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GC-MS Quantification and Identification of Phytochemical Profiling, Potential Antioxidant Activity by DPPH, and Mineral Elements of Eggplant Powder (Solanum Melongena) in Sulaymaniyah City, Iraq

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Abstract

Solanum melongena, the plant used as a food and medical remedy known as eggplant, has anti-inflammatory, antioxidant, and anti-cancer properties. It is one of the vegetables that is most widely cultivated worldwide and has a wide range of vegetable sizes, colors, and shapes. This study looked at thirteen chemical compounds in the plant using gas chromatography and mass spectrometric detection (GC-MS). The gathered information verified that the object included thirteen chemical components, likes 2 — pentanone-4-hydroxy 4- methyl% 2.30, 1,3-dioxolane-4-methanol %0.17, formic acid %0.86, methenamine-methoxy %4.528,1,2,3-propanetriol (glycerin) %40.86, tetradecane%0.16, hexadecane%0.27, octadecane %0.16, myristic acid %3.14, nonadecane%0.44,9,12-octadecadienoic acid linolenic acid %0.43, oleic acid%2.36, and octacosane %0.62. These substances have been found to have strong anti-free radical properties, with eggplant powder's antioxidant activity standing out. These compounds are chemically characterized as antioxidants. Using the effective concentration EC_{50} method, the EC_{50} of eggplant powder (Solanum melongena) was 209 µg/mL. The extracts' DPPH-free radical scavenging activity was assessed, and it increased with concentration, going from 4.8828 (µg/mL) to 1250 (µg/mL). The radical scavenging activity of eggplant powder (Solanum melongena) increased from 0.87 to 76.04 indicating a high correlation between concentration and radical scavenging activity. The composition of the mineral elements in the powdered eggplant was ascertained using an **(**ICP/OES Perkin Elmer 2100), (Inductivity Coupled Plasma Emission Spectrometer). Al, Cu, Mn, Fe, Ba, Cr, Co, and Ni were found to be the principal minerals with high concentrations. However, the concentrations of (Cd 0.8974 µg/L and Pb not detectable) were incredibly low.

Keywords: Antioxidant activity, DPPH, Eggplant powder, GC/MS, ICP/OES, Mineral element.

Introduction

After potatoes and tomatoes, eggplant (Solanum melongena L.) is the third most popularly farmed solanaceous vegetable in terms of commercial and economic importance. It is primarily grown in tropical and subtropical regions, either outdoors or in greenhouses[1-3]. It is one of the most popular vegetables consumed globally. Dark-colored fruits and vegetables are known to be better for human bodies since they are a good source of phenolic components, including flavonoids, anthocyanins, and carotenoids, especially in oriental countries [4]. Plants are plentiful sources of advantageous secondary metabolites that are prized as medicines, antimicrobials, flavors, and fragrances. Antioxidant chemicals, which are included in these herbal ingredients' fatty acids, are crucial for preserving health and enhancing human life. Consequently, there is an increasing interest in discovering plants for use as food and medicine. [5] Because there are so many methods to prepare eggplant and so many

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different ways to utilize it, including fresh, dried, and preserved, it is a crucial part of the human diet. According to reports, eggplant is a good source of vitamins (vitamins B1, B6, and K), dietary fiber, and phytochemicals, particularly important phenolic compounds, which are widely utilized to lower blood cholesterol levels in people.[6] Antioxidants can protect cells in a number of ways, such as by converting reactive oxygen species (ROS) into non-radical species (which depend on the specific antioxidant), stopping the ROS-initiated auto-oxidative chain reaction, and lowering localized oxygen concentrations. Exogenous antioxidants that are present in frequently consumed fruits, vegetables, drinks, cereals, and other dietary products, such as ascorbic acid (vitamin C), tocopherol (vitamin E), carotenoids, and polyphenols, may assist the body's natural defense against oxidative stress. [7] There is interest in extracting phenolic compounds and anthocyanins from various plant materials to create more value-added products because of the many advantages of phenolic compounds. The storage conditions, chemical make-up, and particularly the extraction conditions and choice of extraction techniques all have a significant impact on the extraction efficiency of plant active components. [8] The nutritional value of the eggplant vegetable is well recognized; it contains an adequate number of carbs, proteins, and minerals, including copper, zinc, and iron, as well as vitamins. [9]

Nearly 90% of the bioactive chemicals found in eggplant—including phenolic acids and chlorogenic acids (CAs)—have been shown to have health-promoting properties.[10] The importance of eggplant as a source of polyphenols, which are created during plant growth as a defensive mechanism and result from secondary metabolism, has been acknowledged. This class of compounds includes substances like phenolic acid, flavonoids, anthocyanins, and tannins [11,12]. Due to its high phenol and anthocyanin content, eggplant is one of the top ten vegetables with regard to its ability to absorb oxygen radicals[13-15]. The nutritional value of the eggplant fruit is well recognized; it contains an adequate number of carbs, proteins, and minerals like copper, zinc, and iron, as well as vitamins. Moreover, eggplant has a very high concentration of bioactive substances that are good for you, such phenolic acids. whereas chlorogenic acids make up nearly 90% of these components. The polyphenols present serve as nutraceuticals that guard against respiratory infections, cancer, cardiovascular disease, and safeguard memory function in the brain.[16] The most common type is an elongated, ovoid, or thin type with dark purple skin.[17] Plants store a variety of defensive chemicals in their cells, including, phenolic acids, minerals, pigments, antioxidants, and Osmo protectants. Of these, phytochemicals with properties that include free radical scavenging, anti-mutagenesis, anti-cancer, and anti-inflammatory activities have been the subject of considerable research. Examples include phenolic acids, anthocyanins, and flavonoids.[18] The objectives of this research to identify and quantify the phytochemicals present in eggplant powder by GC/MS analysis. phytochemicals are biologically active found in plant that have potential health benefits and to evaluate the antioxidant activity of eggplant powder using DPPH methods, antioxidants help neutralize harmful free radicals in the body, which can cause oxidative stress and contribute to various diseases, furthermore mineral content in eggplant determine by (ICP/OES Perkin Elmer 2100), (Inductivity Coupled Plasma Emission Spectrometer), mineral elements are essential for various physiological function in the human body, and their presence in the food can contributes to overall nutritional value . By conducting this study, researcher's aim is to gain a better understanding of the phytochemical composition, antioxidant potential, and mineral contents. This information can provide insights in to the potential's health benefit and nutritional value of egg plants as a dietary supplements or ingredients.

Figure 1. Eggplant (Solanum Melongena) powder.

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Materials and Methods

Plant Products

In July 2022, a thin kind of eggplant was bought from a neighborhood store in Sulaymaniyah City. It was removed from the calyx by using a knife to slice it into a circular shape. The samples were dried in an oven for 48 hours at 50 °C, and then they were ground into a soft, fine powder using a grinder (Wiley mill), Fig. 1. The chemicals used in this study were nitric acid, distilled deionized water, ethanol, methanol, DPPH mixture (2,2-diphenyl-1-picrylhydrazyl) radical.

Gas Chromatography-mass Spectrometry (GC-MS) Analysis of Phytochemicals

The chemical composition of a methanolic extract of eggplant was determined using a Trace GC-ISQ mass spectrometer (Thermo Scientific, Austin, TX, USA) with a direct capillary column TG–5MS (30 m × 0.25 mm × 0.25 µm film thickness) as previously described. The column oven temperature was initially held at 50 °C and then increased by 5 °C/min to 250 °C withhold 1 min, then increased to 300 °C at the rate of 30 °C/min. The injector temperatures were kept at 260 °C. Helium was used as a carrier gas at a constant flow rate of 1 mL/min. The solvent delay was 4 min, and diluted samples of 1 µL were injected automatically using an AS3000 autosampler coupled with GC in the split mode. EI mass spectra were collected at 70 eV ionization voltages over the range of *m/z* 50–650 in full scan mode. The ion source and transfer line were set at 250 °C and 270 °C, respectively. The components were identified by a comparison of their retention times and mass spectra with those of the Wiley-09 and NIST-11 mass spectral databases. [19,20]

Assessment of Free Radical Scavenger Activity (DPPH)

The DPPH assay was performed according to the method developed by Siddartha et al. Calculation of the antioxidant potential for scavenging was done using the method that was documented [21]. Various concentrations—1.5 ml of alcoholic extract and a 0.25 Mm DPPH mixture—were diluted in ethanol. This mixture was shaken ferociously to maintain its condition at room temperature. Using a spectrophotometer, the absorbance includes the UV - visible spectrum was measured to determine the degree of DPPH decolorization after 30 minutes. The Eq 1 [22, 23] was then used to analyze the antioxidant activity. Ascorbic acid was used as a positive control.

$$\% \text{ Scavenging Activity} = [(Ac - As) / Ac] \times 100 \quad \ldots\ldots 1$$

Ac stands for the blank's absorption.
As indicates sample absorbance.

Identifying Mineral Elements

A porcelain crucible containing 5 g of eggplant powder was placed in a muffle furnace, where the temperature was raised to 800 ºC and maintained there for 6 hours. The sample was taken out of the furnace and weighed; the amount of ash determined for the sample was 0.156 g%. The remaining ash was dissolved in 1:1 nitric acid, filtered through Whatman No. 42, and the volume was finished with (100 mL of distilled deionized water). All reagents were prepared using ultra-high purity commercial acids and distilled deionized water. The solution was then injected into an (ICP/OES Perkin Elmer 2100), (Inductivity Coupled Plasma Emission Spectrometer) Perkin Elmer 2100 DV in the USA for determination. In parallel analyses, the mineral contents of the samples were measured against reference solutions of known concentration. Samples are typically digested with pure HNO_3 (65-70%), according to the literature,[22] in a microwave oven before the resultant solution is diluted with ultra-pure water. It is a crucial stage in the analysis that results in the sample's matrix are dissociating and there is a simultaneous release of the elements (analytes). It should be emphasized that the degree of ionization of the elements is determined by the plasma's temperature and ionization potential. [24]

Results and Discussion

Analysis of Phytochemicals Using a Mass Spectrometer and Gas Chromatography (GC-MS)

The eggplant powder sample was examined and identified using a GC/MS analyzer; the chromatogram of the phytochemical's components is shown in Fig. 2. The chemicals were described and identified in Table 1 after the GC- MS spectra of the phytochemical compounds were compared with the NIST 2008 library. Table 1 provides the proportional

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percentages of the phytochemical makeup and retention times. Table 1 and Fig. 2 illustrate that in eggplant powder, fourteen phytochemical substances were found like (2-Pentanone, 4-hydroxy-4-methyl % 2.33, 1,3-Dioxolane-4-methanol % 0.17, Formic acid % 0.86, Methenamine, N-methoxy %4528, 1,2,3- Propanetriol Glycerin % 40.86, Tetradecane % 0.16, Hexadecane % 0.27, Octadecane % 0.16, Myristic acid % 3.14, Nonadecane % 0.44, 9,12-Octadecadienoic acid % 0.43, Oleic Acid % 2.36, Octacosane % 0.62). The eggplant powder in this study is rich in glycerin, linoleic acid, oleic acid, 2-Pentanone, and 4-hydroxy-4-methyl linoleic acid (an omega-6 fatty acid). Essential fatty acids include oleic acid and linoleic acid. All bodily systems must have fatty acids to operate normally. Only food sources can provide people with vital fatty acids. These fatty acids are used to create hormones that control the immunological and nervous systems. Polyunsaturated fatty acids are a subclass of essential fatty acids.

Table 1. Chemical composition percentages derived from GC-MS analysis of eggplant powder.

Peak	Retention Time	%Peak Area	Chemical Name	Compound Nature	Molecular Weight	Molecular Formula
1	9.551	% 2.33	2-Pentanone, 4-hydroxy-4-methyl	Ketone	116.16	$C_6H_{12}O_2$
2	13.449	% 0.17	1,3-Dioxolane-4-methanol	Heterocycle compound	104.10	$C_4H_8O_3$
3	15.346	% 0.86	Formic acid	Carboxylic Acid	46.03	CH_2O_2
4	23.176	%4.528	Methenamine, N-methoxy	Amine	61.084	CH_3ONHCH_3
5	25.234	% 40.86	1,2,3- Propanetriol Glycerin)	Alcohol	92.09	$C_3H_8O_3$
6	34.927	% 0.16	Tetradecane	Hydrocarbon	198.39	$C_{14}H_{30}$
7	43.151	% 0.27	Hexadecane	Hydrocarbon	226.41	$C_{16}H_{34}$
8	50.553	% 0.16	Octadecane	Hydrocarbon	254.494	$C_{18}H_{38}$
9	56.177	% 3.14	Myristic acid	Fatty Acid	228.37	$C_{14}H_{28}O_2$
10	59.177	% 0.44	Nonadecane	Hydrocarbon	268.518	$C_{19}H_{40}$
11	61.503	% 0.43	9,12-Octadecadienoic acid	Fatty Acid	280.452	$C_{18}H_{32}O_2$
12	61.703	% 2.36	Oleic Acid	Fatty Acid	282.47	$C_{18}H_{34}O_2$
13	61.852	% 2.07	Oleic Acid	Fatty Acid	282.47	$C_{18}H_{34}O_2$
14	63.069	% 0.62	Octacosane	Hydrocarbon	394.77	$C_{28}H_{58}$

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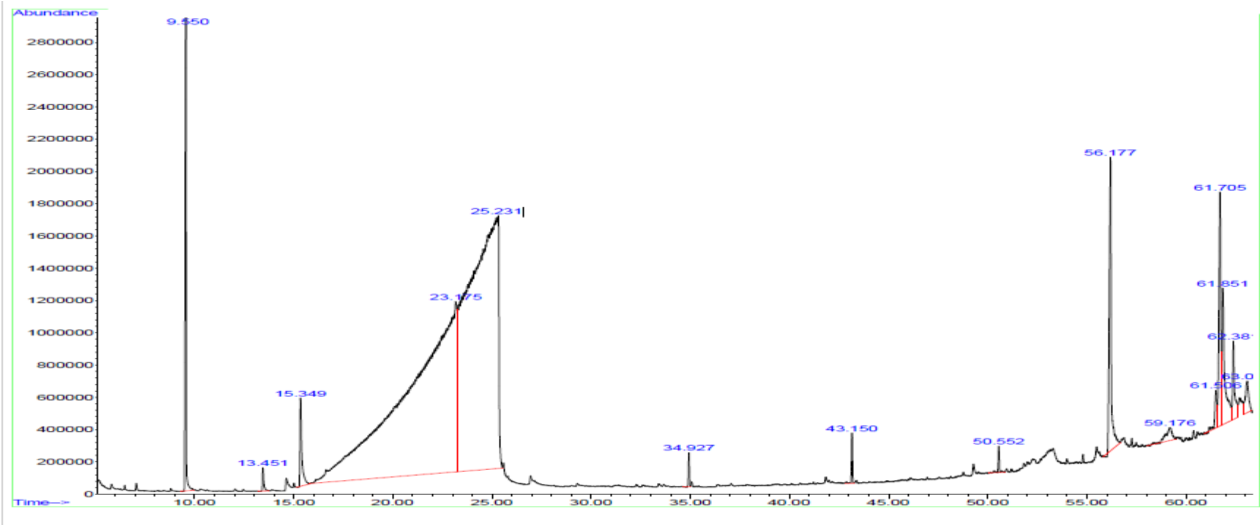

Figure 2. The Eggplant Powder's Acquired GC-MS Chromatogram.

Assessment of Free Radical Scavenger Activity (DPPH)

In order to assess the antioxidant activity of eggplant powder, the DPPH assay protocol was performed. A solution of DPPH (using it as an indicator) when combined with an antioxidant agent produces diphenyl picrylhydrazine in its reduced state (non-radical) at different intensities. The mixture's changes from deep purple to yellow in reaction. The sample's absorbance was calculated at 517 nm in wavelength. As Fig 4 depicts, the dosage response curve for the dimethyl sulfoxide extract's capacity to scavenge free radicals. It can be seen in Fig. 3 that the DPPH's scavenging behavior was graphically evaluated. The amount of DPPH radicals scavenged increased as extract concentration increased. A common metric for evaluating free radical scavenging activity is the EC50 value. It is defined as the amount of extract required to scavenge radicals 50% of DPPH under the experimental conditions employed. A lower EC50 DPPH value is related to higher antioxidant activity. Based on radical scavenging activity, see Table 2, Fig. 3 displays the EC50 DPPH value of eggplant powder extract. The EC_{50} DPPH = 209 (µg/ml), the DPPH scavenging ability of eggplant powder was compared with that of ascorbic acid, a known standard antioxidant. The dosage response curve for the radical scavenging ability is shown in Fig. 3 and 5. With rising extract concentration, the scavenging of DPPH radicals increased, as recorded in Table 3. Additionally, the half maximal inhibitory concentration IC_{50} of ascorbic acid, a common antioxidant, was calculated in this study to be 0.021 mg/ml and was considerably different from eggplant powder. According to Table 3. , Fig. 4 and 6, ascorbic acid's scavenging effect ranged from 11.44% at concentrations of 8.25 g/ml to 80.54% at concentrations of 66µ g/ml, while eggplant powder's scavenging impact ranged from 0.87 to 76.04 at concentrations of 4.8µ g/ml to 1250 µg/ml.

According to the measured absorbance values and calculated percentage inhibition, the antioxidant activity of eggplant powder and standard (Ascorbic acid) is concentration dependent. According to the findings, eggplant powder had good DPPH radical scavenging abilities that were comparable to ascorbic acid at all concentrations studied. The biological mechanism behind the antioxidant property is associated with the removal of free radicals, namely hydroxyl radicals and metal chelation, which have an impact on immune system performance and cell signaling[25, 26]. Eggplant peel is frequently disposed as biowaste, which causes significant losses of organic materials that typically contain high amounts of vital bioactive chemicals. [27]. They possess strong antioxidant abilities and control the inflammatory response linked to various illnesses. [28.] With discovering the pathophysiology of illnesses including diabetes and ailments linked to oxidative stress, interest in herbal plant treatment has grown. A condition that arises from the body's inability to neutralize an excess of reactive oxygen species (ROS) like superoxide, singled oxygen, and H_2O_2 produced by cell metabolism. The body's antioxidant defense system includes innate components superoxide dismutase, catalase, and hydro peroxidase as well as acquired antioxidants from plants. These findings have inspired researchers to look for pharmaceutically effective antioxidants to

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aid the antioxidant defense system in combating various ailments[29].

Table 2. Eggplant powder's antioxidant potential as measured by DPPH.

Conc. (µg/ml)	1250	625	312.5	156.25	78.125	39.0625	19.5312	9.76562	4.8828	Control
OD1	0.236	0.244	0.463	0.588	0.739	0.774	0.813	0.904	0.994	0.992
OD2	0.243	0.238	0.456	0.579	0.723	0.783	0.794	0.912	0.989	0.975
OD3	0.229	0.249	0.471	0.592	0.741	0.788	0.789	0.897	0.998	0.988
Average	0.236	0.243667	0.463333	0.586333	0.734333	0.781667	0.798667	0.904333	0.993667	0.985
RSA% 1	76.04061	75.22843	52.99492	40.30457	24.97462	21.42132	17.46193	8.22335	-0.91371	-0.71066
RSA% 2	75.32995	75.83756	53.70558	41.21827	26.59898	20.50761	19.39086	7.411168	-0.40609	1.015228
RSA% 3	76.75127	74.72081	52.18274	39.89848	24.77157	20	19.89848	8.93401	-1.3198	-0.30457
Average	76.04061	75.26227	52.96108	40.47377	25.44839	20.64298	18.91709	8.189509	-0.87986	0.00
STDEV	0.71066	0.559144	0.761985	0.675972	1.001601	0.720264	1.285511	0.761985	0.457792	0.902355

Table 3. Ascorbic acid's antioxidant potential as measured by DPPH.

Concentration. (µg/ml)	66	33	16.5	8.25
	0.171	0.298	0.455	0.785
	0.173	0.298	0.456	0.784
	0.173	0.3	0.453	0.784
Average	0.172333	0.298667	0.454667	0.784333
RSA1	80.69251	66.35303	48.62627	11.3662
RSA2	80.46669	66.35303	48.51336	11.4791
RSA3	80.46669	66.12721	48.85209	11.47911
RSA%	80.54196	66.27776	48.66391	11.44148
STDEV	0.130376	0.130376	0.172472	0.06518

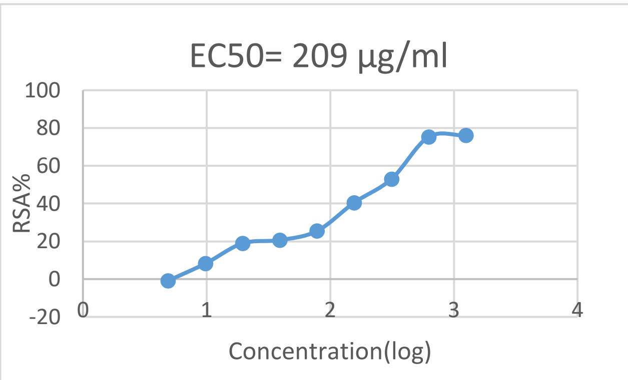

Figure 3. The Percentage of Eggplant Powder's Scavenging Activity on DPPH Radicals.

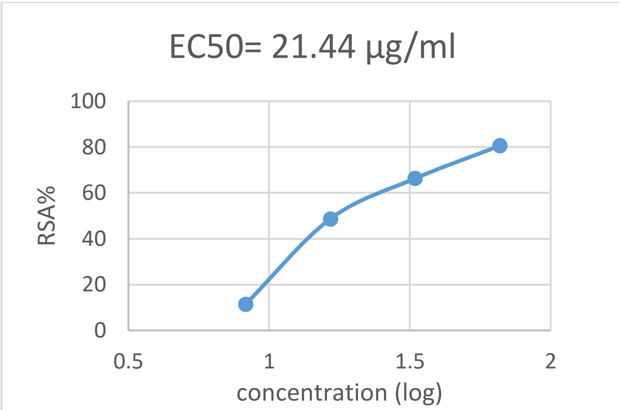

Figure 4. The Percentage of Ascorbic Acid Scavenging Activity on DPPH Radicals.

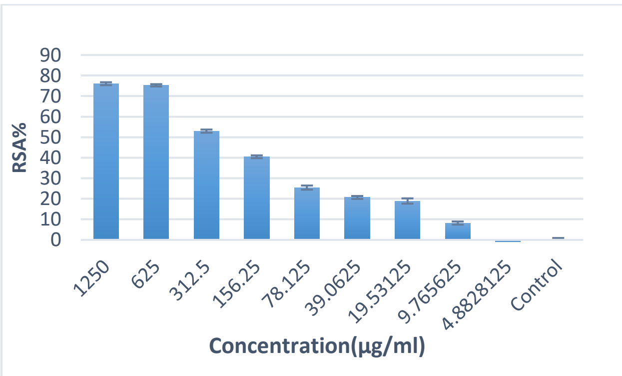

Figure 5. The DPPH Radical Scavenging Activities of Eggplant Powder.

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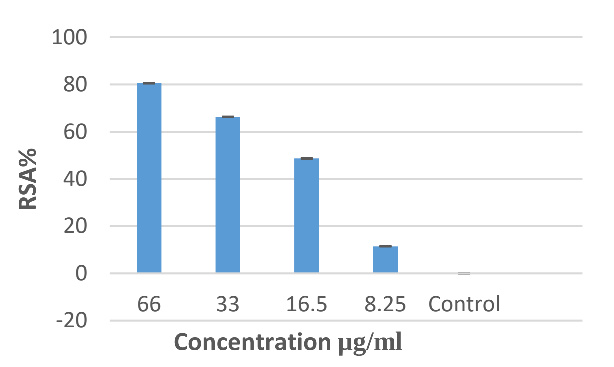

Figure 6. The DPPH Radical Scavenging Activities of Ascorbic acid.

Identifying Mineral Elements

By using ICP/OES, the mineral elements present in eggplant powder were identified. Table 4 lists the minerals that make up eggplant powder. Results showed that eggplant powder has extremely high levels of (Al, Cu, Mn, Fe, Ba, Cr, Co, and Ni) correspondingly. Additionally, very low levels of (Cd, Pb) were found in eggplant. An illustration of the mineral content of eggplant powder is shown in Fig. 7. While (Al) content was highest (87473.33218 µ/L), and (Ca) content was found (20508.6563 µ/L), (Cu and Mn) contents were found (1376.4168 µ/L, 1037.8228 µ/L respectively). While (Cd) was lowest (0.8974 µ/L). Cadmium can enter the body of a person through food, which increases the risk of anemia, hypertension, muscle cramps, osteoporosis, and cancer.[30] Eggplants (Solanum melongena L.) stand out for having high levels of certain minerals like P, K, Ca, and Mg [31].

Table 4. Analysis of Eggplant Powder's Elements Using (OES-ICP Perkin Elmer 2100).

Elements	ppb = µg/L
Al	87473.332
Ni	55.0394
Cu	1376.416
Mn	1037.822
Fe	697.966
Co	61.8748
Ca	20508.65
Cd	0.8974
Cr	62.6907
Ba	488.056
Pb	< LOD

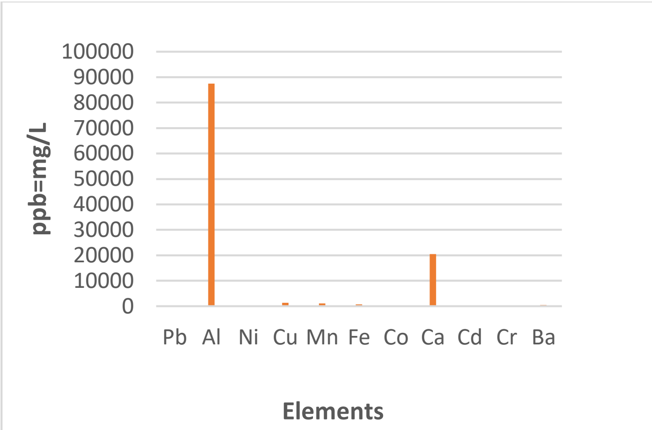

Figure 7. Graphical Representation of Eggplant Powder's Mineral Content

Conclusion

Due to its numerous medicinal uses, eggplant (Solanum melongena) has been used in traditional cultures for ages. Preliminary phytochemical analysis of methanolic extract of Eggplant powder showed the presence of bioactive ingredients, along with the pharmaceutical sector, such as glycerin, ketones like 2-Pentanone, 4-hydroxy-4-methy, amines like Methenamine and N-methoxy, carboxylic acids like formic acid, heterocyclic compounds like 1,3-Dioxolane-4-methanol, essential fatty acids like oleic acid and linoleic acid, and non-essential fatty acids like myristic acid. Based on the results of the DPPH experiment, eggplant powder's enhanced antioxidant capacity suggests that it might be useful in preventing the onset of a number of oxidative stress disorders and as a food additive to boost the antioxidant activity of food could be utilized in food products as a natural antioxidant. It was found that eggplant powder are important sources of minerals highlighting aluminum, calcium, magnesium and iron. This study can serve as a basis for further research into the potential health benefits of consuming Solanum melongena

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Authors' Declaration

- Conflicts of Interest: None.
- I hereby confirm that all the Figures and Tables in the manuscript are mine. Furthermore, any Figures and images, that are not mine, have been included with the necessary permission for re-publication, which is attached to the manuscript.
- Ethical Clearance: The project was approved by the local ethical committee at Sulaymaniyah University.
- No animal studies are present in the manuscript.
- No human studies are present in the manuscript.
- No potentially identified images or data are present in the manuscript.

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استخدام GC-MS للتعيين الكمي والخصائص الكيميائية النباتية، لنشاط مضادات الأكسدة المحتملة بواسطة DPPH، والعناصر المعدنية لمسحوق الباذنجان (Solanum Melongena) في مدينة السليمانية العراق

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الخلاصة

ان مسحوق الباذنجان (Solanum melongen)، هو النبات المستخدم كعلاج غذائي وطبي، له خصائص مضادة للالتهابات ومضادة للأكسدة ومضادة للسرطان. إنها واحدة من الخضروات التي تزرع على نطاق واسع في جميع أنحاء العالم ولديها مجموعة واسعة من أحجام وألوان وأشكال. في هذه الدراسة تم التركيز على اربعة µعشر مركبًا كيميائيًا باستخدام تقنية (GC-MS)، وقد اظهرت النتائج بأن المركب يحتوي على اربعة عشر مكونًا كيميائيًا، مثل:

(2—pentanone-4-hydroxy 4- methyl% 2.30), (1,3-dioxolane-4-methanol %0.17), (formic acid %0.86), (methenamine,N-methoxy %4.528), (1,2,3-propanetriol (glycerin) %40.86), (tetradecane%0.16) ,(hexadecane%0.27), (octadecane %0.16),(myristic acid %3.14), (nonadecane%0.44), (9,12-octadecadienoic acid (linolenic acid) %0.43), (oleic acid%2.36), and (octacosane %0.62).

ان هذه المركبات التي تم تشخيصها لها خصائص قوية مضادة للجذور الحرة، مع نشاط مضاد للأكسدة في مسحوق الباذنجان. تتميز هذه المركبات كيميائيا بأنها مضادات الأكسدة. باستخدام طريقة EC_{50}، تم تقييم نشاط DPPH-free radical المستخلص، وتزداد مع التركيز، من 4.8828 ((µg/ml) إلى 1250 ((µg/ml). تم التأكد من محتوى العناصر المعدنية في مسحوق الباذنجان باستخدام ICP / OES (مطياف انبعاث البلازما المقترن بالحث). حيث تم العثور على Al وCu وMn و Fe و Ba و Cr و Co و Ni كمعادن رئيسية ذات تراكيز عالية. ومع ذلك، كانت تركيزات (الكادميوم والرصاص) منخفضة بشكل غير متوقع.

الكلمات المفتاحية: النشاط مضادات الأكسدة، DPPH ، المسحوق الباذنجان، GC/MS، GC/MS .