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Research Article

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Preparation and Investigation of Egyptian-Cultivated Pumpkin Seed Oil: Antioxidant Activity and Key Constituents via GC-MS Characterization and ADMET Squalene Analysis

Ola M. Yehia[1*]; Abeer A. Khamis[1]; Elsayed I. Salim[2]; Marian N. Gerges[1]

[1]Biochemistry Division, Chemistry Department, Faculty of Science, Tanta University, Tanta, Egypt.
[2]Department of Zoology, Faculty of Science, Tanta University, Tanta, Egypt.

[*]**Corresponding author:** *Ola M. Yehia* **e-mail:** *ola30974631@science.tanta.edu.eg*

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KEY WORDS

Pumpkin seed oil, DPPH, ABTS, GC-MS analysis, Squalene.

ABSTRACT

Pumpkin seed oil, extracted from Cucurbita pepo seeds cultivated in Egypt, possesses significant nutritional and therapeutic potential due to its rich bioactive compound profile. This study aimed to evaluate the chemical composition and antioxidant activity of the oil to support its applications in health and industry. Using Soxhlet extraction, a yield of 38.15% (w/w) was obtained. Gas Chromatography-Mass Spectrometry (GC-MS) analysis revealed the presence of Linoleyl acetate (32.12%), Glycidyl (Z)-9-nonadecenoate (19.45%), and Squalene (3.16%) as major components. The methylated oil analysis identified unsaturated fatty acids, including 9,12-Octadecadien-1-ol (46.17%) and 6-Octadecenoic acid (24.30%). Antioxidant assays, including 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid (ABTS), reducing power, and phosphomolybdate methods revealed a strong radical scavenging and reducing potential in a dose-dependent manner. ADMET analysis of Squalene revealed favorable pharmacokinetics, including compliance with Lipinski's Rule of Five, absence of toxicity or carcinogenicity alerts, and moderate human intestinal absorption. These findings highlight the antioxidant and therapeutic potential of pumpkin seed oil, emphasizing its suitability for applications in functional foods, pharmaceuticals, and cosmetics. Future research should focus on isolating and optimizing key bioactive compounds for targeted applications.

Introduction

Medicinal plants have long been used in traditional healthcare systems, particularly in developing countries, to prevent and manage diseases related to inflammation and oxidative stress. Their therapeutic potential lies in the presence of bioactive compounds with antioxidants and anti-inflammatory properties, making them increasingly popular even in developed nations **(Hlatshwayo et al., 2025).** The pumpkin plant (*Cucurbita pepo*) belongs to the family Cucurbitaceae and is characterized by its climbing or trailing growth habit, large lobed leaves, and vibrant yellow flowers. Its fruits vary in size, shape, and color, depending on the cultivar, and contain numerous flat, oval seeds **(Rezk et al., 2022)**. In Egypt, pumpkin is cultivated on approximately 19,309 hectares, yielding a total production of 417,109 tons annually, highlighting its agricultural and economic significance **(Shehata et al., 2023).**

Pumpkin is a highly versatile plant, valued for its nutritional and health-promoting properties. Different parts of the pumpkin, including the pulp, seeds, leaves, and peels, have significant uses in food and health industries. Pumpkin byproducts, such as peels and seeds, are also excellent sources of bioactive compounds that can enhance nutritional value when effectively utilized **(Rosales et al., 2025)**. Pumpkin seeds, in particular are highly nutritious, containing proteins, vitamin C, potassium, magnesium, and moderate amounts of calcium, zinc, and copper, which are essential for maintaining good health. They are also rich in fibers, carotenoids, antioxidants, and phytosterols, which contribute to their anti-inflammatory, antidiabetic, and neuroprotective properties **(Aziz et al., 2023)**. Furthermore, they are a valuable raw material in the food industry, used to enhance the nutritional profile of products such as flour and protein-rich meals **(Villamil et al., 2023)**.

Pumpkin seed oil is a natural product extracted from the seeds of Cucurbita pepo and other pumpkin varieties. It is typically prepared using methods such as cold pressing, solvent extraction, and supercritical CO_2 extraction **(Hu et al., 2024)**. The oil holds great importance due to its numerous health benefits and wide applications in the food, pharmaceutical, and cosmetic industries **(Šamec et al., 2022).** It is widely used to support cardiovascular health, alleviate symptoms of benign prostatic hyperplasia, regulate hormones, and reduce the impact of oxidative stress-related conditions such as diabetes and neurodegenerative diseases. Its antioxidant and moisturizing properties also make it a valuable ingredient in skincare products, sunscreens, and therapeutic formulations **(Hu et al., 2023)**.

The oil is rich in unsaturated fatty acids, particularly linoleic acid (omega-6) and oleic acid (omega-9), with smaller amounts of saturated fatty acids like palmitic and stearic acids. It also contains bioactive compounds such as tocopherols (vitamin E), carotenoids (lutein, zeaxanthin, and β-carotene), phytosterols (β-sitosterol and Δ7-sterols), polyphenols, and minerals like magnesium, potassium, and zinc **(Singh and Kumar, 2023)**. These components collectively contribute to its antioxidant, anti-inflammatory, and therapeutic properties. For instance, tocopherols and polyphenols help combat free radical damage, carotenoids support eye and immune health, and phytosterols promote cholesterol regulation and prostate health **(Batool et al., 2022).** Despite its recognized benefits, the complex composition of pumpkin seed oil makes it challenging to identify the compounds responsible for its therapeutic effects. The interactions among its bioactive components, such as fatty acids, tocopherols, carotenoids and squalene are not fully understood, requiring further research to optimize its applications in health, nutrition, and industry.

This study aims to identify the major chemical components in pumpkin seed oil from seeds cultivated in Egypt. Determining the most abundant compounds could enable their isolation and targeted use in therapeutic and industrial applications, enhancing the oil's value and paving the way for more effective functional foods, supplements, and pharmaceuticals.

Materials and Methods

Chemicals and reagents

The chemicals used in this study were of a high analytical grade and sourced from reputable suppliers.

Plant material

Pumpkin seeds were freshly purchased in March 2024 from Khedr El Attar store, El Moez Street, Hussain, Egypt. The pumpkins were cultivated in Beheira Governorate during the preferred planting season, from late March to April. The seeds were stored in a refrigerator at 4°C to maintain their quality. Prior to oil extraction, the seeds were sun-dried for two days to reduce moisture content and prepare them for processing.

Oil preparation

The 100 g of pumpkin seeds sun-dried for two days showed a reduced weight to 96.5g. The seeds were coarsely powdered using a mortar and pestle. The extraction method was performed utilizing a Soxhlet apparatus with 250 mL of n-hexane as the extraction solvent Following a previously described method with some modifications **(Chari et al., 2018)**. The solvent was heated at its boiling point (76°C), and the extraction process was carried out for 4 h, allowing for continuous solvent evaporation and condensation. Glass wool was used in the side arm to prevent solid particles from entering the solvent. After the process, the n-hexane was removed using a rotavapor, leaving the extracted pumpkin seed oil in the flask. The oil yield was determined by calculating the weight of the extracted oil, which was derived from its volume and density. The percentage of oil concentration was then calculated using the formula:

$$\text{Oil Yield (\%)} = \left(\frac{\text{Weight of oil (g)}}{\text{Weight of seeds after drying (g)}}\right) \times 100$$

The recovered oils were stored in a vial at 4°C until further examination was required.

Studying of pumpkin seed oil components

Oil methylation

Fatty acid methyl esters were prepared for GC-MS analysis following a previously described method **(Hagos et al., 2023)**. One gram of extracted oil was mixed with 6 mL of 2% methanolic KOH in a 50 mL round-bottom flask. The mixture was heated at 60-70 °C for 1 h with constant stirring, then cooled to room temperature. After adding 40 mL of n-hexane, the solution was transferred to a separatory funnel, and the organic layer was separated, dried over anhydrous sodium sulfate, and filtered through Whatman filter paper. The solvent was removed using a rotary evaporator to concentrate the methyl esters, which were stored at 4°C until GC-MS analysis.

Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

The chemical composition of pumpkin seed oil was analyzed using a Shimadzu HS-GC-MS system (QP2020) equipped with a mass spectrometer detector (MS) and a fused silica capillary column. Two samples were prepared: crude pumpkin seed oil extracted using a Soxhlet apparatus with n-hexane as the solvent, and methylated pumpkin seed oil prepared by converting the extracted oil into fatty acid methyl esters. The GC-MS conditions for both analyses included an initial column oven temperature of 50°C for 3 min, followed by an increase at a rate of 5°C/min to 300°C, with a final hold of 10 min. The injection temperature was 280°C, and the injection mode was split with a ratio of 15:1. Helium was used as the carrier gas with a column flow rate of 1.37 mL/min. The ion source and interface temperatures were set at 220°C and 280°C, respectively, with a mass range of 35–500 m/z and a solvent cut time of 2 min. For the methylated sample, 1 µL was injected, and the retention times and mass spectra of the detected compounds were identified using the NIST17 library.

Antioxidant assays

Evaluation of DPPH radical scavenging activity

The free radical scavenging activity of pumpkin seed oil and squalene were evaluated using the DPPH radical, following a previously reported method **(Molyneux, 2004)**. Briefly, 0.1 mL of various concentrations of the oil sample was mixed with 3.9 mL of DPPH solution (0.003% w/v). The mixtures were shaken thoroughly and left to stand in the dark at room temperature for 1 h. After the reaction period, the decrease in absorbance of the solution was measured at 515 nm utilizing a Shimadzu UV-1800 UV-Vis spectrophotometer.

Assessment of ABTS radical scavenging activity

The ABTS radical scavenging activity of pumpkin seed oil and squalene were assessed spectrophotometrically by measuring the reduction in absorbance of the ABTS radical at 734 nm **(Re et al., 1999).** The activated ABTS solution was prepared by mixing 14 mM ABTS with 4.9 mM potassium persulfate and incubating in the dark for 16 h. In the assay, 0.1 mL of the sample was mixed with 3.9 mL of ABTS solution. The mixture was incubated for 6 min at room temperature, and the absorbance was recorded.

Reducing power assay

The reducing power of pumpkin seed oil and squalene were evaluated following a previously described method **(Ayoola et al., 2008)**. Briefly, 1.25 mL of 1% w/v potassium ferricyanide solution was mixed with 1.25 mL of phosphate buffer (0.2 M, pH 7.6) and 0.5 mL of the oil. The mixture was incubated at 50°C for 30 min. After incubation, 1.25 mL of 10% w/v trichloroacetic acid was added to the mixture, which was then centrifuged at 3000 rpm for 10 min. Following centrifugation, 1.25 mL of the supernatant was diluted with distilled water (1:2), and 0.25 mL of 0.1% w/v $FeCl_3$ solution was added. The absorbance of the resulting solution was measured at 700 nm.

Phosphomolybdate assay

The total antioxidant capacity of pumpkin seed oil and squalene were determined using the method outlined in a previous study **(Khan et al., 2012).** Briefly, 2 mL of phosphomolybdate reagent was combined with 0.2 mL of the oil. The mixture was thoroughly mixed and incubated at 95°C for 90 min. After incubation, the absorbance of the solution was measured at 695 nm.

In Silico ADMET study

The ADMET characteristics were analyzed utilizing the ADMETlab 2.0 platform (available at https://admetmesh.scbdd.com/). The SMILES formats for compound were obtained from the PubChem database and submitted to the platform for analysis. ADMETlab 2.0 provided a comprehensive evaluation of the compounds' pharmacokinetics and toxicological profiles, supporting their stability for further research.

Results

Isolation of pumpkin seed oil

Approximately 40 mL of oil was obtained, which corresponds to a weight of 36.8 g, based on the density of pumpkin seed oil (0.92 g/mL). The oil concentration was calculated as 38.15% **(w/w)**. The extracted oil was dark green in color and stored in a refrigerator at 4°C to maintain its stability until further analysis.

Analysis of Pumpkin Seed Oil Using GC-MS

The Gas Chromatography-Mass Spectrometry (GC-MS) analysis of the pumpkin oil sample identified multiple chemical constituents with varying retention times and relative abundances **Fig. (1).** The most dominant compound was Linoleyl acetate (retention time: 43.259min), comprising 32.12% of the total detected components. This was followed by Glycidyl (Z)-9-nonadecenoate (19.45%, retention time: 43.383min) and E, E, Z-1,3,12-Nonadecatriene-5,14-diol (12.00%, retention time: 58.574min). Other notable compounds include Myristic acid glycidyl ester (9.23%, retention time: 40.358min) and 9,12-Octadecadien-1-ol, (Z, Z)- (8.55%, retention time: 36.788min). Additionally, other constituents such as Squalene (3.16%, retention time: 48.996 min), Hexadecanoic acid, methyl ester (1.65%, retention time: 33.558min), and Pentatriacontane (0.90%, retention time: 50.255min) were also detected **Table (1)**.

Analysis of Methylated Pumpkin Seed Oil Using GC-MS

The GC-MS analysis of the methylated pumpkin seed oil sample revealed the presence of several fatty acid methyl esters (FAMEs) and other volatile compounds **Fig. (2)**. The chromatogram exhibited distinct peaks, with the most abundant compound being 9,12-Octadecadien-1-ol, (Z, Z)- (retention time: 36.799min) contributing 46.17% of the total area. This was followed by 6-Octadecenoic acid, methyl ester, (Z)- (retention time: 36.965 min) with an area percentage of 24.30%, and Hexadecanoic acid, methyl ester (retention time: 33.557min) with 9.05% of the total area. Minor components included Heptadecanoic acid, 16-methyl-, methyl ester (4.45%, R.T.: 37.512min) and 1H-Pyrazole, 4,5-dihydro-3,5,5-trimethyl- (0.78%, R.T.: 3.486min). Details of the identified compounds, their retention times, and relative abundances are summarized in **Table (2)**.

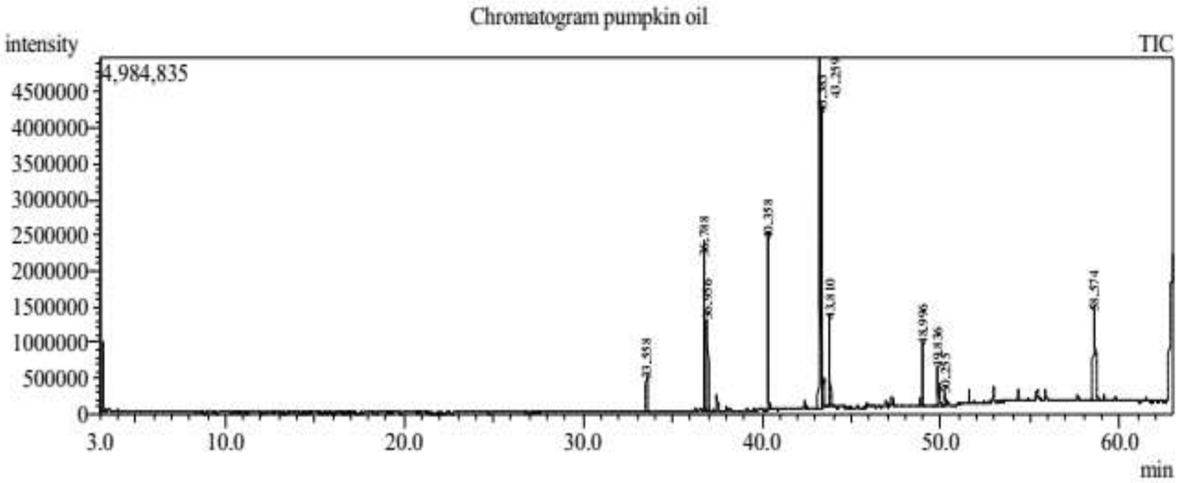

Fig. (1): Total Ion Chromatogram (TIC) of the pumpkin oil sample

Table (1): Major compounds identified in the pumpkin oil sample by GC-MS analysis.

Retention Time (min)	Compound Name	Area %
33.558	Hexadecanoic acid, methyl ester	1.65
36.788	9,12-Octadecadien-1-ol, (Z,Z)-	8.55
36.956	6-Octadecenoic acid, methyl ester, (Z)-	4.53
40.358	Myristic acid glycidyl ester	9.23
43.259	Linoleyl acetate	32.12
43.383	Glycidyl (Z)-9-nonadecenoate	19.45
43.810	Myristic acid glycidyl ester	4.66
48.996	Squalene	3.16
49.836	9,12-Octadecadien-1-ol, (Z,Z)-	3.75
50.255	Pentatriacontane	0.90
58.574	E,E,Z-1,3,12-Nonadecatriene-5,14-diol	12.00

Table (2): major compounds identified in the methylated pumpkin oil sample by GC-MS analysis.

Retention Time (min)	Compound Name	Area %
3.117	Toluene	12.53
3.312	Hexane, 2,5-dimethyl-	0.67
3.440	Heptane, 4,4-dimethyl-	0.35
3.486	1H-Pyrazole, 4,5-dihydro-3,5,5-trimethyl-	0.78
33.557	Hexadecanoic acid, methyl ester	9.05
36.799	9,12-Octadecadien-1-ol, (Z,Z)-	46.17
36.965	6-Octadecenoic acid, methyl ester, (Z)-	24.30
37.050	6-Octadecenoic acid, methyl ester, (Z)-	0.85
37.512	Heptadecanoic acid, 16-methyl-, methyl ester	4.45

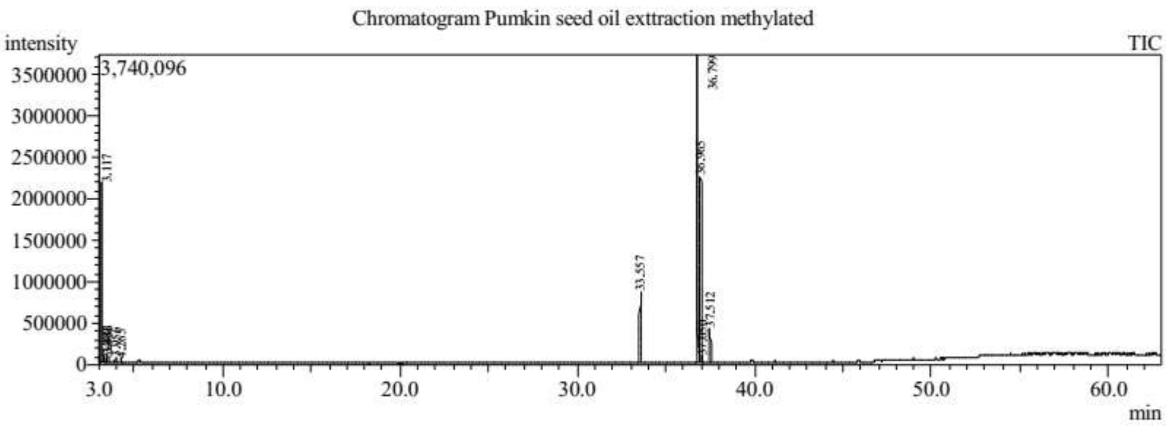

Fig. (2): Total Ion Chromatogram (TIC) of the methylated pumpkin oil sample

Antioxidant assays

The effect of pumpkin seed oil and/ or Squalene on DPPH radical scavenging activity

The DPPH scavenging activity of both pumpkin seed oil and squalene showed a progressive increase with increasing sample volumes. For pumpkin seed oil, the scavenging activity was minimal at lower volumes (e.g., 3.95% at 5 µl) and reached a maximum of 58.45% at 150 µl, with an IC_{50} value of 88.88 µl **Fig. (3A)**. Similarly, squalene exhibited low scavenging activity at smaller volumes (e.g., 5.12% at 0.5 µl) and achieved its highest activity of 58.45% at 2.5 µl. The IC_{50} value for squalene was calculated to be approximately 2.1 µl **Fig. (3B)**.

The effect of Pumpkin Seed Oil and/or Squalene on ABTS radical scavenging activity

The ABTS radical scavenging activity demonstrated a proportional increase with the sample volume for both pumpkin seed oil and squalene. For pumpkin seed oil, the activity was minimal at smaller volumes but gradually increased, reaching a maximum of approximately 80% at 150 µL, with an IC50 value of 73.78 µL **Fig. (4A)**. In parallel, squalene showed a steady rise in scavenging activity, starting from lower volumes and peaking at around 65% at 2 µL, as illustrated in **Fig. (4B)**.

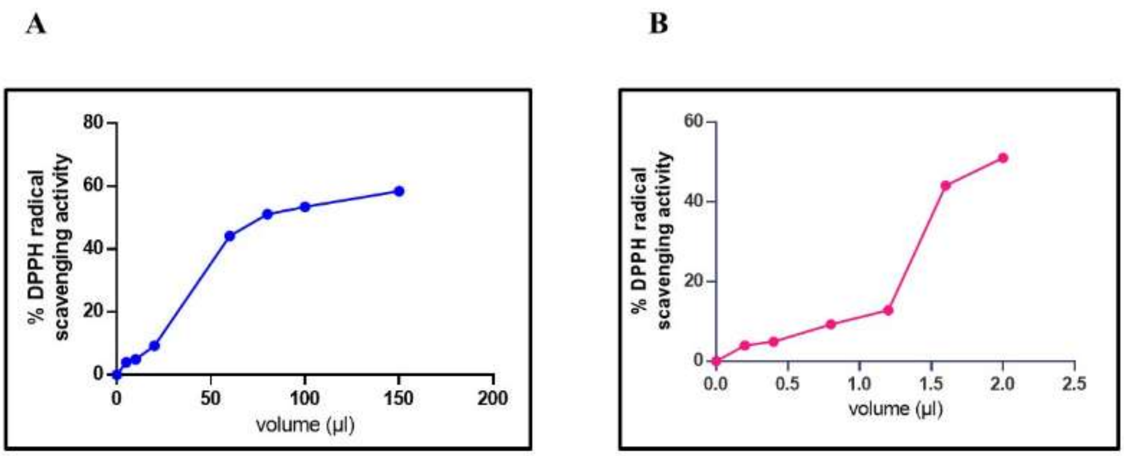

Fig. (3): % DPPH radical scavenging activity of (A) extracted pumpkin seed oil (B) Squalen**e**

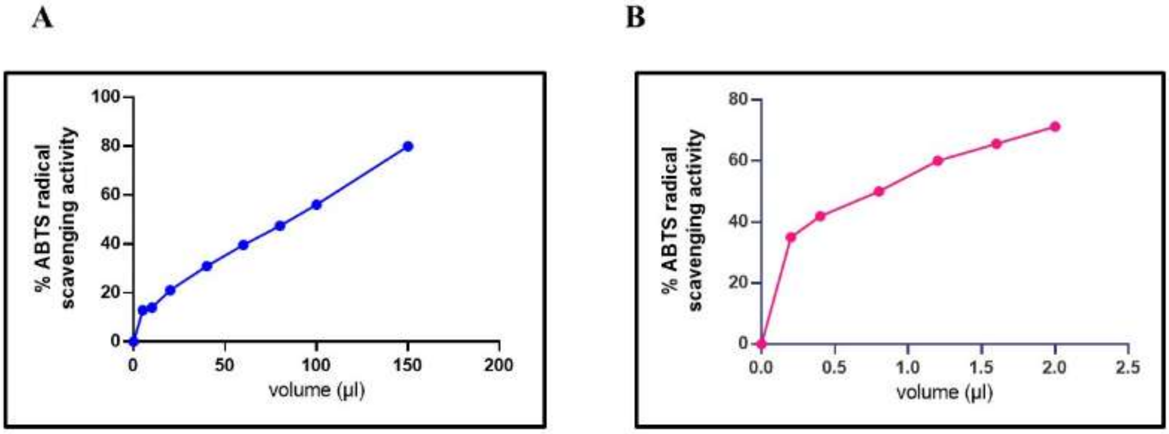

Fig. (4): % ABTS radical scavenging activity of (A) extracted pumpkin seed oil (B) Squalene

Evaluation of reducing power for Pumpkin Seed Oil and/or Squalene

The reducing power of both pumpkin seed oil and squalene increased consistently with the sample volume. For pumpkin seed oil, the highest reducing power was observed at 150 µL, as indicated by the absorbance at 700 nm **Fig. (5A**). Similarly, squalene exhibited a gradual rise in reducing power with increasing volume, reaching its peak at 2 µL, as shown in **Fig. (5B)**.

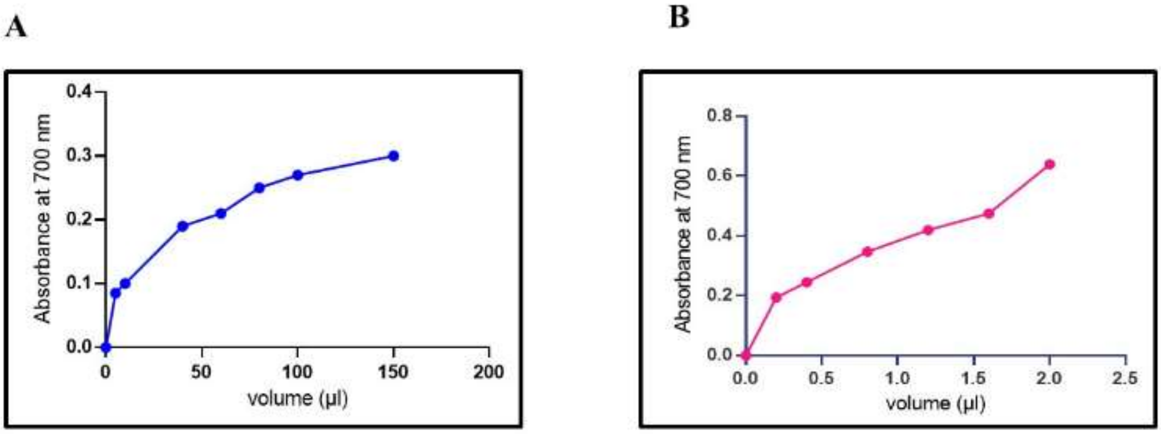

Fig. (5): Reducing power of (A) extracted pumpkin seed oil (B) Squalene.

Total antioxidant capacity of Pumpkin Seed Oil and/or Squalene

The total antioxidant capacity of pumpkin seed oil and squalene was determined using the phosphomolybdate assay, revealing a volume-dependent increase in antioxidant activity for both samples. For pumpkin seed oil, the activity rose from approximately 10 at 25 µL to 30 at 75 µL, and ultimately to 60 at 150 µL, demonstrating a clear proportional relationship between volume and antioxidant capacity **Fig. (6A**). Likewise, squalene displayed a steady increase in antioxidant activity, reaching a maximum of around 40 at 2 µL, as shown in **Fig. (6B)**.

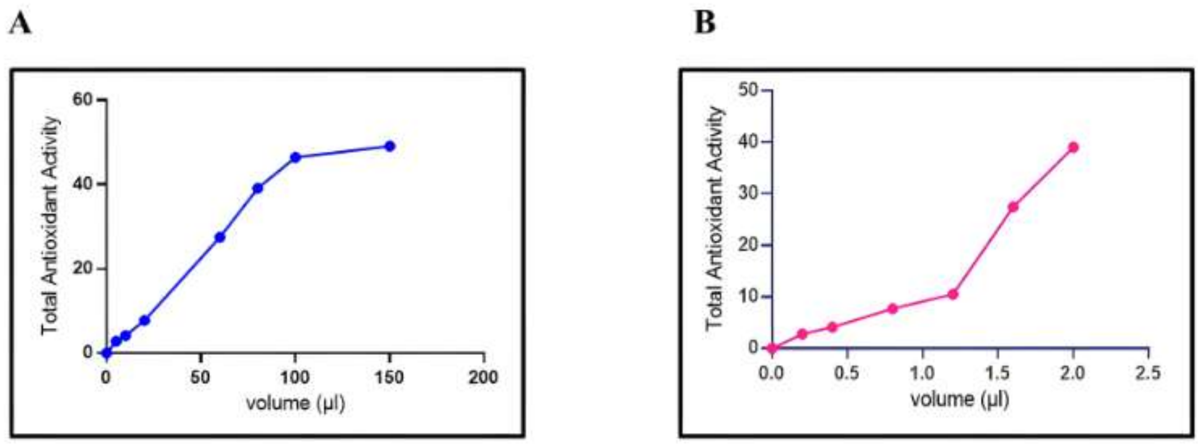

Fig. (6): Total Antioxidant Activity of (A) extracted pumpkin seed oil (B) Squalene.

ADMET features

The ADMET properties of Squalene were analyzed to assess its pharmacokinetics and safety profiles **Fig. (7)**. Squalene satisfied Lipinski's Rule of Five with no violations. It showed moderate human intestinal absorption (HIA) scores and was unable to cross the blood-brain barrier (BBB), ensuring good CNS safety. It demonstrated a favorable safety profile with no AMES toxicity or carcinogenicity alerts. The findings highlight squalene's pharmacokinetics advantages and safety profile, as summarized in **Table (3)**.

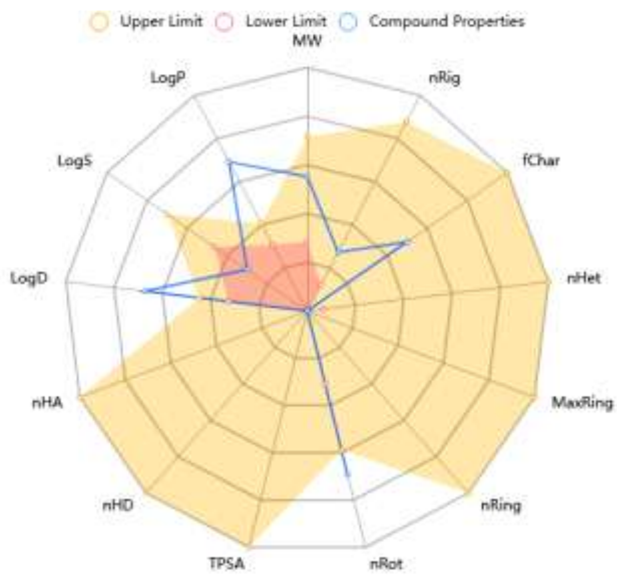

Fig. (7): ADMET profile of squalene

Table (3): ADMET properties of squalene

Property	Optimal Range	Squalene
Molecular Weight (g/mol)	≤ 500	410.39
Topological Polar Surface Area, Å² (TPSA)	≤ 140	0.0
Partition Coefficient (LogP)	≤ 3	10.065
Number of Rotatable Bonds (nROT)	0~11	15
Hydrogen Bond Acceptors (HBA)	0–12	0
Hydrogen Bond Donors (HBD)	0–7	0
Human Intestinal Absorption (%)	≥ 30% (High)	54.8%
Blood-Brain Barrier (BBB)	No	No
AMES Toxicity	Non-toxic	Non-toxic
Carcinogenicity	Non-carcinogenic	Non-carcinogenic

Discussion

Pumpkin seeds are highly nutritious, rich in essential nutrients such as omega-3 fatty acids, magnesium, and zinc, making them a valuable addition to a healthy diet. They are known for their ability to support heart health, regulate cholesterol levels, and reduce the risk of chronic diseases due to their antioxidant and anti-inflammatory properties **(Bashir et al., 2025)**. Beyond the seeds, pumpkin seed oil has gained significant attention for its high content of bioactive compounds, offering numerous therapeutic benefits. This oil is versatile and used as a culinary ingredient and a dietary supplement, further highlighting the nutritional and medicinal value of pumpkin seeds and their oil **(Alsalamah et al., 2025).**

The method of extraction significantly influences the yield and composition of pumpkin seed oil, affecting its nutritional quality, bioactive compound content, and overall therapeutic potential **(Can-Cauich et al., 2019).** The yield of pumpkin seed oil in this study was 41.45% (v/w), extracted using an organic solvent (hexane). In contrast, a previous study reported a yield of 7.8%, where the extraction was carried out using chloroform via maceration **(Ikpa and Chidozie-Ikpa, 2024).** Additionally, the yield obtained in this study is relatively high compared to the values reported in other countries, such as Uganda (35.6%) **(Kukeera et al., 2015)**, Algeria (33.5%) **(Benalia et al., 2015)**, and Bangladesh (23%) **(Amin et al., 2019)**. However, it is slightly lower than yields reported from Ethiopia (43.6% to 50.5%) **(Hagos et al., 2023)** and some studies in Nigeria, where values ranged from 42.1% to 55.8% **(Uba, 2019, Yusuf et al., 2021, Bwade et al., 2013).** These variations in oil yield can be attributed to differences in pumpkin seed varieties, environmental conditions, and extraction methods, including the type of solvent and procedures used.

In this work, GC-MS analysis of pumpkin seed oil identified various chemical constituents, with Linoleyl acetate being the most abundant, followed by Glycidyl (Z)-9-nonadecenoate and E, E, Z-1,3,12-Nonadecatriene-5,14-diol. These findings differ from a study conducted by **(Ikpa and Chidozie-Ikpa, 2024)**, where cis-Vaccenic acid was the dominant compound, highlighting the influence of seed variety and environmental factors on the chemical composition. Prior studies discussed that compounds such as linoleyl acetate and glycidyl esters are not typical natural constituents of edible oils but they can appear due to thermal degradation, transesterification, or solvent or derivatization artifacts during GC–MS analysis **(Cheng et al., 2017, Arisseto et al., 2018, Mat Shukri, 2023).** Interestingly, the presence of squalene in this study aligns with prior findings which emphasized its therapeutic potential due to antioxidant and cholesterol-lowering properties **(Alsalamah et al., 2025)**. Another study identified tocopherols, β-carotene, squalene, and polyphenolic compounds as the major bioactive compounds present in all pumpkin seed oil samples **(Singh and Kumar, 2023)**. The use of hexane as the extraction solvent may have contributed to preserving a diverse range of bioactive compounds, as supported by **(Can-Cauich et al., 2019)**, which noted the impact of extraction methods on the retention of bioactive molecules. These variations underscore the importance of extraction techniques and regional factors in shaping the chemical profile of pumpkin seed oil.

In the current study, the GC-MS analysis of Methylated oil revealed the dominance of unsaturated fatty acids, particularly 9,12-Octadecadien-1-ol (Z, Z)- and 6-Octadecenoic acid, methyl ester (Z)-, which aligns with previous studies that identified linoleic acid (C18:2, omega-6) as the most abundant fatty acid in pumpkin seed oil, accounting for over 50% of the total fatty acids **(Hagos et al., 2023, Benalia et al., 2015)**. This supports the high prevalence of polyunsaturated fatty acids (PUFAs) in pumpkin seed oil, which are considered essential for human health. Moreover, the presence of Hexadecanoic acid, methyl ester, in this study agrees with its identification as a significant saturated fatty acid in other pumpkin seed oils, such as those from Algeria and Kenya, which reported palmitic acid as a major component **(Benalia et al., 2015, Karanja et al., 2014)**. These variations in fatty acid composition can be attributed to differences in seed variety, geographic origin, and environmental conditions, further emphasizing the nutritional value of pumpkin seed oil as a rich source of essential fatty acids.

Antioxidant compounds safeguard cells from the harmful effects of reactive oxygen species (ROS) and are recognized for their effectiveness in preventing various modern diseases, such as cancer and heart disease **(Kulaitienė et al., 2018)**. In this study, DPPH and ABTS were used to assess the antioxidant potential of the extracted pumpkin seed oil. Both methods demonstrated a dose-dependent increase in scavenging activity, with IC_{50} values of 88.88 µL and 73.78 µL, respectively, and these findings are consistent with **(Boujemaa et al., 2020)**. Furthermore, the antioxidant properties of pumpkin seed oil have been demonstrated in a prior study where its administration was shown to mitigate the adverse effects of aflatoxin exposure. The biochemical parameters of the group treated with pumpkin seed oil were significantly improved, approaching those of the control group **(Eraslan et al., 2013).**

Similarly, squalene has shown significant antioxidant properties, contributing to its protective effects against oxidative stress. While an earlier study **(Warleta et al., 2010)** found no evidence of activity against stable radicals such as DPPH and ABTS, more recent findings have highlighted its antioxidative potential. For instance, squalene exhibited 32% scavenging activity in the DPPH assay, significantly reduced ROS levels, and upregulated antioxidant genes such as *sod* and *gpx4b*, protecting zebrafish from oxidative damage **(Zhang et al., 2023).** These findings are supported by a study showing squalene’s ability to reduce inflammatory markers like *tnfa* and *cox2* while enhancing antioxidant defenses, making it a promising compound for combating oxidative stress **(Ibrahim and Naina Mohamed, 2021).**

Among the various bioactive compound identified in the pumpkin seed oil, squalene (3.16%) stands out as a particularly valuable compound due to its well- documented biological and therapeutic properties. While other compounds, such as Linoleyl acetate (32.12%) exhibits antioxidant activity **(Ge et al., 2024)** and Glycidyl (Z)-9-nonadecenoate (19.45%), was present in higher concentrations without a clear defined biochemical role, squalene’s multifunctionality and industrial relevance give it a distinct advantage. It is a potent antioxidant with cholesterol-lowering, anti-inflammatory, and antimicrobial properties, making it especially relevant for cardiovascular health, immune support, and skin protection **(Dasari et al., 2025).** Additionally, its extensive application-ranging from pharmaceutical to cosmetics- highlights its importance as a bioactive compound worthy of further investigation, while other compounds, including E,E,Z-1,3,12-Nonadecatriene-5,14-diol and unsaturated fatty acids (such as 9,12-Octadecadien-1-ol and 6-Octadecenoic acid, methyl ester), enhance the oil's overall nutritional and therapeutic potential, their narrower application scope and lesser scientific focus make squalene a more promising candidate for standalone studies **(Siano et al., 2016).**

To further reinforce the suitability of squalene for therapeutic applications, an ADMET analysis was conducted to evaluate its pharmacokinetics and safety profiles. The result confirmed that squalene complies with Lipinski's Rule of Five, indicating favorable drug-likeness and oral bioavailability. Its moderate intestinal absorption (54.8%) is consistent with expectations for lipophilic compounds and supports its potential for oral formulations. Furthermore, its inability to cross the blood-brain barrier (BBB) ensures a high level of safety for systematic therapies by eliminating risks of CNS toxicity. The absence of AMES toxicity and carcinogenic alerts further highlights its non-toxic nature, supporting its long-term safety for pharmaceutical and nutraceutical applications. These results align with squalene's established reputation as a safe and effective compound widely used as a vaccine adjuvants **(Mendes et al., 2022).**

Despite the findings of this study, pumpkin seed oil has not yet been widely applied or tested in broader experimental settings. However, identifying the chemical compounds present within the oil provides a foundation for future research. This could pave the way for studying the efficacy of individual compounds, such as squalene, which holds significant potential for various applications.

Limitations and Future Prospectives

The study did not include toxicity or safety assays for the oil or its major constituents. These analyses are essential for ensuring safe applications in food, pharmaceutical, and cosmetic industries. Further research should focus on detailed toxicity assessments and pharmacokinetic evaluations.

Additionally, while this study focused on the extraction and characterization of pumpkin seed oil, further investigations should prioritize bioactive components with well-defined roles and industrial relevance, such as squalene, to explore their individual properties and potential applications.

Conclusion

Pumpkin seed oil extracted from *Cucurbita pepo* cultivated in Egypt demonstrated a high yield and a rich composition of bioactive constituents, particularly linoleyl acetate, glycidyl (Z)-9-nonadecenoate, and squalene. The oil exhibited strong antioxidant potential, as evidenced by its dose-dependent radical scavenging and reducing activities in DPPH, ABTS, reducing power, and phosphomolybdate assays. Moreover, ADMET analysis of squalene indicated favorable pharmacokinetic properties and safety profiles, supporting its potential use in nutraceutical and pharmaceutical formulations. Overall, the findings confirm that pumpkin seed oil is a valuable natural source of antioxidants with promising applications in health-promoting products, medications, and functional foods. Future research should focus on isolating and characterizing the individual bioactive constituents responsible for the antioxidant activity of pumpkin seed oil to better understand their mechanisms of action. *In vitro* and *in vivo* studies are recommended to validate the observed findings and assess the oil's safety, efficacy, and bioavailability in biological systems. Additionally, exploring the synergistic effects of pumpkin seed oil with other natural antioxidants could enhance its therapeutic potential.

Declarations

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Consent for publication

Not applicable

Availability of data and materials

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تحضير وتحليل زيت بذور اليقطين المزروعة في مصر: النشاط المضاد للأكسدة والمكونات الرئيسية باستخدام التحليل الطيفي GC-MS وتحليل ADMET للسكواليين

علا محمود يحيى [1*]، عبير عبد الحميد خميس[1]، السيد إبراهيم سالم[2]، ماريان نبيل جرجس[1]

[1] شعبة الكيمياء الحيوية، قسم الكيمياء، كلية العلوم، جامعة طنطا، طنطا، مصر.

[2] قسم علم الحيوان، كلية العلوم، جامعة طنطا، طنطا، مصر.

يمتلك زيت بذور اليقطين المستخرج من بذور نبات *Cucurbita pepo* المزروعة في مصر إمكانات غذائية وعلاجية كبيرة نظرًا لاحتوائه على مجموعة غنية من المركبات النشطة بيولوجيًا. هدفت هذه الدراسة إلى تقييم التركيب الكيميائي والنشاط المضاد للأكسدة للزيت لدعم تطبيقاته في مجالات الصحة والصناعة. باستخدام طريقة الاستخلاص بجهاز سوكسليت، تم الحصول على نسبة عائد بلغت 38.15% (وزن/وزن). كشف تحليل الكروماتوغرافيا الغازية-مطياف الكتلة (GC-MS) عن وجود مكونات رئيسية مثل أسيتات اللينوليل (32.12%)، غليسيديل-9-(Z) نوناديكينوات (19.45%)، وسكواليين (3.16%). كما أظهر تحليل الزيت الميثيلي وجود أحماض دهنية غير مشبعة، بما في ذلك 9،12-أوكتاديكايدين-1-أول (46.17%) و6-أوكتاديكينويك أسيد (24.30%). أظهرت اختبارات النشاط المضاد للأكسدة، والتي شملت اختباري 2،2-ثنائي فينيل-1-بيكريل هيدرازيل (DPPH) و2،2'-أزينو-بيس (3-إيثيل بنزثيازولين-6-سلفونيك أسيد)(ABTS) ، بالإضافة إلى اختباري القدرة الاختزالية وطريقة الفسفوموليبدات، قدرة قوية على إزالة الجذور الحرة وزيادة النشاط الاختزالي بطريقة تعتمد على الجرعة. أظهر تحليل ADMET للسكواليين خصائص دوائية ملائمة، بما في ذلك الامتثال لقاعدة ليبنسكي الخماسية، وعدم وجود إشارات للسمية أو التسرطن، وامتصاص معتدل في الأمعاء. تسلط هذه النتائج الضوء على الإمكانات المضادة للأكسدة والعلاجية لزيت بذور اليقطين، مما يعزز ملاءمته لتطبيقات الأغذية الوظيفية، والصناعات الدوائية، ومستحضرات التجميل. ويجب أن تركز الأبحاث المستقبلية على عزل وتحسين المركبات النشطة الرئيسية لاستخدامات محددة.