubated at a temperature of 37 degrees Celsius in a 96-well plate. The preincubation process was done at 37 C ^{o}. It was then put back in the incubator where it was allowed to incubate with the addition of twenty liters of 1% soluble starch as substrate after half an hour. Ten minutes of constant pressure heating was applied to the mixture when 100 liters of DNS color reagent were introduced into the mixture. A Multiplate Reader was used to record an absorbance reading at 540 nanometers. The measurement was done to determine the absorbance of the final mixture. The concentrations of acarbose that were used as standards were between 0.1 and 0.5 mg/ml. A material that was not subjected to any experimental procedures was also developed so as to act as a control. Moreover, the experiment was done three times in each instance.

Inhibition of Alpha- Glucosidase Assay

An analysis was done to determine the inhibitory effect of the extract and fractions on alpha-glucosidase. The analysis was conducted in the traditional manner, and some modifications were made here and there. A 96 well plate was preheated at 37 degrees Celsius in 15 minutes to preincubate a reaction mixture. The reaction mixture was composed of fifty liters of phosphate buffer, ten liters of alpha-glucosidase, and twenty liters of different extracts and fractions with the concentration of half a milligram per milliliter. The phosphate buffer was 99.9 percent in concentration and with a pH of 6.8. The preincubation was done in 37 degrees Celsius. A substrate was then added to the mixture in the next step with twenty liters of five millimolar P-NPG. The mixture was then incubated at 37 degrees Celsius after that another twenty minutes. The process was stopped by the addition of a sodium carbonate 0.1 M solution in fifty liters. This test was performed on a real basis by use of a multiplate reader with a wavelength of 405 nm to measure the absorption of newly released nitrophenol. Acarbose was used as a standard, and the concentration of the tested sample was 0.5 mg/mL.

Animals in Research and the Care of Drugs

The volunteers used in the research were healthy male albino rats of a weight of 200-250g. The animals were kept in polypropylene cages with normal laboratory food under a controlled environment at a temperature of 23+2 C^{o} and allowed to acclimatize to the laboratory atmosphere eight hours before the studies. The animals were supplied by a facility that deals with animal breeding. The rats were maintained in well-ventilated cages and fed a normal rat food as they were confined in normal conditions (23+3 C ^{o}, 55-70 ^{o} of humidity, 12 h dark/light cycle). The total number of rats was twelve and each group was made up of four rats. The animals belonging to group I acted as control group and were used with maize oil as a vehicle. In every trial such as the *Origanum majorana* methanol fraction, Di-(2-ethylhexyl) phthalate 100mg/kg was the positive control.

Statistical Analysis of Data

The statistical analysis was done using the GraphPad Prism 5 Statistical Package. The data was assessed through a one-way analysis of variance (ANOVA) and Bonferonni test. In the case of the three tests, the in vitro data of the IC50 were reported as the mean value, with or without the standard error of the mean. The results of the quantification of phytochemicals were presented as the mean with the standard deviation and the free radical scavenging abilities were presented as a percentage. Statistical analysis was done at a level of P=<|human|>A P= level of P=.05 was applied.

Results and Discussion

Origanum majorana L. is one of the most important representatives of the Lamiaceae family that locals commonly call sweet marjoram. This plant has a long history of medical usage in terms

of herbs and medications. Though it was native to the Mediterranean region, it has since grown in the entire world especially in Europe, North Africa, and Asia due to its therapeutic benefits. The forerunners of the world human settlements knew its potential and how it could be used to complement food in numerous ways. The key feature of this plant is that it has numerous pharmacological functions as a potent medication such as anti-inflammatory, hepatoprotective, and menstrual cycle regulating activity among others. Its pharmacognostic properties are due to all its pharmacological components. The aerial parts of the plant are believed to have been subjected to a wide range of phytols, phenolics and other substances that have medicinal value. The extract, dried leaves or essential oil of this plant is also used as a good antibacterial medicine. The essential oil of the leaves of the sweet marjoram has been shown to be responsive to a number of medical conditions such as respiratory and gastrointestinal diseases. The marjoram leaf is a sweet plant with a long history of medicinal use, which includes the treatment and treatment of diabetes, catarrh, and anxiety. It is a great source of vitamins A and C, and as a healthy addition, it is used traditionally to treat anxiety, fungal infections, and bacterial infections with its essential oils extracted out of its leaves [8, 9]. Although numerous works have been found on the biologically important compounds isolated in the sweet marjoram leaf essential oil and other extracts, there is no published evidence that could identify the compounds present in the methanolic extract. To enhance its future medical use to the benefit of humanity, the study at hand embarked to research and identify the chemicals present in the methanolic-extract of leaves by the use of GC-MS. The *O. majorana* leaf extract was subjected to the GC-MS, and twenty chemicals were identified and classified. The properties of the GC-MS graph (peak area, retention time) were used to locate the phytochemicals of the solvent, and their molecular formula were confirmed with the WILEY8 of the Schimadzu GCMS-QP2010 Ultra. The table gives a summary of the key chemicals found in the sweet marjoram leaf extracts as determined by gas chromatography mass spectrometry (GC-MS) analysis. Twenty bioactive compounds were used in the current study: Bis(2,4-dimethylpentadienyl)ruthenium(II), Pentacosanoic acid, methyl ester, 10-Octadecenoic acid, trans, Triacontanoic acid, octadecyl ester, Docosanedioic acid, dimethyl ester, 1,1'-Bicyclopentyl, 2-hexadecyl, Phenol, 2,6-bis(1,1-dimethylethyl), 6,10,14-Trimethylpentadecan-2-ol, n-Hexadecanoic acid methyl ester, 9-Octadecenoic acid (9Z)-, methyl ester, L-Proline, 1-acetyl, Oleoyl chloride, Dimethyl 2-methylisophthalate, Butanoic acid, 3-hexenyl ester, (E)-, 2,4,7-trimethyl-1H-indene, tau-Cadinol, 2-Octanol, 2,6-dimethyl, 7-Pentadecanone, 7-Pentadecanone, Phytol, and 2,2,3,5-Tetramethylhexane.

Table 1. GC-MS of a leaf extract of *Origanum majorana*.

Compound	Molecular Formula	Molecular Weight	Compound	Molecular Formula	Molecular Weight
Bis(2,4-dimethylpentadienyl)ruthenium(II)	$C_{14}H_{22}Ru$	291.4 g/mol	L-Proline, 1-acetyl	$C_{16}H_{31}NO_7Si$	377.5 g/mol
Pentacosanoic acid, methyl ester	$C_{26}H_{52}O_2$	396.7 g/mol	Oleoyl chloride	$C_{18}H_{33}ClO$	300.9 g/mol
10-Octadecenoic acid, trans	$C_{18}H_{34}O_2$	282.5 g/mol	Dimethyl 2-methylisophthalate	$C_{11}H_{12}O_4$	208.21 g/mol
Triacontanoic acid, octadecyl ester	$C_{48}H_{96}O_2$	705.3 g/mol	Butanoic acid, 3-hexenyl ester, (E)-	$C_{10}H_{18}O_2$	170.25 g/mol
Docosanedioic acid, dimethyl ester	$C_{24}H_{46}O_4$	398.6 g/mol	2,4,7-trimethyl-1H-indene	$C_{12}H_{14}$	158.24 g/mol
1,1'-Bicyclopentyl, 2-hexadecyl	$C_{26}H_{50}$	362.7 g/mol	tau-Cadinol	$C_{15}H_{26}O$	222.37 g/mol
Phenol, 2,6-bis(1,1-dimethylethyl)-	$C_{23}H_{38}O$	330.5 g/mol	2-Octanol, 2,6-dimethyl	$C_{12}H_{24}O_2$	200.32 g/mol
6,10,14-Trimethylpentadecan-2-ol	C18H38O	270.5 g/mol	7-Pentadecanone	$C_{15}H_{30}O$	226.4 g/mol
n-Hexadecanoic acid methyl ester	$C_{17}H_{34}O_2$	270.5 g/mol	Phytol	$C_{20}H_{40}O$	296.5 g/mol
9-Octadecenoic acid (9Z)-, methyl ester	$C_{36}H_{70}O_4$	566.9 g/mol	2,2,3,5-Tetramethylhexane	$C_{10}H_{22}$	142.28 g/mol

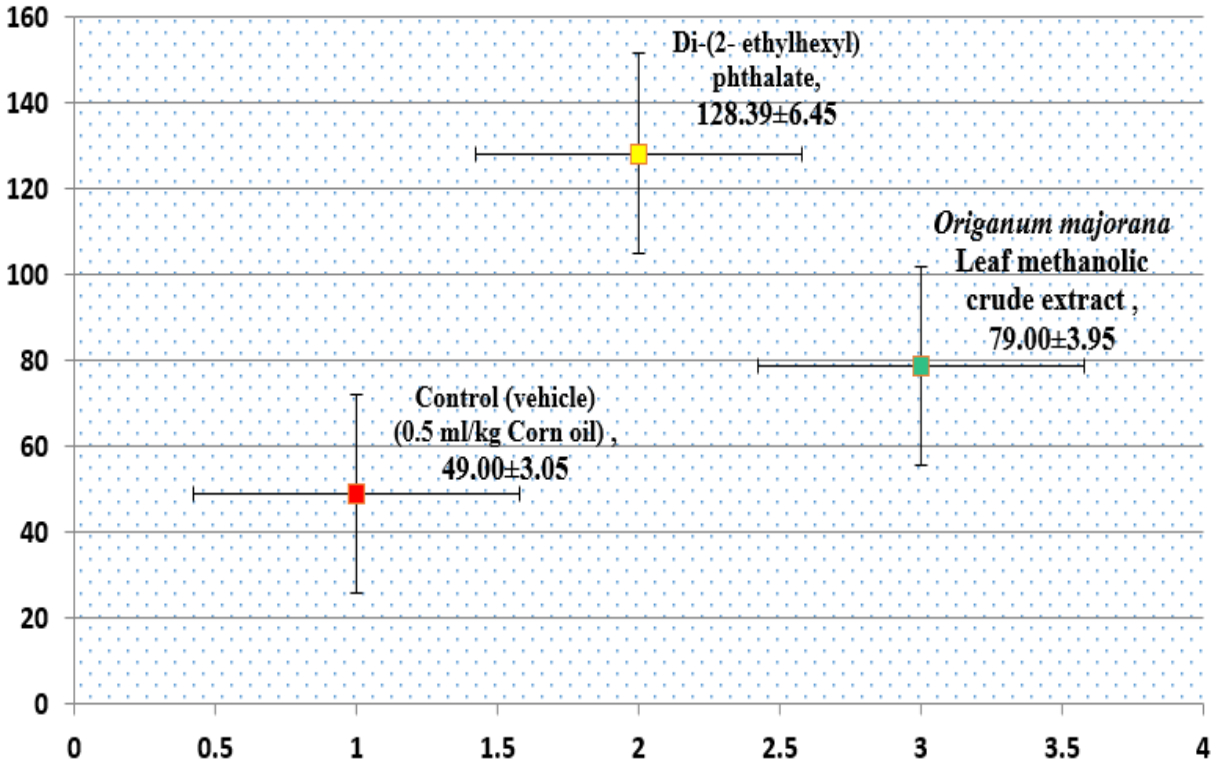

Figure 1. Oral administration of bioactive secondary metabolites of *Origanum majorana* Leaf methanolic crude extract, Di-(2- ethylhexyl) phthalate and Control on serum serum enzymes Serum Glutamic-Pyruvic Transaminase.

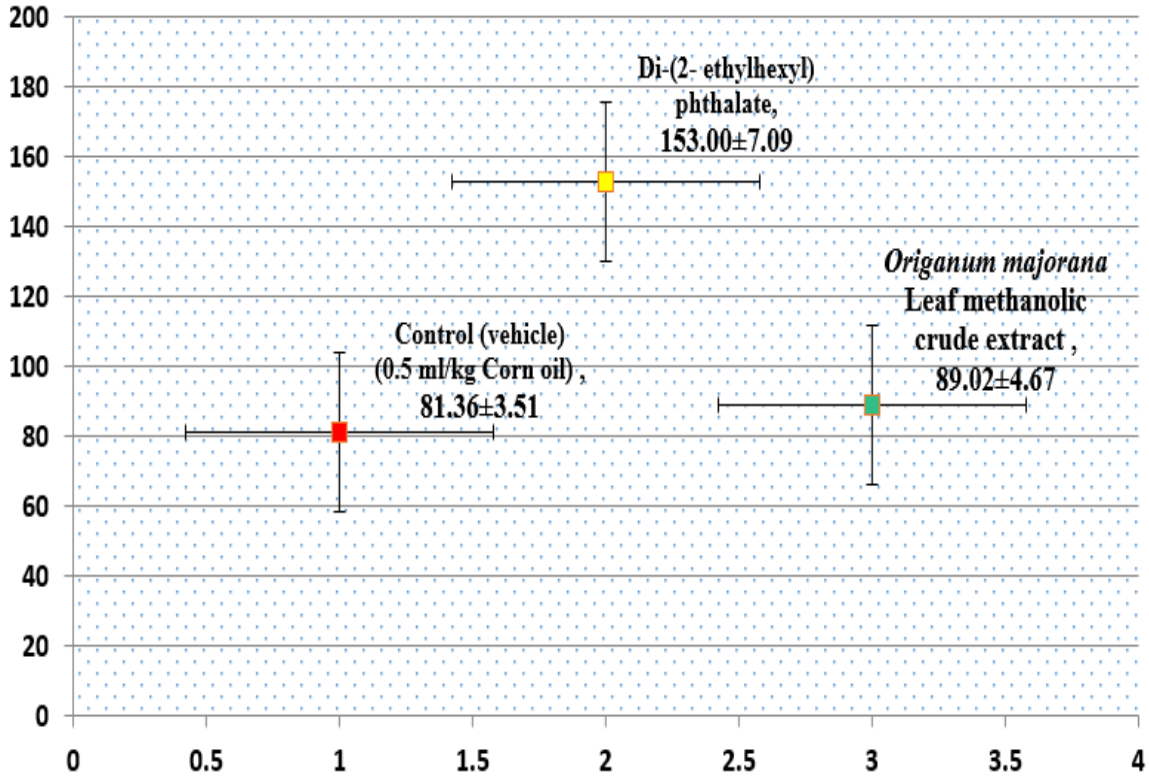

Figure 2. Oral administration of bioactive secondary metabolites of *Origanum majorana* Leaf methanolic crude extract, Di-(2- ethylhexyl) phthalate and Control on serum serum enzymes Serum Glutamic-Oxaloacetic Transaminase.

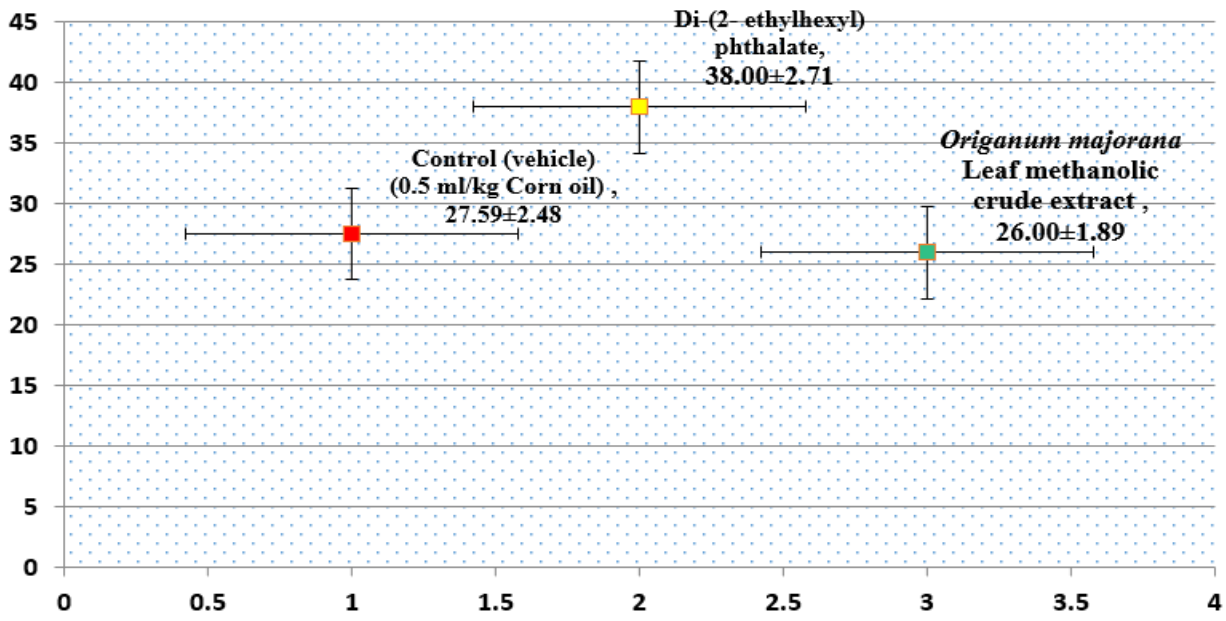

Figure 3. Oral administration of bioactive secondary metabolites of *Origanum majorana* Leaf methanolic crude extract, Di-(2- ethylhexyl) phthalate and Control on serum serum enzymes Alkaline phosphatase.

The hypothesis aimed at investigating the impact of *Origanum majorana* Leaf methanolic crude extract on serum enzymes (SGPT, SGOT, and ALP) in the laboratory rats through in vitro experimental test. The extract administered orally to the rats gave the following results (79.00±3.95, 89.02±4.67, and 26.00±1.89) with *Origanum majorana* Leaf methanolic crude extract, (128.39±6.45, Figures 1, 2 and 3. Liver is an important organ that assists the body in maintaining homeostasis, generation of energy, and the removal of toxic chemicals and medicines. Hepatic cholestasis is the accumulation of bile acids in the liver caused by a disproportion between the production of the bile in the liver, its secretion, or flow. Hepatic oxidative stress, hepatocyte death, inflammation, and the production of reactive oxygen species (ROS) are caused by pathological concentrations of bile acids in the blood stream. Two cases of acquired causes of intrahepatic cholestasis are hepatitis and drug-induced liver injury. Hepatic cholestasis has symptoms of itchy skin, hyperlipidemia, and jaundice. When unattended to, the conditions may also result in liver cirrhosis, fibrosis and eventual liver failure. Ursodeoxycholic acid has not been reported to enhance the symptoms of cholestasis in some patients, and hence, it is the only medicine that has been approved by FDA to treat this illness currently [9, 10]. There is no currently effective medication that can manage the progression of the liver issues even though the disorders are a global problem with high rates of death and morbidity. The side effects of the new drugs that are employed in the treatment of the chronic liver diseases are also found. Research has shown the antiviral, antiseptic and antifungal properties of ursolic acid of OM and its essential oil especially thymol and carvacrol. Isolated ursolic acid and rosmarinic acid, of OM also possess fat-reducing and liver-protective properties [11-13]. In the animal studies, the bile duct ligation, which is an in vitro model of the development of hepatic cholestasis, has been proven to produce alterations in the liver functioning parameters by causing secondary biliary cirrhosis as well as oxidative stress. The studies of the rats prove that methanolic extract of OM shields the liver against CCl4 intoxication and decreases AST, ALT, and ALP. This may be due to its protective effect by inhibiting cytochrome P450 that inhibits the production of free radicals. Liver fibrosis starts with inflammatory processes and evolves partly because of immunological reactions. Inflammatory cells are one of the primary causes

of the cytokines that initiate the inflammatory reactions and, subsequently, tissue fibrosis [14, 15]. Our findings are consistent with a recently conducted research which reported an increase in the expression of hepatic TNF- 2 and TGF- 2 genes in liver damage. Our results indicated that OM extract reduced the expression of the hepatic TGF- B gene whereas BDL- V increased the gene. TNF-A expression was not significantly different as compared to that of BDL-V, however. TGF-b is a profibrotic cytokine which suppresses the breakdown of the extra cellular matrix, and increases collagen synthesis by amplifying the inhibition of metalloproteinases [16–18]. Consequently, OM extract could suppress inflammatory response in rats by the control of oxidant status and/or by the decrease in the proinflammatory sign (TGF-b) [19]. The activity towards alpha-amylase was recorded as (76.00±4.90, 58.52±3.86, 31.00±2.18 and 27.06±1.15) respectively depending on the type of extract (Methanolic crude extract, Ethanolic fraction, Ethyl acetate fraction and Acarbose, respectively). The following measurements of the inhibitory potency against alpha-glucosidase activity in Figures 4 and 5 were measured, (48.05±3.61, 35.00, 22.09 and 17.00), respectively.

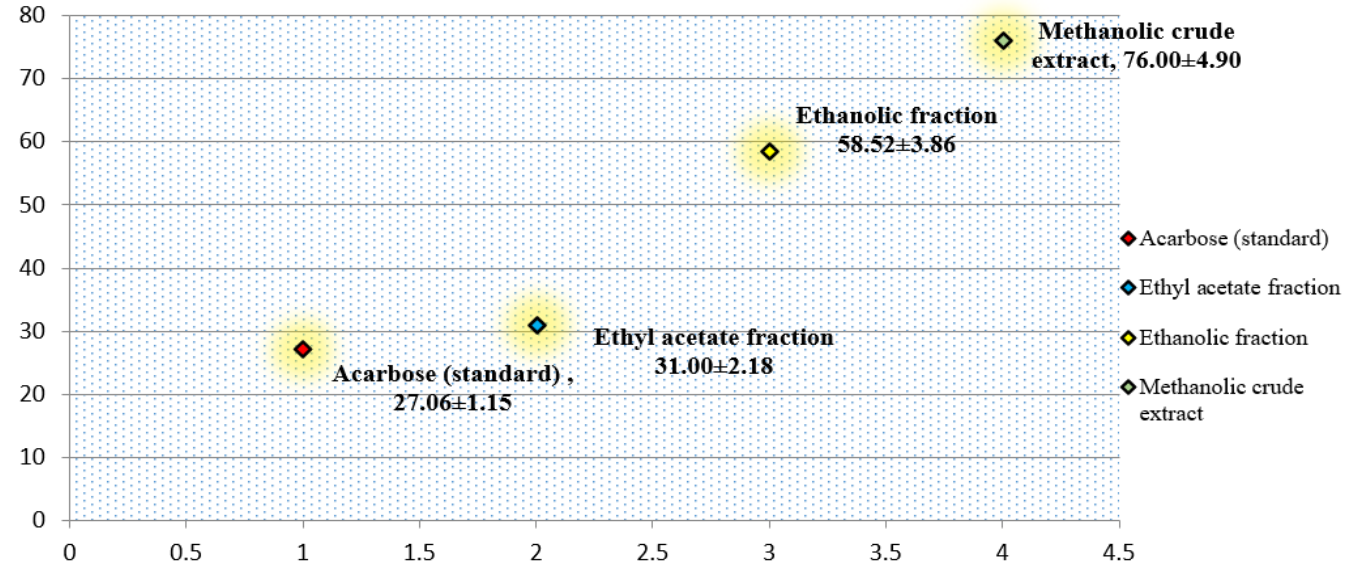

Figure 4. IC50 values of α-amylase inhibition by *Origanum majorana* (Methanolic crude extract, .Ethanolic fraction, Ethyl acetate fraction, and Acarbose as (standard)

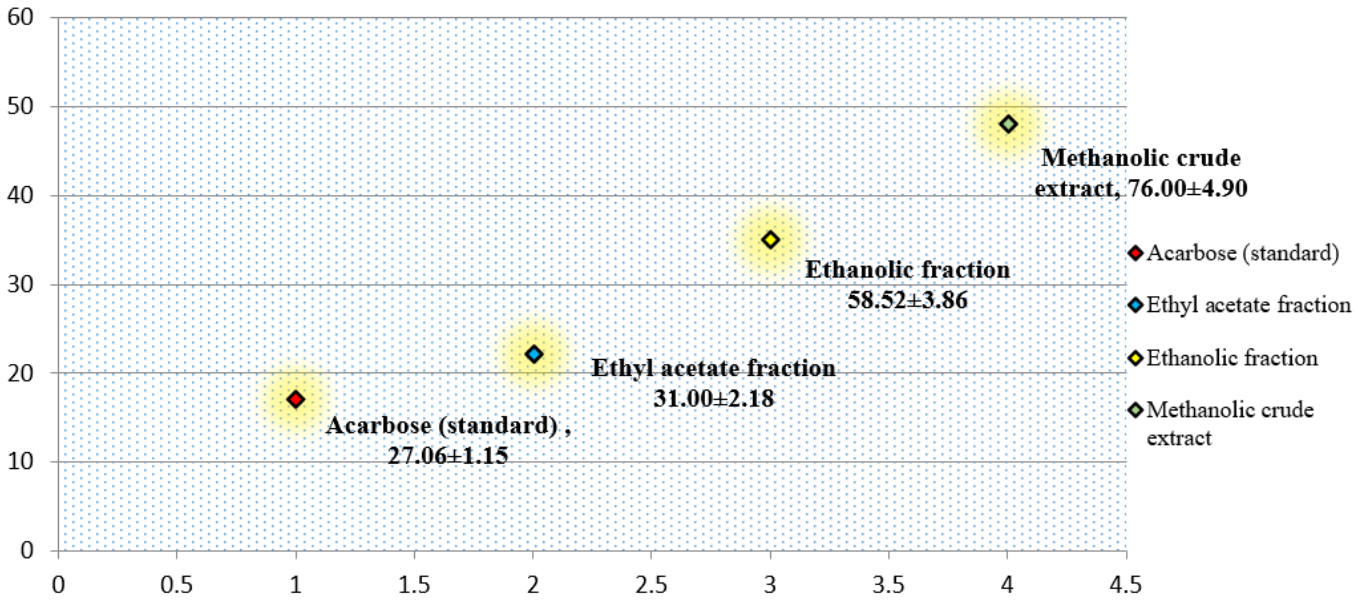

Figure 5. IC50 values of α-Glucosidase inhibition by *Origanum majorana* (Methanolic crude extract, Ethanolic fraction, Ethyl acetate fraction, and Acarbose as (standard)

Conclusion

The GC-MS analysis showed that the medicinal effects of *Origanum majorana* are attributable to the fact that the secondary metabolites occur in the plant. In some way, the use of alternative drugs with naturally occurring ingredients as a formulation of pharmaceutical businesses may be a sustainable approach. The present paper has determined that the *Origanum majorana* L. leaves contain such clinically significant chemicals that are of interest both pharmacologically and industrially as can be guessed based on the above discussion. The findings of this research will guide the future research on the safety and efficacy of these drugs in application in human beings particularly in the management of diabetes and inflammation. It is also considered that these substances, or their derivatives, can be demonstrated to offer an alternative to chemical-based therapies of various disorders.

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