

Original Research Article

Analysis Bioactive Compounds of *Apium graveolens* and *Lepidium sativum* using GC/MS Technique and Exploration of Its Antioxidant (Nitric oxide, Peroxynitrite, and Hydroxyl Radical Scavenging) Activity

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Abstract: In the process of identifying and quantifying its volatile organic compounds (VOCs), the GC-MS is commonly used in the study of the *Apium graveolens* otherwise known as celery. *Lepidium sativum* commonly referred to as garden cress is a herb that has drawn the attention of many GC-MS studies in order to establish the chemical make up of it. Gas chromatography-mass spectrometry (GC-MS) is among the efficient techniques and it entails separation and identification of substances based on the boiling points and mass spectrums. This enlightens the chemical compounds of celery that might have been the source of any possible therapeutic or aromatic use. The GC/MS test established *Lepidium sativum* and *Apium graveolens*. It was identified by the mass spectrometry and comparing the sample spectral pattern with the samples in the library of Chem. station. This research was carried out to determine the molecular formula and structure of phytochemicals of plants *A. graveolens* and *L. sativum*. As a result, the GC-MS analysis is used as the beginning of the analysis of the active principles in *A. graveolens* and *L. sativum*. The results of this research study have been utilized in manufacturing of most of the present-day drugs. Ascorbic acid, beta- Ocimene, Neophytadiene, and 2-hydroxy-3 -(2-methylphenoxy) propanoic acid, 2-amino-2-deoxy-D-mannitol, 9-Octadecenamide, Furancarboxaldehyde, Cyclopentene-1, 3 -dione, are among bioactive substances that are present in the two plants. The presence of phytochemicals of *A. graveolens* and *L. sativum* may be explained by the disease control. *Apium graveolens* leaves extract (Methanol, Ethanol fraction and standards) and its antioxidant activity against Nitric oxide, peroxynitrite, hydroxyl radicals and Hydroxyl radical scavenging. The extracts were recorded as different types and were (695.07±30.05), (644.71±28.04) and Gallic acid (standard) (851.15±35.99) respectively in the *Apium graveolens* and *Lepidium sativum* against the Peroxynitrite scavenging respectively. Compared to the Hydroxyl radical scavenging potential, recorded (295.06±23.09), (223.00±16.05) and Mannitol (standard) (527.91±30.08) respectively in *Apium graveolens* and *Lepidium sativum* respectively. At the same time note (35.09±2.34), (19.00±2.07), and Curcumin (standard) (66.54±4.69) respectively in *Apium graveolens* and (57.00±2.09), (33.91±2.08) and Curcumin (standard) (70.00±4.08) respectively in the Nitric oxide radical scavenging potential.

Keywords: Bioactive Compounds, *Apium Graveolens*, *Lepidium Sativum*, GC/MS Technique, Nitric Oxide, Peroxynitrite, Hydroxyl Radical Scavenging.

INTRODUCTION

Herbs usage is not a new phenomenon at all, as it has been used since culinary seasoning up to the point of a medicine and even preservation of a food. The right diet is that diet that can greatly reduce the risk of being infected with certain ailments. The celery, *Apium graveolens* had a variety of different nutrients including carbs, flavonoids, steroids, alkaloids, glycosides, phenols, furocoumarins, volatile oils, sesquiterpene alcohols, fatty acids, and low concentration of most chemicals. The previous researches carried out into the pharmacological sector have revealed that the *Apium graveolens* possessed vast spectrum of pharmacological effects that encompassed the gastro intestinal tract, the

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cardiovascular, cytotoxicity, microbes, helminths, cholesterol, inflammation and the central nervous system [1-3]. A chemical equation has compounds in it. The initial phytochemical analysis of the *Apium graveolens* methanol extract revealed that the seed had glycosides, alkaloids, flavonoids, carbohydrates and steroids. Celery can prevent heart disease, jaundice, liver disease, rheumatic disease, gout and obstruction of the urethra. The paper has established that Celery leaf ethanol extracts enhance fertility and sperm production in rats. One of the ways the celery can assist the heart is by reducing blood sugar, cholesterol and high blood pressure. Experiments claim that celery contains anti-inflammatory and antifungal properties [4, 5]. Its grains will aid in the treatment of bronchitis, asthma, skin chronic ailments (such as psoriasis), vomiting, fever, and tumors. The phenolic compounds (e.g., thymol, eugenol, carvacrol) and other components (e.g., linalool, myrcene, sabinene, menthol and camphene) are found in greater quantity in plant extracts and are associated with the antimicrobial properties. Extending the oxidative stress of lipids, preserving well-being and preventing coronary heart ailment and malignancy are all ways in which phenolic chemicals can enhance nutritional strength and quality of food. The trend in favor of functional foods with defined health-related outcomes has opened the question of the food manufacturers that have traditionally valued the significance of the antioxidant chemicals. In addition to terpenoids, phthalides, furocoumarins, phenolic acids and flavonoids, celery is also very rich source of bioactive chemicals.

It has a strong and rich aroma and taste due to so many different factors, including the time of the year, location and the type of celery [6-8]. It is possible to see the smell of many kinds of celery differently. The centre of interest is the aromatic molecules as far as the comparison of the quality of the flavors of the processed and fresh produce is concerned. Another issue that affects the sensory of the vegetable to a large extent is celery due to the differences in concentration of volatile organic makeup (VOC) which changes as the plants develop. In order to increase the autologous competitive power, plants synthesized volatile organic compounds (VOCs) via the secondary metabolic pathways. They are low boiling compounds that are small molecule compounds which attract pollinating insects and seed dispersers and deter parasites, pathogens and herbivores. Volatile organic compounds can be synthesizing by the whole plant; roots, stems and leaves to flowers and fruit to seeds. The volatility organic compounds (VOCs) can be classified in terms of the ways they are formed as esters, phenols, alcohols, amino acid and terpenes [9, 10]. The most terpene is D-limonene which is the majority of volatile organic compounds (VOCs) in celery extracts with HS-SPME-GC-MS and the aroma of the celery seeds is a derivative of phthalide. Celery is subject to factors that influence it to affect its fragrance properties. An example of one such scenario was the unstable contents and sensory characteristics of celery which differed significantly among years of harvest. Celery with high temperatures contained large amounts of sesquiterpenes. Machine olfaction, i.e. the recognition of grape cultivar, an electronic-nose (e-nose) technology and VOC emissions of plant organs, makes it possible to detect the agricultural products of fruits and vegetables with significant speed. In order to enrich the richness and quantify the concentration of plant volatile organic compounds (VOC) the traditional techniques use solvent extraction and steam distillation but the newer techniques use the methods of extraction like head-space-solid phase micro extraction (HS-SPME), enzyme-aided extraction, and the micro-wave-assisted extraction [11-13]. The HS-SPME approach is also simple to manage and does not entail the use of a lot of equipment. It is also quick, solvent is not required and is easy as opposed to solvent extraction. Sampling, enrichment and injection is also a part of it. It is a quick and environmentally conscious method of obtaining organic volatility and semi-volatility compounds without use of organic reagents and is highly efficient in reducing the odor of the same. The HSSPME and GC-MS were used to extract and analyze the volatiles in the eureka lemon pulp and the Xiangshui lemon pulp since the former had more alcohols, aldehydes and esters. *Lepidium sativum* is also commonly used in the traditional Iraqi medicine to cure a lot of diseases by the doctors. It can be useful in cases of lumbago or other symptoms of the loin which are caused by rheumatism [14, 15].

Garden cress shares an aroma and taste akin to those of mustard and watercress that share a comparable peppery, acid aroma (genetically related). Common names containing the word cress are used to describe garden cress, depending on the region of the world: pepper cress, pepperwort, garden pepper cress, mustard and cress, peppergrass and poor man pepper. The upper portion of such annual plant can give several branches and can extend to a height of 60 cm (about 24 inches) high. Its application is possible in asthma, cough, dysentery, diarrhea, skin diseases and blood disorders. In the example of the injuries, skin troubles and eye illnesses, eat some fresh fruit. Hot and dry leaves in this plant have aphrodisiac and diuretic properties, anti-inflammatory properties, spleen-disease properties, chest pain, bronchitis, rheumatism, and muscle pain. Root is applied in instances of secondary syphilis and tenesmus. Some of the conditions that are also treated with the assistance of this plant include asthma, cough and bleeding piles [16, 17]. The weakly stimulant and diuretic leaves are also administered in the management of scorbutic diseases in addition to liver problems. The plant seeds possess aphrodisiac, diuretic, laxative, tonic, galactagogue and emmenagogue effects. They are used topical in case of trauma and strains. Our study was aimed at analyzing the antioxidant property and bioactive contents of *Apium graveolens* and *Lepidium sativum*.

MATERIALS AND METHOD

Apium graveolens *Lepidium Sativum* Preparation of Extracts

The leaves were bought at a vegetable shop in Iraq in Babylon where they were utilized in making the celery leaves extract. The leaves were dried in the shade and ground to powder and stored in the fridge (4C C) till extraction.

After 24 hours of powdered celery, the contents of 50 g powdered celery leaves were combined with the Soxhlet device, and 200 mL of distilled water were combined. The second step was to filter the mixture using Whatman No. 1 filter paper followed by the use of rotary evaporator to concentrate the filtrate. Finally, the concentrated extract was kept at 4°C until it was needed. It was diluted using distilled water and a required amount of the extract was added. A total of (290) mL of 80 per cent methanol (50 g) of dried powdered leaves of celery was extracted by adding overnight. The liquid was then filtered with the aid of a vacuum pump. Methanol extraction of the residue was repeated three or four times until the residue was emptied. Drying in a vacuum was by rotary evaporation at 50°C on methanol. The lyophilized extract that is free of methanol will be stored in the dark until it is needed. *Lepidium sativum* seeds were ready in the form of fine powder since they were thoroughly washed with tap water and the distilled water and dried under the shade and subsequently crushed into fine powder. The dried fine powder resulting, after a gram of it had been heated up in one hundred milliliters of distilled water, allowed to infuse, after which, thirty minutes, then cooled down, and filtered.

Identification and Isolation of Vital Compounds

The following conditions were employed in the identification of the *Apium graveolens* and *Lepidium sativum* under the GC/MS Technique (Hp. 5890A): The HP7673 was in combination with packed capillary column (50m x 0.2mm x 0.3 thickness film carbowax 20M) and was combined with helium as carrier gas with the rate set at 20 cm/sec. The split ratio was 100: 1 where the diluted samples were injected by an automatic injector (2.0 100:1) at 150°C in ethyl alcohol (1:10V/V). Increase in temperature of the oven was set at a slow pace of 200°C after 60 minutes with a starting point of 60°C and an Increment of 2.8°C/min. In order to identify what was in the sample, we matched the spectra with the library of Chem. Station which is the place where all the data of over 43,000 different chemicals are stored by using the mass spectrometry.

Statistical Analysis

The SIEVE software (Thermo Fisher Scientific, USA) was used in the processing and extraction of the detection data. Upon conversion, the data were then converted and edited using the Microsoft excel 2010 in order to avail a two dimensional data list that contained the parameters of retention time, molecular weight of metabolites and the peak intensity. The relative content of the metabolites was then obtained by the ratios of the areas of the metabolites to the internal standard. The imported data was then taken through the multivariate statistic analysis using SIMACA-p software 14.1 (MKS Umetrics AB, Umeå, Sweden). After the weight normalization and unit variance (Uv) standardization, the partial least squares regression (PLS) was utilized to determine the contribution made by x -variables (36 components) to the y -variables (antioxidant capacity) by the combination of all data of the four celery petioles and leaves. The one-way analysis of variance (ANOVA) was for the differences in the levels of major antioxidants, and the antioxidant capacities of each group with the aim of recognising the significant difference ($P < 0.05$). All the numbers are provided as means + SD. Statistical analysis was done using SPSS 24.0 software (SPSS Inc., Chicago, IL).

RESULTS AND DISCUSSION

They can be also used in medication studies, quality assurance and traditional use of plants since the GC-MS analysis offers the complete chemical profile of the plant which identifies and quantitatively measures the bioactive compounds present in the plant. The technique enables one to form a precise chemical fingerprint because of the separation and identification of compounds which is very sensitive to detect even small proportions, and also very selective to differentiate compounds because of the spectrum libraries. The GC-MS identify the following phytochemicals in *Apium graveolens*; n-hexadecanoic, Caryophyllene, beta-Ocimene, Neophytadiene, Ascorbic acid, 2-hydroxy-3-(2-methylphenoxy) propanoic acid, 3-(2-methylpiperidin-1-yl)propyl 4-cyclohexyloxybenzoate, Oleic acid, (-)-beta-Pinene, +trans-Limonene oxide, Part of the analysis advantages of a plant with GC-MS include Flashed volatile fragrance compounds to non-volatile metabolites following derivatization, GC-MS can consistently name and quantify numerous drugs. Chemical fingerprinting technique is also beneficial in identifying and determining quality of plants particularly medicinal plants because plants generate exclusive and exhaustive chemical profiles. New products and drugs: GC-MS can be applied to develop new drugs, nutraceuticals and cosmetics since it identifies the definite bioactive chemicals. It could also be useful in determining the efficacy of the active constituent of the traditional medicines. In the management of quality, GC-MS identifies the quantities, and concentration of the required chemicals in the plant based products to ensure that the products are standard and in good quality. The analysis will assist the researchers to know the effect of any change in the environment of the plants or pathogenesis through the identification of new molecules or data to be incorporated into the examination of the metabolomics [18]. High quality selectivity and sensitivity: The assay is applicable with complex samples of plants because the assay is able to detect chemicals in low concentrations and differentiate them. It is feasible because it has great spectral libraries since the mass spectrometry of the unknown can be compared with already known one in the database to identify a specific chemical molecule very quickly and precisely [19].

Table 1: GC-MS analysis of phytochemical profile of *Apium graveolens*

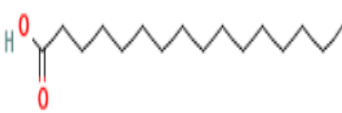
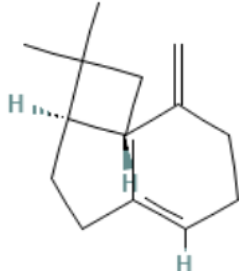
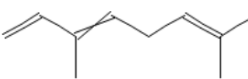
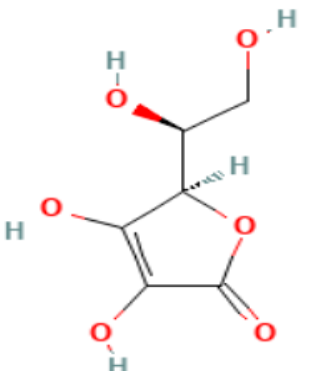
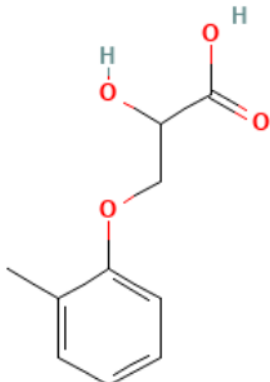
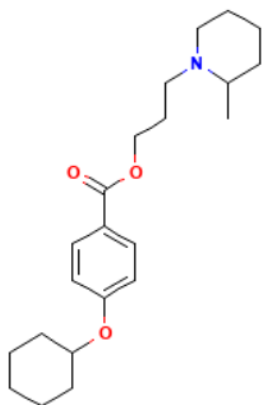
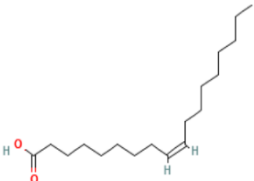
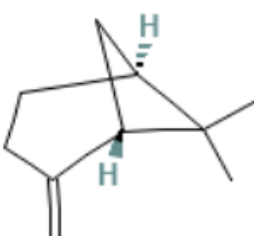
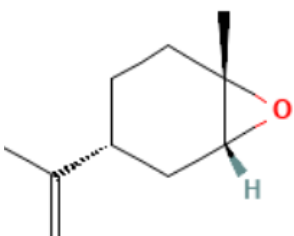
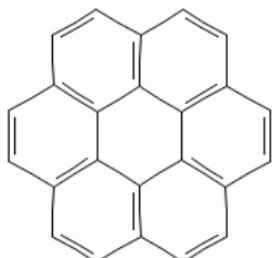
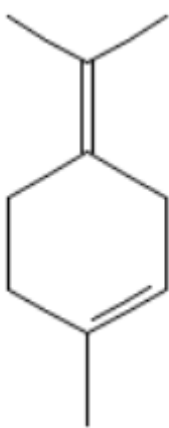
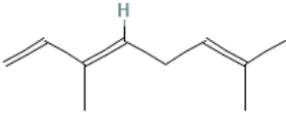
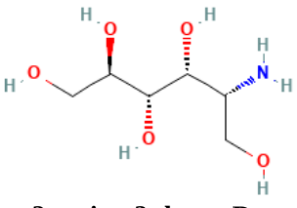
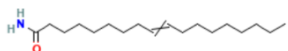
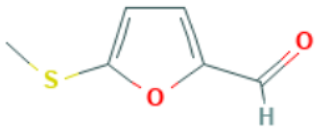
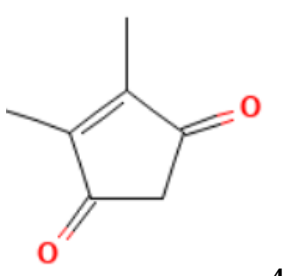
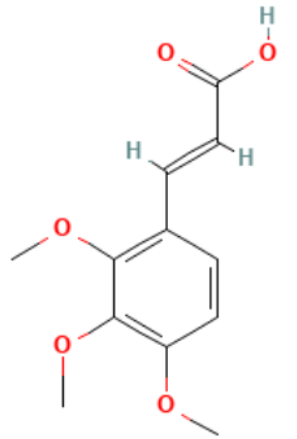
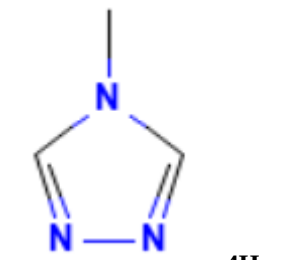
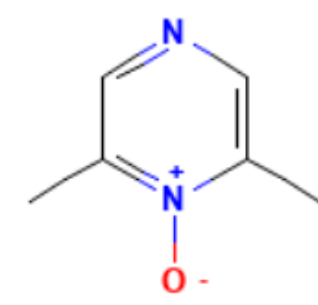
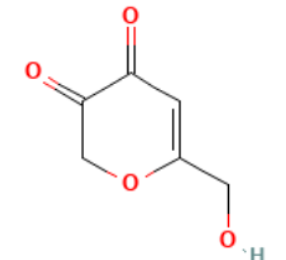
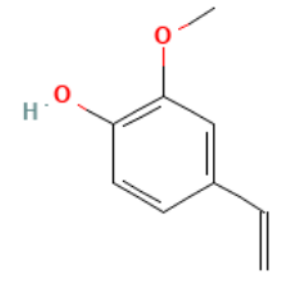
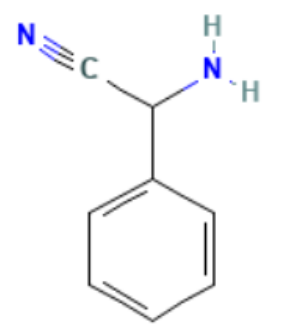
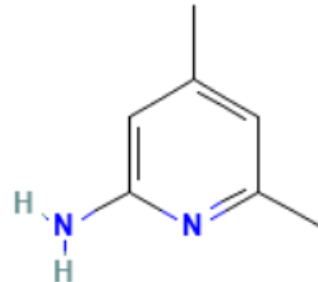
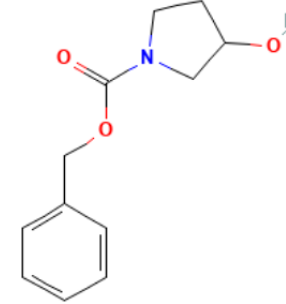
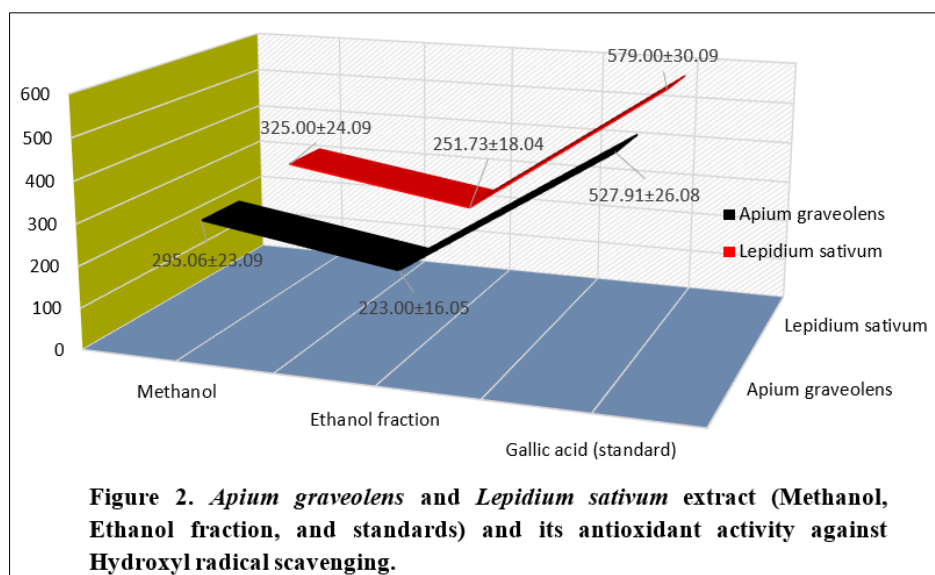
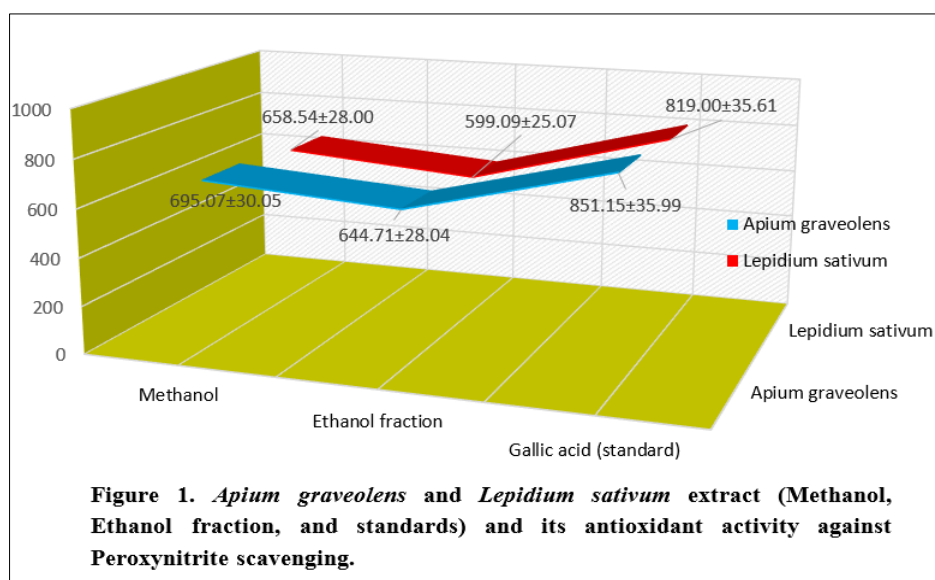
 n-hexadecanoic, MF: C₁₆H₃₂O₂ , MW: 256.42 g/mol	 Caryophyllene, MF: C₁₅H₂₄ , MW: 204.35 g/mol	 beta-Ocimene, MF: C₁₀H₁₆ , MW: 136.23 g/mol	 Neophytadiene, MF: C₂₀H₃₈ MW: 278.5 g/mol
 Ascorbic acid, MF: C₆H₈O₆ MW: 176.12 g/mol	 2-hydroxy-3-(2-methylphenoxy)propanoi c acid, MF: C₁₀H₁₂O₄ MW: 196.2 g/mol	 3-(2-methylpiperidin-1-yl) propyl 4-cyclohexyloxybenzoate , MF: C₂₂H₃₃NO₃ MW: 359.5 g/mol	 Oleic acid, MF: C₁₈H₃₄O₂ MW: 282.5 g/mol
 (-)-beta-Pinene, MF: C₁₀H₁₆ MW: 136.23 g/mol	 (+)-trans-Limonene oxide, MF: C₁₀H₁₆O MW: 152.23 g/mol	 Carnone, MF: C₂₄H₁₂ MW: 300.4 g/mol	 alpha-Terpinolene, MF: C₁₀H₁₆ MW: 136.23 g/mol
 trans-Ocimene, MF: C₁₀H₁₆ MW: 136.23 g/mol			

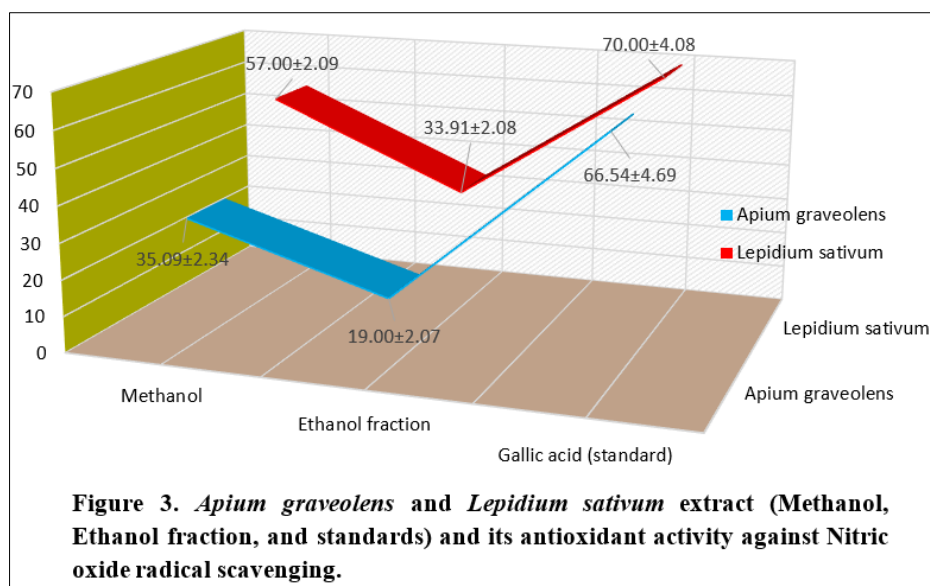
Table 2: GC-MS analysis of phytochemical profile of *Lepidium sativum*

 2-amino-2-deoxy-D-mannitol, MF: C₆H₁₅NO₅ MW: 181.19 g/mol	 9-Octadecenamide, MF: C₁₈H₃₅NO MW: 281.5 g/mol	 2-Furancarboxaldehyde, 5-(methylthio)-, MF: C₆H₆O₂S MW: 142.18 g/mol	 4-Cyclopentene-1,3-dione, 4,5-dimethyl-, MF: C₇H₈O₂ MW: 124.14 g/mol
 trans-2,3,4-Trimethoxycinnamic acid, MF: C₁₂H₁₄O₅ MW: 238.24 g/mol	 4H-1,2,4-Triazole, 4-methyl-, MF: C₃H₅N₃ MW: 83.09 g/mol	 Pyrazine, 2,6-dimethyl-, 1-oxide, MF: C₆H₈N₂O MW: 124.14 g/mol	 2H-Pyran-3,4-dione, 6-(hydroxymethyl)-, MF: C₆H₆O₄ MW: 142.11 g/mol
 o-methoxy-p-vinylphenol, MF: C₉H₁₀O₂ MW: 150.17 g/mol	 2-Amino-2-phenylacetonitrile, MF: C₈H₈N₂ MW: 132.16 g/mol	 2-Pyridinamine, 4,6-dimethyl-, MF: C₇H₁₀N₂ MW: 122.17 g/mol	 benzyl 3-hydroxypyrrolidine-1-carboxylate, MF: C₁₂H₁₅NO₃ MW: 221.25 g/mol

Lepidium sativum or garden cress is an annual herb whose traditional use and possible medical use are numerous. Its seeds and sprouts contain particularly large concentrations of nutrients and bioactive chemicals thus it is a crop of choice in agriculture in regards to medical and as well as culinary purposes in the globe. The *Lepidium sativum* extract has the potential to be used successfully to control and prevent diabetes mellitus and prognosis of the disease. The *L. sativum* plant seeds are edible and can be utilized as food supplement or nutritional supplement; they can be used in the body to maintain more calories and oxidation of nutrients. The initial indication is that the chitosan-TPP nanoparticles whenever loaded to the lectin proteins could be antitumor. Also, in addition to others, tested the extract of *Lepidium sativum* on its effect on hepato-carcinoma and liver injury when used as a therapeutic agent [20-23]. The advantages of celery (*Apium graveolens*) in the example of free radicals neutralization are many; one of the methods of utilizing the components of the celery plant is via antioxidants e.g. flavonoids, phenolic acids and essential oils. This activity facilitates liver activity by reducing

oxidative stress and consequently preventing cellular damage and inflammation, is neuroprotective and reduces the possibility of chronic diseases such as cardiovascular disease [24, 25]. *Apium graveolens* Leaves extract (Methanol, Ethanol fraction, and standards) and antioxidant activity against Nitric oxide, peroxy nitrite, Hydroxyl radicals and Hydroxyl radical scavenging. Peroxynitrite scavenging Figure 1 recorded different types of extracts, (695.07±30.05), (644.71±28.04) and Gallic acid (standard) in *Apium graveolens* and *Lepidium sativum* respectively. In *Apium graveolens* they have recorded (295.06±23.09), (223.00±16.05) and Mannitol (standard) (527.91±26.08) respectively in the Hydroxyl radical scavenging potential Figure 2. In the meantime record (35.0932.34), (19.0032.07) and Curcumin (standard) 66.544.69 respectively in *Apium graveolens* and in *Lepidium sativum* respectively of Nitric oxide radical scavenging potential Figure 3. *A. graveolens* is a superb natural antioxidant that would counteract the occurrence of free radicals and fight against the oxidative stress induced by the abundance of bioactive chemicals i.e., essential oils, phenolic acids, flavonoids. The fruits flies that had been subjected to paraquat have shown research evidence that the antioxidant properties could be applied to neurodegenerative diseases by lowering the level of inflammation and oxidative stress. The extracts have as well been discovered to provide protection against liver injuries in certain conditions such as liver toxicity occasioned by acetaminophen [26]. Among them, the mechanism of action is to lower inflammatory indices and signs of oxidative stress in the liver. When the celery takes in the form of the regular intake the natural elements in it can actually mitigate the risk of acquiring a disease like the cardiovascular disease and cancer as these are capable of lowering the oxidative damage. As a result of its anti-inflammatory effects, which include antioxidant chemicals, *A. graveolens* may be used in decreasing the inflammatory reaction of the body [27]. The components of the plant may have varying antioxidant power. An example is that ascorbic acid and other bioactive chemicals are also abundant in celery leaves, and essential oils are abundant in celery seeds.





The application of *L. sativum* in the traditional medicine has a long history of application in controlling different medical conditions. A preliminary phytochemical study of *L. sativum* as a conventional methodology identified flavonoids, coumarins, sulfur glycosides, triterpenes, sterols and some imidazole alkaloids. The second constituents of this plant are glucosinolates. The *L. sativum* alkaloids are imidazole alkaloids, which are rarely found, the lepidine. Various pharmacological research studies are yet to be done on *L. sativum* despite the extensive history of traditional and edible use of the plant. Thus far, glucosinolates and alkaloids are not studied in phytopharmacologically [28]. *Lepidium sativum* or garden cress is a great source of vitamins, flavonoids and Phenols whose antioxidant and anti-inflammatory effects and are used in the prevention of oxidative stress on cells. The oil that is produced at the plant can improve the body antioxidant enzyme activity and the research has proved that the extracts of the plants possess high antioxidant activity in various tests. *L. sativum* have ascorbic acid (vitamin C), phenols, flavonoid, sulforaphane and other antioxidants. The radicals are unstable chemicals and capable of harming the cells, it has been shown that the extracts of the plant and its oil have the capacity of scavenging the radicals. *L. sativum* oil has been found to increase the antioxidant position and activities of animal antioxidant enzymes. Antioxidant effect *L. sativum* positively contributes to the overall health maintenance of the cells and prevents developing chronic diseases and it is by the alleviation of oxidative stress. The antioxidants present in *L. sativum* can be also viewed as an additional health benefit and it has been known to have anti-inflammatory effects [29]. The study has established the antioxidant chemicals that are contained in *L. sativum* to protect the liver and other body organs.

CONCLUSION

Food and medicinal value of herbs are an established and welcome surprise. The reason is that herbs can be utilized as good preservatives because it has antioxidants. Herbs in dairy products are capable of enhancing the well-being and health of humans. She is supposed to be given herbal products that have the potential health benefits in respect of regulatory standards of safety, efficacy, quality testing and marketing authorization procedures. It is not to be negative in any way. The scientific community is forced to conduct an accurate research and report their findings. In the present study, the phytocompounds were identified in terms of molecular formula and structure of two plants *A. graveolens* and *L. sativum*. The GC-MS analysis is the first way of determining the nature of the active ingredients of these plants. The research has resulted in the discovery of valuable information in the development of most current drugs. The disease control can also be caused by the presence of biologically active compounds whose presence is shown by beta-Ocimene, Neophytadiene, Ascorbic acid, 2-hydroxy-3-(2-methylphenoxy) propanoic acid, 2-amino-2-deoxy-D-mannitol, 9-Octadecenamide, Furancarboxaldehyde, and Cyclopentene-1,3-dione, which are the phytochemicals in *A. graveolens* and *L. sativum*.

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