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Original Research Article

Screening of Bioactive Natural Compounds of Ethanolic Leaves Extract *Origanum vulgare* Using GCMS Technique and Evaluation of Its [α-amylase and α- glucosidase] Inhibitory Activity

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Abstract: The purpose of metabolite profiling is to measure as many metabolites as possible from predefined structural groups which include organic acids as well as amino acids and carbohydrates. The volatile compounds function as excellent indicators to identify their original substances which enables assessment of product quality. Precursor levels in immature plant stages cause minimal aroma volatile synthesis which gets gradually controlled towards the optimal harvest moment. Many crucial primary and secondary metabolites isolated from Origanum vulgare leaves play essential roles in better human dietary nutrition. A powdered form of Origanum vulgare leaves originated from dried and processed leaves of each plantlet. The soxhlet device contained 100g of the powder while hot percolation proceeded using 400 ml of methanol as the solvent for about 10 hours. The process started by applying vacuum to transform the solvent mixture into a semi-solid paste before placing it in a desiccator for the removal of residual solvent quantities. The main phytochemicals identified were 1,7-Diepi-8,15-cedranediol, M.W.:238.37 g/mol, 1-methyl-4-(6-methylhepta-2,5-dien-2-yl)cyclohexene, M.W.: 204.35 g/mol, Methyl palmitate, M.W.: 270.5 g/mol, Neocembrene, M.W.: 272.5 g/mol, Phytolaccagenic acid, 516.7 g/mol, Terpilene, 136.23 g/mol, n-Heneicosane, M.W.: 296.6 g/mol, Farnesylacetone, 262.4 g/mol, 5-Methyl-2-isopropylphenol, 150.22 g/mol, Pentadecan-2-one, 226.40 g/mol, Dolcymene, 134.22 g/mol, Dipentene, 136.23 g/mol, (+)-Dihydrocarvone, 152.23 g/mol, (-)-trans-Caryophyllene, 204.35 g/mol, l-isoleucine, 131.17 g/mol, Linalyl isobutyrate, 224.34 g/mol, palmitic acid, 256.42 g/mol, and γ-muurolene, 204.35 g/mol. According to the type of extract (Methanolic rude extract, Hexane fraction, Ethanol fraction, Water fraction and acarbose (Standard) recorded (97.09 ± 0.71, 42.08 ± 0.40, 59.91 ± 0.32, 73.95 ± 0.51 and 18.19 ± 0.11) respectively inhibitory potency against α-amylase. While recorded (63.47±0.49, 51.13±0.35, 40.04±0.31, 32.06±0.25, and 17.02±0.16) respectively inhibitory potency against α- glucosidase activity. The inhibitory effects shown by methanol and ethanol fraction were significantly ($P<0.05$) more potent than that observed with acarbose in percent inhibition of α-glucosidase.

Keywords: Bioactive Natural Compounds, *Origanum vulgare*, GCMS Technique, α-amylase, α- glucosidase.

INTRODUCTION

The flavoring herb Origanum vulgare L. operates under the name oregano and exists for global culinary purposes within multiple dishes that include salads and pizza and sausages. Ethnopharmacological preparations have used Oregano (Origanum vulgare) as a medicinal herb since centuries for the treatment of ailments including convulsive coughs, digestive disorders and menstrual problems and bronchitis and asthma. This plant demonstrates properties as a carminative, diaphoretic, expectorant, stimulant, anti-oxidant, anti-inflammatory and anti-microbial agent with additional medical applications. Organic business activities value oregano as a natural source of active compounds because it serves as one of the world's leading spice plant exports and consumption rates [1-3]. The bushy herbaceous perennial plant Origanum vulgare originates from Europe and central Asia with its biological components comprising vitamins and tannins as well as resins sterols flavonoids and phenolic glucosides and essential oil. Utmost flavoring properties in

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oregano stem from its aromatic substances whose chief components exist in essential oil. Phytochemical investigations of Origanum vulgare essential oils and its related species identify large component diversity with notable differences in chemical makeup and concentrations between individual plants. A number of factors, including heredity, geographical distribution, plant component investigated, time of collection, extraction procedures, ambient conditions, etc., could be contributing to this heterogeneity [4, 5]. Previous research indicates that individuals with high levels of essential oil typically accumulate large amounts of phenolic monoterpenes, such as carvacrol, thymol, and their biosynthetic precursors, γ-terpinene and p-cymene. In contrast, plants with low levels of essential oil tend to have high levels of sesquiterpenes, such as germacrene D, (E)-β-caryophyllene, γ-muurolene, and caryophyllene oxide [6, 7], acyclic monoterpenoids, such as linalool and/or linalyl acetate, β-ocimene, or myrcene, and/or bicyclic monoterpenoids, primarily sabinene and cis-/trans-sabinene hydrate. Research questions and work focuses determine which of three plant metabolite analytical methods will be used: metabolic profiling or metabolite target analysis or metabolic fingerprinting [8]. Determining absolute metabolite concentrations is not required under metabolic profiling because the technique only measures relative levels of metabolites. Targeted metabolite analysis enables the determination of specific absolute concentration levels of pathway-relevant metabolites through specialized extraction practices paired with tailored separation and detection systems. Metabolic fingerprinting represents a third concept in metabolome analysis that does not focus on metabolite identification but detects all detectable metabolites for inter-sample comparison through single non-specific analysis of mixed metabolites. The analytical strategy determines the need for various instrumental platforms with different setups to reach data acquisition excellence. There is no single methodology that can measure the entire metabolome in a single step due to the diversity of structural classes of metabolites, which range from primary metabolites like organic acids, amino acids, and carbohydrates to very complex secondary metabolites like phenolics [9–11], alkaloids, and terpenoids. To identify every metabolite in a complicated mixture, several methods must be combined. Despite their extreme differences, two samples could have the same metabolite profile when employing the same technique. Consequently, the suite of variations in the metabolite profiles can only be uncovered by utilising a variety of instrument platforms and methodologies. To investigate extremely complex mixtures, a number of extraction techniques and instrument platforms have been developed; each must be selected based on specific interests. These include of gas chromatography (GC), liquid chromatography (LC)–mass spectrometry (LC–MS), nuclear magnetic resonance (NMR) [12,13], and Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR-MS or FT-MS) in combination with mass spectrometry (MS). One of the most advanced, reliable, and sensitive instrument platforms for metabolite analysis is the combination of GC with electron impact ionisation (EI) mass spectrometry (MS), which may be the oldest hybrid technique in analytical chemistry. GC-MS is frequently referred to be the "gold standard" in metabolomics because it provides strong quantification techniques [14–17], excellent chromatographic separation power, and the capacity to identify metabolites with high fidelity. Since GC-MS based techniques were among the first to be used for target analysis and metabolite profiling, they provided defined procedures for machine setup, data mining, and interpretation. It provides the most cost-effective procurement, operating, and maintenance costs as compared to other instrument platforms. The usage of GC-MS as a platform for metabolomics is further supported by EI spectrum libraries that are both publically and commercially available. Several traditional remedies have been advocated as potential additions to the current therapeutic alternatives for the treatment of diabetes. Worldwide, people with diabetes manage a wide range of symptoms with traditional plant medicines. Healthcare providers and the general public alike are looking into natural medicinal items as potential alternatives to synthetic pharmaceuticals because of the widespread agreement that plant remedies are safer and more cost-effective than contemporary pharmaceuticals [18, 19]. Consequently, research on compounds originating from traditional therapeutic herbs has become increasingly important [20]. The objectives of this study were to examine the inhibitory activity of [α-amylase and α- glucosidase] and to screen for bioactive natural components in an ethanolic extract of the leaves of Origanum vulgare.

MATERIALS AND METHODS

Raw Material and Chemicals

The extraction process using hydro-distillation lasted for 6 hours through a Clevenger-type apparatus on 500 grams of fresh, unharmed Origanum vulgare Linn. leaves obtained from Herbarium of medicinal plants in Diwaniyah Governorate. Gas chromatography with retention index (R.I.) analysis served to identify the single peaks through matching with authentic laboratory standards or published findings having similar R.I. The obtained mass spectra from GC-MS analysis led to identification through comparisons that integrated NBS 54 K.L as well as WILEY8 spectrometry database and published literature.

Homogenization and Extraction

The plant tissue needs homogenization to obtain a fine powder as a prerequisite before metabolites extraction because the solvent requires access to tissue cells through this process for effective extraction. The extraction process of plant metabolites needs optimization to minimize losses that happen because metabolites get chemically degraded or converted enzymatically. Null derivatized solutions composed of extraction solution alone should run together with all tested samples to discover chemical contaminants that need to be eliminated from subsequent analysis. The reference

sample contains all metabolites from real samples thus it allows normalization of metabolite levels in real samples. PCA analysis enables an inference of data set quality through cluster patterns of pooled quality control samples.

Gas Chromatography-Mass Spectrometry Analysis

The HP5873 Mass Spectrometer and HP6890 Gas-Chromatograph were used to conduct this experiment. The column underwent a heating program that progressed from 50 °C to 300 °C at a rate of 6 °C/min, with a final hold time of 5 min. We used an ionisation energy of 70 eV and a temperature of 150 °C for the MS Quad. Solvent delay began scanning molecules in the 50–550 amu range after 3 minutes. By comparing the mass spectra of the compounds detected with the ones in the NIST2.0 library, which is software developed by the National Institute of Science and Technology in the United States, we were able to identify the compounds in the oil sample [21, 22]. By dividing the overall area by each compound's area and then multiplying by 100, we were able to get their percentage area. In addition, the retention indices (RIs) were determined by utilising the linear equation derived from a standard alkane C8-C20 calibration curve. These RIs were subsequently compared to values reported in the literature to validate the components' identities.

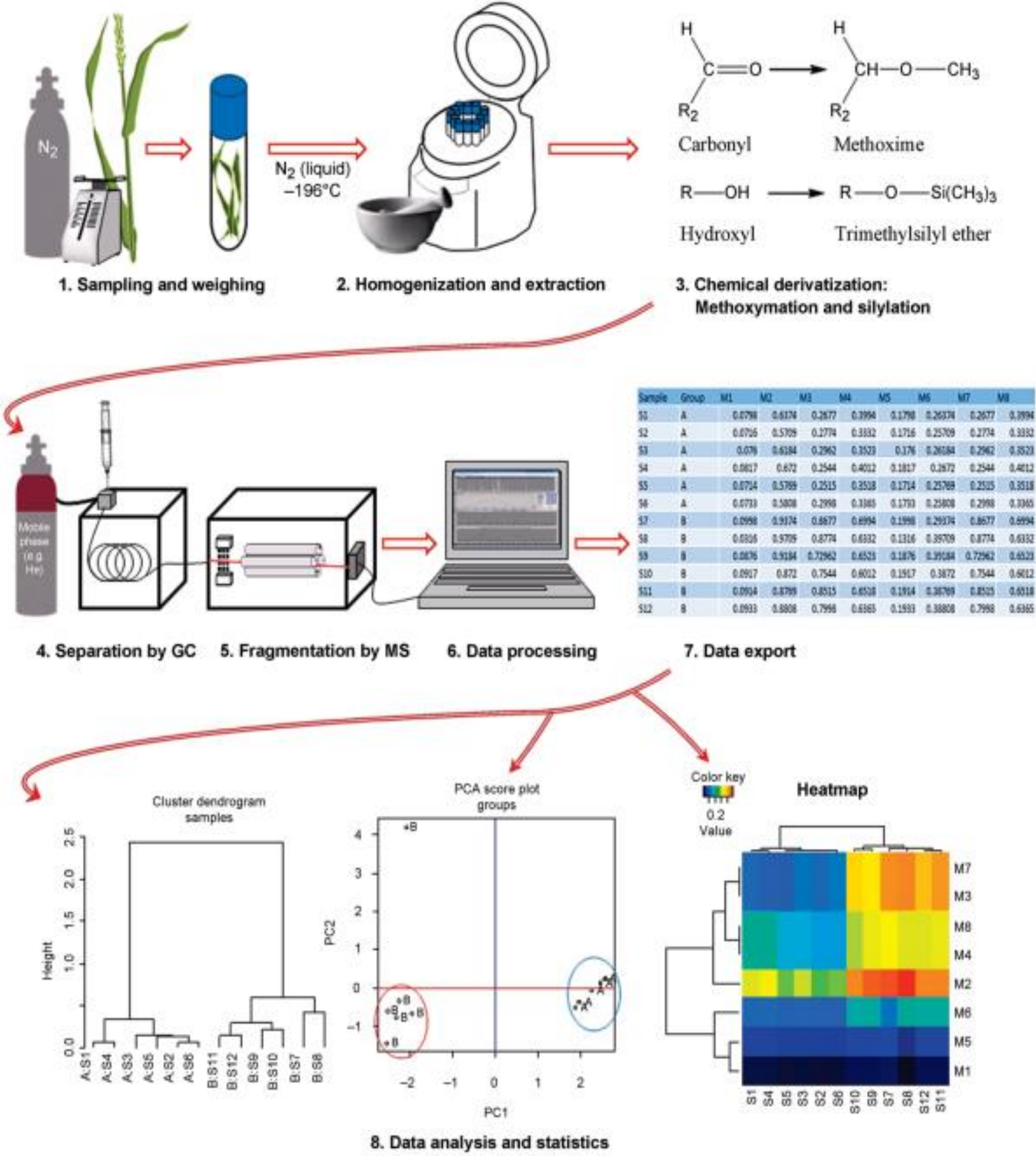

Figure 1: A GC-MS experiment's workflow, outlining its overall approach and experimental steps

Preparing and Exporting Data

Software packages for effective in silico data preprocessing include the commercial software packages AnalyzerPro (SpectralWorks) and Masshunter (Agilent) together with Xcalibur (ThermoFisher Scientific) and AMDIS (National Institute of Standards and Technology, Gaithersburg, MD, USA (NIST)) which is freely available. By analyzing the peak areas of component peaks the software measures relative amounts which typically use m/z values from internal standards for standardization. Before statistical analysis becomes possible data normalization deals with the experimental errors in the sample preparation process alongside changes in analytical instrument sensitivity. The correct peak identification through retention time index (RI) analysis employs either alkanes or fatty acid methyl esters to determine compound matching based on standard comparison. Each compound identification process works through comparing its mass spectrum and retention index properties against a database to avoid inaccurate peak assignments that happen when retention times fluctuate in the analytical run period [23-25]. Modern software packages include built-in capabilities for automated RI evaluation of all compounds while performing automated mass spectral deconvolution.

α- amylase inhibitory assay

Several minor changes were added to the standard procedure for measuring α-amylase inhibitory effect of the extract and fraction solutions. A mixture of twenty millilitres α-amylase at two international units per millilitre was used to combine with two hundred millilitres solution containing extract or fractions at concentrations of 0.5 milligrammes per millilitre and 500 millilograms of 6.8 phosphate buffer reaching one hundred millimolar phosphate concentration. The mixed solution received aliquot distribution into 96-well plate after 20-minute incubation at 37 °C. Most conditions for preincubation were maintained under 37°C to support the incubation process. Next the mixture was relocated to a 37 degrees Celsius incubator where it remained for thirty minutes before receiving thirty milliliters of 1% soluble starch solution prepared in 100 mM phosphate buffer with pH 6.8. A 10-minute boiling period at constant pressure occurred when 100 litres of DNS colour reagent were combined with the solution. The Multiplate Reader (Multiska Thermo Scientific version 1.00.40) read absorbance at 540 nanometres wavelength. The absorbance measurement technique served to determine the concentration level of the final solution mixture. Experimental researchers utilized standard acarbose concentrations between 0.1 and 0.5 mg/ml as their control solutions. The synthesis of an unprocessed material proceeded simultaneously with the experimental compounds (extracts and fractions). The experiments were each conducted three times. Probability of inhibition appeared as the result which used the calculated formula. The measurement of IC50 relied on drawing the inhibition data to determine the enzyme activity changes at different fraction concentrations.

The percentage of inhibition could be determined by applying the following formula:

$$\% \text{ Inhibition} = (Abs_{control} - Abs_{extract}) / Abs_{control} \times 100$$

α- Glucosidase Inhibitory Assay

Based on the results of the analysis, we know that the extract and fractions block α-glucosidase. Otherwise, the analysis was conducted following the standard technique, with a few little adjustments. Prior to preparation, the serum samples were pre-cooled in a 96-well plate at 37 degrees Celsius for 15 minutes. In addition to the fifty litres of 6.8 phosphate buffer solution, there were twenty litres of the nine separate extracts and first nine fractions at a concentration of 0.500 mg/mL, ten litres of the purified alpha-glucosidase at a concentration of one unit/mL, and fifty litres of the mixture. A temperature of 37 degrees Celsius was used for pre-incubation. A further twenty minutes were spent incubating the mixture at 37 degrees Celsius. Following the addition of twenty litres of P-NPG as a substrate, which had a five millimolar concentration. A 50-liter solution of sodium carbonate, with a concentration of 0.1 M, was added to halt this process. Using a multiplate reader and a wavelength measurement of 405 nm, this experiment actually calculated the absorption of the newly released nitrophenol. The sample under review contained 0.5 mg/mL of acarbose, which was utilised as a standard measurement. In order to compare the read results, these three tests were performed about three times. In addition, a separate experiment was run at the same time but without the chemical being studied. To make sure the results are as accurate as possible, the studies were repeated three times. The inhibitory activity of α-glucosidase was measured in terms of the percentage of inhibition using the following expression:

$$\% \text{ Inhibition} = (Abs_{control} - Abs_{extract}) / Abs_{control} \times 100$$

A control represents the control's absorbance, while A extract represents the fractions. The amounts of fractions needed to inhibit enzyme activity to 50% were represented by the IC50 values that were computed from the visual representations.

Statistical analysis

Statistical testing was conducted through GraphPad Prism 5 Statistical Package based in USA. The subsequent research steps relied on one-way analysis of variance (ANOVA) together with the Bonferroni test for data interpretation. The in vitro IC50 values were presented as mean ± standard error mean in triplicate determination.

RESULTS AND DISCUSION

The research community has documented many applications which demonstrate how GC–MS-based metabolomics assists in studying metabolites together with their regulatory pathways that change following genetic or environmental disturbances. Metabolomics has already contributed to several detailed research areas as documented in studies [26, 27]. The biochemical information collected through GC-MS allows scientists to monitor organism classification through gene and environment stimuli and reveal the similarities and differences between experimental conditions. The biochemical responses produced by people with shared genomes will diverge when their environments alter. Research using metabolomics successfully distinguishes genetically comparable organisms which grow in various locations and under different environmental conditions [28-31]. Metabolomics provides valuable chemical plant assessments needed for genetic modification risk assessments. Genetically modified foods and crops go through safety evaluations that use a substantial equivalence framework where existing crops serve as the baseline reference standard and they establish comparisons between baseline characteristics and those of new food items. 1,7-Diepi-8,15-cedranediol, M.W.:238.37 g/mol, 1-methyl-4-(6-methylhepta-2,5-dien-2-yl)cyclohexene, M.W.: 204.35 g/mol, Methyl palmitate, M.W.: 270.5 g/mol, Neocembrene, M.W.: 272.5 g/mol, Phytolaccagenic acid, 516.7 g/mol, Terpilene, 136.23 g/mol, n-Heneicosane, M.W.: 296.6 g/mol, Farnesylacetone, 262.4 g/mol, 5-Methyl-2-isopropylphenol, 150.22 g/mol, Pentadecan-2-one, 226.40 g/mol, Dolcymene, 134.22 g/mol, Dipentene, 136.23 g/mol, (+)-Dihydrocarvone, 152.23 g/mol, (-)-trans-Caryophyllene, 204.35 g/mol, l-isoleucine, 131.17 g/mol, Linalyl isobutyrate, 224.34 g/mol, palmitic acid, 256.42 g/mol, and γ-muurolene, 204.35 g/mol. Plant metabolite profiling under variable conditions relies strongly on GC-MS-based metabolomics due to its extensive benefits over alternative analytical systems including operational stability and strong separation strength and measuring accuracy [32-35]. The future development of GC–MS technology for plant metabolomics needs our acceptance of innovative advancements which can advance its present performance. Several significant advancements can result from new GC–MS developments such as increasing both detectable and identifiable compounds along with raising analytical speed levels. Multidimensional or GC–MS systems show successful exploitation in various stages of metabolic research applications. The analysis of volatiles through this technology operates successfully in wine inspection and the oil compositional analysis of various origins and the fragrance sector. GC-MS technology faces two significant hurdles for better derived data because it detects only approximately 400 metabolites which restricts interpretation capability within biological and biochemical contexts. The analysis of a biological system requires testing multiple metabolites to gain complete understanding [36-39]. GC–MS serves as an additional analytical technology that works alongside LC–MS systems and capillary electrophoresis (CE)–MS devices and NMR spectroscopy equipment. A holistic review of the system requires analyzing metabolomics data together with genomic sequences and transcriptomic and proteomic expression levels and metabolic fluxes and anatomic parameters as well as measuring physical manifestations after introducing environmental or genetic factors. Diabetes mellitus demonstrates high blood glucose levels as its main indicator and represents one of the major death-causing conditions worldwide [40]. Alpha-glucosidase (α-glucosidase) represents the essential enzyme for sugar absorption together with hydroxylating and digesting functions among humans. The inhibition of α-glucosidase provides a beneficial method to control type 2 diabetes disease. The trend favors treating diabetes using natural resources. Multiple bioactive compounds and nutraceutical substances serve to regulate and control diabetes symptoms according to scientific research. Phenolic metabolites provide an effective treatment method to reduce pre- or post-diabetic disease occurrences. A complete knowledge of polyphenol significance for food and human health demands detailed identification as well as characteristic assessment of phenolic metabolites. The strong antimicrobial and antioxidant properties of food herbs and spices now attract both industry research organizations. From historic times onward people have kept utilizing herbs and spices as food elements in continuous practice. Medical research found that herbs and spices preserve food quality because they increase flavor while having sensory and organoleptic effects. The health-promoting effects of phenolic compounds in plant-based products currently generate substantial research interest according to studies [44, 45]. Multiple scholars have focused their studies on antioxidant properties and phenolic compositions of common herbs and spices because various research show culinary herbs serve as natural food sources of antioxidant phenolic compounds. Several possible health benefits reported for phenolic compounds have made them an important research target for desirable phytochemicals vertebrate human health [46-49]. Historical records show that culinary spices together with herbs have served various medical functions which include treating joint inflammation along with aches and sprains and fractures. These products find applications in cosmetic manufacturing as well as pharmaceutical industries and culinary production and animal feed production. Herbal and spice utilization across the world has resulted in increased manufacturing numbers.

1,7-Diepi-8,15-cedranediol, M.W.:238.37 g/mol	**1-methyl-4-(6-methylhepta-2,5-dien-2-yl) cyclohexene, M.W.: 204.35 g/mol**	**Methyl palmitate, M.W.: 270.5 g/mol**	**Neocembrene, M.W.: 272.5 g/mol**
Phytolaccagenic acid, 516.7 g/mol	**Terpilene, 136.23 g/mol**	**n-Heneicosane, M.W.: 296.6 g/mol**	**Farnesylacetone, 262.4 g/mol**
5-Methyl-2-isopropylphenol, 150.22 g/mol	**Pentadecan-2-one, 226.40 g/mol**	**Dolcymene, 134.22 g/mol**	**Dipentene, 136.23 g/mol**
(+)-Dihydrocarvone, 152.23 g/mol	**(-)-trans-Caryophyllene, 204.35 g/mol**	**l-isoleucine, 131.17 g/mol**	**Linalyl isobutyrate, 224.34 g/mol**
palmitic acid, 256.42 g/mol	**γ-muurolene, 204.35 g/mol**		

The inhibitory effectiveness of fractions of Origanum vulgare against the activities of α-amylase and α-glucosidase: The anti-α-amylase and anti-α-glucosidase activities of the Origanum vulgare are demonstrated by the figures in Figures 2 and 3, respectively. Both the dosage and the percentage of Origanum vulgare were found to have an effect on the inhibitory activities in the enzymatic inhibitor assay. At the highest dose being studied, the inhibitory effect was most pronounced, whereas at the lowest dose, it was least so. The inhibitory potency against α-amylase was found to be (97.09±0.71, 42.08±0.40, 59.91±0.32, 73.95±0.51 and 18.19±0.11), respectively, depending on the type of extract (Crude extract, Hexane fraction, Ethanol fraction, Water fraction, and acarbose (Standard). The inhibitory potency against α-glucosidase activity was (63.47)±0.49, (51.13)±0.35, (40.04)±0.31, (32.06)±0.25, and (17.02±0.16) correspondingly. In terms of % inhibition of α-glucosidase, the inhibitory effects demonstrated by methanol and the ethanol fraction were noticeably stronger than those seen with acarbose (P<0.05).

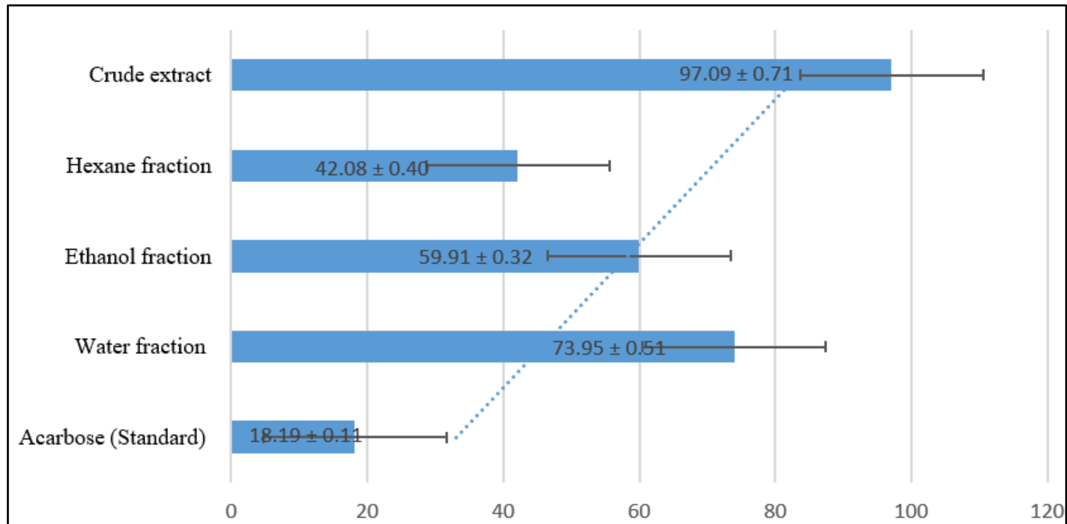

Figure 2. Inhibitory potency of *Origanum vulgare* Crude, Ethanol fraction, Hexane fraction, Water fraction and acarbose as standard against α-amylase activity

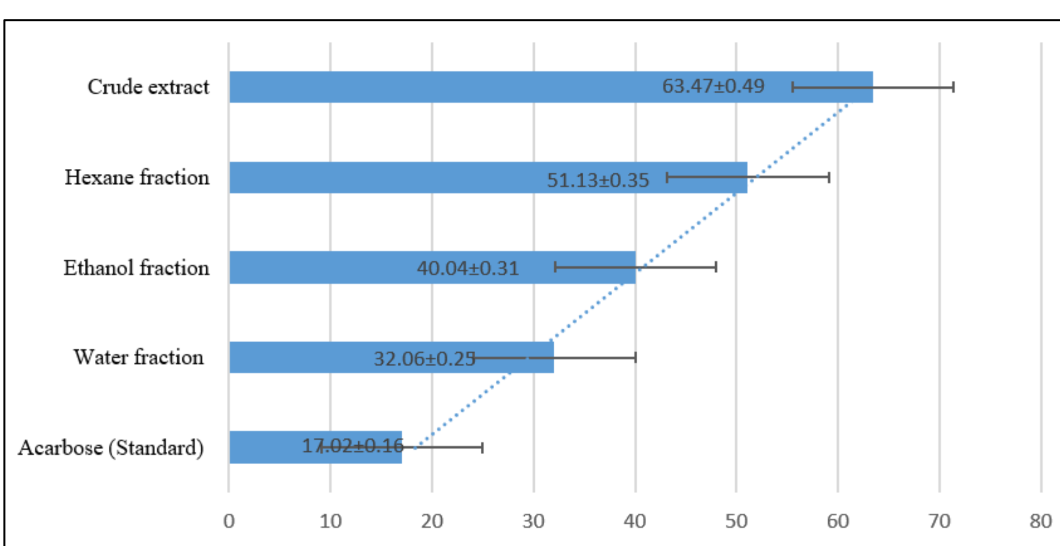

Figure 3. Inhibitory potency of *Origanum vulgare* Crude, Ethanol fraction, Hexane fraction, Water fraction and acarbose as standard against α-glucosidase activity

Blood glucose levels remain elevated continuously in individuals who have metabolic disorders which comprise diabetes mellitus. The condition stems from insulin production flaws as well as functional insulin abnormalities or combined defects with insulin production. Metabolic abnormalities of carbohydrates, lipids and proteins emerge due to a combination of insulin shortage and poor tissue response. Several health complications of short duration and longer duration are directly connected to diabetic conditions. Diabetic ketoacidosis together with malignant hyperthermia-like syndrome with rhabdomyolysis and hyperosmolar hyperglycemia poses quick risks of severe damage or death to patients.

Long-term health consequences from type 2 diabetes normally result in high blood pressure, lipid disorders, retinopathy, kidney diseases along with non-alcoholic fatty liver disease and cardiovascular and atherosclerotic disorders as well as neurological pathologies [50, 51]. Polyuria, increased thirst, hyperphagia, unexplained weight loss, and blurred vision are the most common symptoms of hyperglycemia. Amylose is a linear polysaccharide made of α-(1→4) glycosidic links, while amylopectin is a branching polysaccharide composed of α-(1→6) glycosidic bonds. Starch is the most abundant carbohydrate in meals. One of the most important digestive enzymes is pancreatic α-amylase (EC 3.2.1.1). The majority of starch digestion in humans is carried out by this calcium-based metalloenzyme, which functions as a catalyst and aids in the hydrolysis of the α-1,4 glycosidic linkages of polysaccharide molecules such amylose, amylopectin, glycogen, and other maltodextrins. The last step of digesting carbohydrates is catalysed by another digestive enzyme, α-glucosidase or maltase (EC 3.2.1.20), which acts on 1,4-alpha bonds to produce glucose. Starch is the primary fuel source for humans in their diet. The enzymes α-glucosidase and α-amylase convert dietary sugars and starch into glucose. Saliva and pancreatic juice include α-amylase metalloenzymes, which belong to the glycoside hydrolase family 13 (GH13). A metal cation serves as a co-factor in the active site of a wide variety of enzymes known as methanoenzymes. The hydrolytic activity are just one of several processes that these enzymes promote. Chloride ions stimulate α-amylase, a metalloenzyme that relies on a crucial calcium ion for structural stability [52, 53]. Structural studies have revealed that members of this family do in fact possess similar three-dimensional structures, and while the overall homology of their amino acid sequences is modest, they do contain brief patches of highly conserved residues. The different isozymes are regulated to be expressed exclusively in the pancreas or salivary glands; they are members of a multigene family situated on chromosome one.

CONCLUSION

The present research identified major compounds within the plant species Origanum vulgare. Phytolaccagenic acid, Terpilene, n-Heneicosane, Farnesylacetone, 5-Methyl-2-isopropylphenol, Pentadecan-2-one, Dolcymene. The metabolic condition known as diabetes exists as a persistent medical illness that affects blood sugar because insulin production decreases or because insulin resistance builds up. A method to treat early-stage diabetes involves reducing the blood glucose levels which rise after eating food. The bioactivity of gut-glucose inhibiting carbohydrate-digestive enzymes known as α-amylases can be successfully controlled to improve glucose absorption rates. The enzymes' inhibitors regulate glucose absorption rates thereby reducing post-meal plasma glucose amounts. New evidence based on traditional herbal medicine shows that plant extract components questionably provide substantial therapeutic benefits for Type 2 Diabetes management and associated problems. Numerous scientists have extensively studied plant extract and natural compound inhibitory properties against the α-amylase enzyme but several knowledge gaps remain. Most research studies the plant extract inhibitory effects under laboratory conditions while also identifying bioactive compounds. More scientific analysis combined with research is required to understand natural extracts and compounds pharmacological activities together with active ingredient synergistic behavior and applying them safely. Prospective research will provide essential information about suitable doses of active natural compounds and extracts which provide therapeutic benefits while minimizing side effects.

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