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Antimicrobial Efficacy of Pumpkin (Cucurbita maxima) Seed Oil: A multifunctional Ingredient for Health and Food Preservation

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Abstract

The antimicrobial efficacy, nutritional value, and physicochemical characteristics of pumpkin seed oil (PSO) were investigated. PSO demonstrated significant antimicrobial activity at 100% concentration, with inhibition zones of 0.95 mm (*Bacillus subtilis*), 0.85 mm (*Staphylococcus aureus*), 0.65 mm (*Salmonella typhi*), 9.0 mm (*Rhizopus*), and 8.0 mm (*Saccharomyces cerevisiae*). At lower concentrations (75% and 50%), PSO also showed antimicrobial activity, notably against *Saccharomyces cerevisiae* (12.0 mm, at 75%), *Aspergillus flavus* (10.0 mm at 50%), and *Escherichia coli* (0.75 mm at 50%). Nutritional analysis of pumpkin seeds revealed a low moisture content (4.34%) and significant amounts of crude protein (33.81%), crude ash (6.65%), crude oil (37.95%), and carbohydrates (13.25%). Additionally, the seeds were rich in essential minerals, containing 0.27% Ca, 0.08% Mg, 0.02% Na, 0.21% K, 0.22% P, 0.15% Zn, 0.04% Fe, and 0.24% Cu. The physicochemical parameters of the oil, such as a refractive index of 1.62, density of 0.97 g/cm^3, viscosity of 57.0 poise, and acid value of 2.09 mg NaOH/g, indicates excellent stability and high unsaturation. PSO was found to be rich in essential fatty acids, mainly linoleic acid (78.9%) and palmitic acid (14.8%). The study highlights the potential of PSO as a natural agent in combating antibiotic-resistant bacteria, a stable, nutrient-rich oil with significant antimicrobial properties, making it a promising candidate for various applications in the food and health industries.

Keywords: Pumpkin; Antimicrobial; Inhibition zone; Fatty acids; Physicochemical properties; Nutritional value; Bioactive compounds.

1 .Introduction

Plants are natural sources of many components that can cure various diseases. In modern lifestyle, the use of synthetic drugs has become important, but their adverse effects on human beings are always present [1]. The role of plants, especially fruits and vegetables, is highly recognized for promoting good health and reducing the risk of diseases. The increasing danger posed by ARB has prompted the search for natural

antimicrobial substances that provide both effectiveness and safety. PSO has gained considerable attention for its potential antimicrobial properties, which have been studied in terms of nutritional properties and biological activities [2,3]. Pumpkin seeds (Cucurbita maxima) have long been valued in many cultures for their nutritional benefits. They are a rich source of essential fatty acids, proteins, vitamins, and minerals [4–7]. Studies have shown that PSO is not just a source of nutrition, but also contains bioactive compounds that have health-promoting properties. These properties include anti-inflammatory, antioxidant, and antimicrobial activities [8]. The antimicrobial activity of PSO has been well-documented in studies.

Despite being often considered underutilized and regarded as waste it is now gaining attention in the fields of agriculture, nutraceuticals, and the food industry due to its containing a significant amount of bioactive compounds that offer substantial health benefits [7,9]. Recent studies have focused on exploring the health-promoting properties of these nutrient-rich seeds, which can be used in various food applications [10–14]. Historically, PSO has been utilized in traditional medicine in several countries, including China, Yugoslavia, Brazil, India, Mexico, Argentina, and America, for the treatment and prevention of chronic diseases [15]. Pumpkin seeds are a rich source of oil, comprising approximately 30-50%, as well as fatty acids such as linoleic, oleic, stearic, and palmitic acids. They also contain carotenoids (β-carotene, α-carotene), vitamins (α, β, γ, δ-tocopherol), and minerals [16–18]. β-carotene helps protect the skin from sun damage and acts as an anti-inflammatory agent, while α-carotene is believed to slow down the aging process, reduce the risk of cataract development, and inhibit tumor growth. Vitamin E, in the form of tocopherols, protects cells from oxidative damage by inhibiting the oxidation of unsaturated fatty acids in the cell membrane. Pumpkin seeds, commonly consumed as snacks, are also rich in zinc [19], polyunsaturated fatty acids [20–22], and phytosterols like β-sitosterol.[23] .

Despite the acknowledged health benefits of pumpkin seeds, they are underutilized in certain regions, such as Sudan, where they are mostly discarded or used as snacks. However, these seeds possess properties that are beneficial for managing conditions like hypertension and diabetes [24,25]. The antimicrobial activity of pumpkin seeds also makes them suitable for use as natural preservatives and in alternative therapies [26]. Therefore, the primary objective of this study is to evaluate the effectiveness of PSO as an antimicrobial agent against selected pathogenic microorganisms, with a specific focus on its ability to combat ARB. Additionally, the study aims to establish a correlation between the antimicrobial properties of PSO and its physical and chemical characteristics, as well as its fatty acid profile. We hypothesized that PSO demonstrates significant antimicrobial activity against a wide range of pathogens, including ARB. The antimicrobial effectiveness of PSO is influenced by its physicochemical properties, particularly its high concentration of unsaturated fatty acids and bioactive compounds. PSO can be used as a natural preservative in food products, thereby enhancing food safety and prolonging shelf life. The research findings from this study will provide valuable insights into the potential of PSO as a

natural antimicrobial agent, applicable in both the food industry and healthcare settings. By integrating the antimicrobial effectiveness of PSO with its nutritional and physicochemical profiles, this study aims to contribute to the development of functional food products and natural therapeutic agents.

2. Materials and Methods

2.1. Raw materials

Pumpkin fruits (*C. maxima*) were purchased from The Central Market (Khartoum State, Sudan), and utilized for the experiment without further storage.

2.2. Pumpkin seeds preparation

The seeds were isolated from pumpkin fruits, husked to remove impurities, and dried at 45 ºC for 30 hours using (Memmert Type U40 oven dryer, West German). The dried seeds were then ground into powder using a mortar. The powdered seeds were mixed with n-hexane in a volumetric flask using an orbital shaker (Orbitek, Scigenics Biotech, India). After 24 h, the mixture was centrifuged at 4000 rpm for 20 min using (MPW-56 laboratory centrifuge, German), and the supernatant was collected. The solvent was then removed using a rotary vacuum evaporator (RE 100-Pro, D LAB, China), and the recovered oil was kept at −18 °C until further use [7].

2.3. Antimicrobial activity test

The experiment followed the guidelines of the National Committee for Clinical Laboratory Standards [27] to determine antimicrobial activity. The disc diffusion method was employed using pure cultures of pathogenic bacteria. Nutrient agar 28 g was suspended in 100 mL of distilled water, heated to boiling to completely dissolve the medium, and then sterilized through autoclaving at 15 Ibs pressure and 121 °C for 15 min. The surface of the nutrient agar plate was covered with broth culture for 18 h, excess liquid was removed, and the inverted plate was left to dry on the bench for 1 h before being incubated for 30 min. Different concentrations of the oil sample were prepared for dilution. Filter paper discs were impregnated with the oil sample and placed on the surface of the plate with enough space between them. The plates were then incubated at 37 °C for 24 h. After incubation, the plates were examined for zones of inhibition, which indicated bacterial growth suppression around the sample discs. The diameter of the inhibition zone was measured. If no inhibition zones were observed around other discs, it indicated that the organism was resistant to the sample extraction disc.

2.4. Proximate analysis of pumpkin seeds

2.4.1. Moisture determination

Moisture content was measured using a moisture analyzer instrument (MAC 50/1/WH). Approximately, 5 g of powdered seeds were placed in the dish of the instrument, closed, and dried at 45 ºC until constant weight, and then the result was recorded [28].

2.4.2. Determination of oil content

Oil content was determined using the Soxhlet apparatus following the method reported by [29]. An empty, clean, and dry round bottom flask was weighed. 5 g of powdered seeds were placed in a clean extraction thimble and covered with cotton

wool. The thimble was placed in an extractor. Extraction was carried out for 8 h at the range of 40 to 60 °C with 200 mL hexane. The heat was regulated to obtain at least 15 siphoning per hour. After 8 h, the solvent was evaporated using a rotary evaporator. The flask was placed in an oven at 105 °C until completely dry, then cooled in a desiccator and weighed. The crude oil was calculated by using the formula (1).

$$\text{Crude oil content (\%)} = \frac{(W2-W1)}{Ws} \times 100 \quad (1).$$

Where:

W_1 = Weight of extraction flask.

W_2 = Weight of extraction flask with oil.

W_S = Weight of sample.

2.4.3. Total ash

Ash content was determined using the AACC International Method 08-16.01 with slight changes. Briefly, about 3 g of the sample was placed in a clean, dry crucible and pre-ashed in a fume hood for 30 min. The crucible was then ignited in a muffle furnace at 550 °C for 3 h until light grey ash was obtained. The crucible was removed from the furnace to a desiccator, cooled, and weighed. The ash content was calculated by using the equation (2).

$$\text{Ash content (\%)} = \frac{(W2-W1)}{Ws} \times 100 \quad (2).$$

Where:

W_1 = Weight of empty crucible.

W_2 = Weight of crucible with ash.

W_S = Weight of sample.

2.4.4. Crude fiber

Fiber content was determined according to [30] with minor modifications. About 2 g of defatted pumpkin seeds powder was taken in a 250 mL conical flask. 200 mL of boiling sulfuric acid (H_2SO_4) was added and heated for 30 min, then the sample was filtered using filter paper No. 41. Then, the residue was transferred to a 250 mL conical flask, and 200 mL of KOH solution was added and boiled for 30 min. The liquid was filtered, and the residue was dried at 105 °C in an oven for 2 h, cooled to room temperature, weighed, and ashed at 600 °C in a muffle furnace for 2 h. The residue was cooled at room temperature and weighed. The fiber content was calculated by using the formula (3).

$$\text{Crude Fiber content (\%)} = \frac{(W1-W2)}{Ws} \times 100 \quad (3).$$

Where:

W_1 = Weight of crucible with dry residue before ashing.

W_2 = Weight of crucible with ash.

W_S = Weight of sample.

2.4.5. Crude protein

Crude protein was determined by using the micro-Kjeldahl method according to [31].

2.4.5.1. Digestion of protein:

About 0.2 g of the sample was weighed and placed in a 50 mL Kjeldahl digestion flask. Then, about 1 g of catalyst mixture (96% anhydrous sodium sulfate and 3.5% copper sulfate) was added, followed by 3.5 mL of concentrated H_2SO_4. The contents were heated on an electric fume for 2 h until the color changed to blue-green. The tubes were then removed from the digester, allowed to cool, and transferred to the Kjeldahl distillation apparatus.

2.4.5.2. Distillation

The digested sample was transferred to the distillation unit, and 15 mL of 40% NaOH was added. The ammonia was received in a 100 mL conical flask containing 10 mL of 2% boric acid, and 3-4 drops of methyl red were added. The distillation continued until the volume reached 50 mL.

2.4.5.3. Titration

The contents of the flask were titrated against 0.1N HCl. The titration reading was recorded, and the crude protein was calculated by using the formula (4).

$$\text{Crude protein (\%)} = \frac{(TF\text{-}B)\times N\times 0.014\times 100\times 6.25}{Ws}\times 100 \quad (4).$$

T_F = Titer figure.

B = Blank.

N = Acid normality.

W_S = Weight of sample.

0.014 = mL equivalent of N atom.

6.25 = Protein factor.

2.4.6 Total carbohydrate content

Total carbohydrates were determined by difference according to [32] with minor changes. The total carbohydrate content was calculated according to the equation

$$\text{CHO} = 100 - (Moisture\% + Crude\ oil\% + Ash\% + Crude\ fiber\% + Crude\ protein\%) \quad (5).$$

2.5. Minerals content of pumpkin seeds

Minerals content was estimated using an atomic absorption spectrophotometer (1100 B Perkin-Elmer, Germany). Around, 3 g of pumpkin seeds powder was taken and pre-ashed in a fume hood at 250 °C for 15 min, then transferred to a muffle furnace at 600 °C for 2 h. 10 mL of HCl solution was added and cleaned with distilled water, then filtered using filter paper.

2.5.1. Phosphorus

A 5 mL of solution was placed in a 25 mL volumetric flask. 5 mL of yellow detector and 15 mL of distilled water were added to complete the flask. After 30 min, the reading of sorption was taken in a spectrophotometer.

2.5.2. Calcium and magnesium

About 5 mL of the sample was taken from the solution and placed in a 50 mL volumetric flask, then filled with distilled water. 10 drops of buffer solution and 3 drops of EBT detector were added to 5 mL of the diluted solution, which turned blue and was titrated against EDTA from a burette.

$$\text{Ca + Mg} = [V_{EDTA}\times N_{EDTA}/5\text{mL} \times 1000]\times 50/5\text{mg}/1 \quad (6).$$

2.5.3. Calcium

A 5 mL of the diluted solution was placed in a volumetric flask, and 10 drops of NaOH solution 4N and 50 mL of peroxide detector were added and titrated against EDTA. $Ca = [V_{EDTA} \times N_{EDTA}/5mL \times 1000] \times 50/5$ mg/L (7).

[Ca+Mg]-[Ca] (8).

2.6. Physiochemical properties of PSO

2.6.1. Refractive index

To determine the refractive index, the method outlined by [33] was followed. The refractive index of the oil sample was measured by using a refractometer. The process began by cleaning the chamber of the refractometer with a solvent. The apparatus was then placed in sunlight and calibrated using distilled water. After that, the oil sample was placed in the chamber and subsequently closed. The refractometer's disc was moved back and forth until the crossbar was properly aligned and recorded at 35 °C.

2.6.2. Viscosity

The viscosity of the oil was measured with the MAAKE viscometer 6plus, which is manufactured by Thermo Electron Corporation, Thermo Electron (Karlsruhe) GmbH. The viscometer has the following specifications: type - 387-0100, NO - 387200612208.100, voltage - 240V, frequency - 60Hz, and current - 0.2A. It has an IP rating of 20 and was made in the EEC.

2.6.3. Density

The density bottle was first cleaned with a solvent and subsequently placed in the oven for 15 min and the weight of the empty bottle was then measured. Then, the bottle was filled with distilled water and the weight was recorded. After emptying the distilled water and cleaning the bottle once more, it was filled with an oil sample, and the final weight was measured. The density was then calculated at 30 °C using the methods specified in AOAC (1990), as demonstrated in the equation (9).

$$\text{Crude protein (\%)} = \frac{A\text{-}B}{\text{A-C}} \quad (9).$$

A ≡ Weight in g of density bottle with oil.

B ≡ Weight in g of density bottle.

C ≡ Weight in g of density bottle with water.

2.6.4. Color value

The color was determined using a Lovibond tintometer type D. Values of red, yellow, and blue color were examined following the AOAC methods (1990). The oil sample was filled in a clean glass cell measuring 1×15 cm and placed in the tintometer. The light source was turned on, and the color of the oil sample was compared to the filters in the instrument by looking through the eyepiece. The yellow color was adjusted to 11.0, and then the slides were readjusted until a color match was achieved.

2.6.5. Peroxide value

The peroxide value of the oil sample was determined using the AOAC method (1990). Approximately 2 g of the oil sample was transferred into a 250 mL conical flask. Then, 15 mL of acetic acid and 10 mL of chloroform were added. The solution was gently swirled to dissolve the oil. Afterward, 1 mL of saturated potassium iodide

was added, and the flask contents were shaken and left to stand in the dark for 10 min. The starch indicator was then added. The contents were titrated with 0.01 mL of sodium thiosulfate until the yellow color almost disappeared. The amount of sodium thiosulfate required (a) was recorded, and the process was repeated for the blank. The number of 0.01 mL sodium thiosulfate required for the blank (b) was recorded. The peroxide value was calculated by using the equation (10).

$$\text{Peroxide value} = (a-b)\times M/W_S\times 1000 \quad (10).$$

Where:

a ≡ Titration of the sample.

b ≡ Titration of blank.

M ≡ Molarity of sodium thiosulfate.

W_S ≡ Weight of the sample.

2.6.6. Acid value

Approximately, 2 g of oil sample was dissolved in a solvent mixer. Then, 25 mL of ethanol, 25 mL of diethyl ether, and 0.1 mL of phenolphthalein were added to the solution. The solution was then titrated with sodium hydroxide until the pink endpoint appeared. The determination was carried out according to the British Standard Institution (1958) and calculated using the equation (11).

$$\text{Acid value} = (a-b)\times N(NaOH)\times M_W(NaOH)/W_S \quad (11).$$

a ≡ Reading of oil.

b ≡ Reading of the blank.

N ≡ Normality of (NaOH).

M_W ≡ Molecular weight of (NaOH).

Ws ≡ Weight of the sample.

2.6.7. Saponification value

The determination of the saponification value (SV) was carried out using the AOAC method (1990). To start, 2 g of oil sample was weighed and transferred into a 500 mL conical flask. Afterward, 20 mL of 0.5N KOH was added, and a cooling pipe was fixed onto the flask. The mixture was gently heated for 1 h, with frequent checks, and then allowed to cool. After cooling, 1 mL of phenolphthalein indicator was added, and the solution was titrated with 0.5 mL HCL. The volume was recorded using equation (11).

2.6.8. Iodine value

2 g of the oil sample was weighed and transferred into a 250 mL conical flask. Then, 10 mL of carbon tetrachloride (CCl_4) was added to dissolve the sample. Then, 20 mL of Wij's solution was added, and the mixture was allowed to sit in a dark place for 30 min. After that, 15 mL of 10% potassium iodide and 100 mL of distilled water were mixed in, followed by the addition of a starch indicator. The mixture was then titrated with 0.1N Na_2SO_3. Finally, the result was recorded by using equation (12).

$$\text{Iodine value} = (b-a)\times 1.269/W_S \quad (12).$$

2.6.9. Unsaponifiable matter

The unsaponifiable value was determined following the AOAC method (1990). Firstly, 2 g of the oil sample was weighed in a 250 mL conical flask. Then, 30 mL of

alcoholic KOH solution was added to the flask. The mixture was boiled under reflux for 1 h until saponification was complete. After that, the condenser was rinsed with 10 mL of ethyl alcohol. The warm saponification mixture was transferred to a separating funnel and made alkaline by adding 1 mL of 3 N KOH solution. The mixture was then washed with 50 mL of diethyl ether. The diethyl ether layer was separated and washed with 20 mL of aqueous KOH (0.5 N) followed by 20 mL of distilled water. The diethyl ether layer was collected in weighed dishes, evaporated, and the residue was dried at 80 °C in an oven. The unsaponifiable value was determined using the following equation (13).

$$\text{Unsaponifiable matter \%} = M_2\text{-}M_1/W_S \times 100 \quad (13).$$

$W_1 \equiv$ weight of the empty dish.

$W_2 \equiv$ weight of dish with sample.

$W_S \equiv$ weight of the sample.

2.7. Determination of fatty acids profile

2.7.1. Sample preparation

PSO (2 g) was taken in test tubes and 7 mL of alcoholic NaOH was added by dissolving 2 g NaOH in 100 mL methanol. After that, 7 mL of alcoholic H_2SO_4 1%, along with 99 mL methanol was added and the mixture was shaken by vortex for 3 min. The solution was then left overnight. After that, 2 mL of supersaturated NaCl and 2 mL of normal hexane were shaken for 3 min, resulting in the formation of 2 layers. The hexane layer, which contains the fatty acid, was collected, while the lower layer, the aqueous layer, was left behind. Then, 5 µL of the collected hexane was taken and diluted with 5 mL of diethyl ether. Additionally, 1 g of sodium sulfate was used as a drying agent. The mixture was then filtered through a syringe filter with a pore size of 0.45 µm. The filtered solution was transferred directly to a GC/MS vial, and 1 ml was injected directly into the GC-MS for analysis.

2.7.2. GC-MS analysis

The qualitative and quantitative analysis of PSO was performed using the GC-MS technique with the GC-MS-QP2010Ultra instrument. The instrument was set to the following parameters: a column (Rtx-5 ms-30 m Length × Diameter 0.25 µm), sample injection in split mode, helium as the carrier gas with a flow rate of 1.61 mL/min, a temperature program starting from 60 °C with a rate of 10 C/min up to a final temperature of 300 °C, held for 5 min. The injection port temperature was set at 300 °C, the ion source temperature at 200 °C, and the interface temperature at 250 °C. For the analysis, the sample was scanned in the range of m/z 40-500 charges to ratio using the scan mode. The total run time was 29 min. Component identification was achieved by comparing their retention times and mass fragmentation patterns to those available in the National Institute of Standards and Technology (NIST) library.

2.8. Statistical analysis

The experimental runs were conducted in triplicate, and the data obtained were presented as mean values ± standard deviations (SD). The recorded data underwent one-way ANOVA with Tukey's test ($P<0.05$) using Statistic software (version 8.1, USA) to determine significant differences between the mean values. The figures in

this study were drawn using GraphPad Software (GraphPad Prism 8.0.0, California, USA).

3. Results and discussion

3.1. Antimicrobial efficacy of PSO

Results of antimicrobial activities of PSO against various pathogenic microorganisms are presented in **Fig. 1**. At a 100% concentration, PSO revealed significant activity, with zones of inhibition 0.95±0.07 mm against *Bacillus subtilis*, 0.85±0.07 mm against *Staphylococcus aureus*, 0.65±0.07 mm against *Salmonella typhi*, 9.0±1.41 mm against *Rhizopus*, and 8.0±1.41 mm against *Saccharomyces cerevisiae*. At lower concentrations (75% and 50%), PSO also showed a similar trend, particularly against *Saccharomyces cerevisiae* (12.0±2.82 mm at 75% concentration), *Aspergillus flavus* (10.0±0.41 mm at 50% concentration), and *Escherichia coli* (0.75±0.07 mm at 50% concentration). No inhibitory effect was observed against *Aspergillus niger*. A study by Pandey et al [34] showed that PSO is an excellent antimicrobial agent, especially against *Bacillus subtilis* and *Staphylococcus aureus*, highlighting its potential as a natural antimicrobial agent [1]. These findings highlight PSO's relevance in food preservation and as an alternative treatment for bacterial infections, while its selective efficacy suggests the need for targeted applications. Further research is warranted to explore the specific mechanisms underlying its antimicrobial action and to optimize its application in various industries [35].

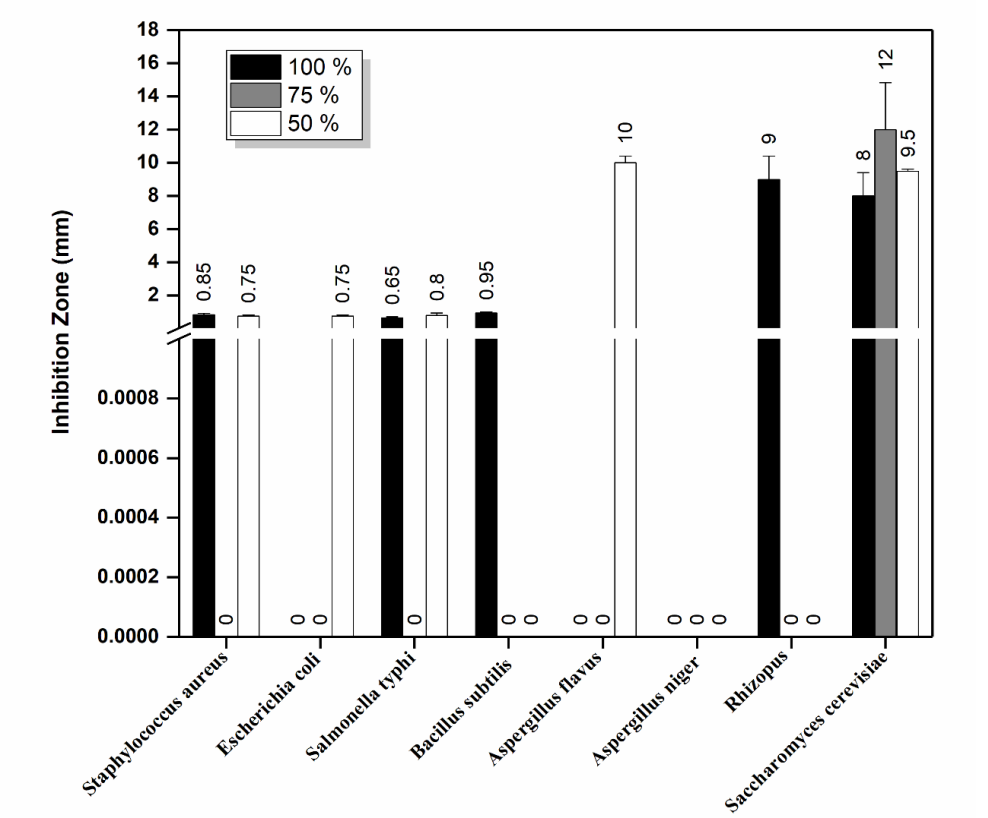

Fig. 1. Antimicrobial activity of pumpkin seed oil against various microorganisms, showing the diameter of inhibition zones at different concentrations (100%, 75%, and 50%)

3.2. Chemical composition of pumpkin seeds

The proximate compositions of pumpkin seeds are shown in **Fig. 2**. The moisture content of the seeds was 4.34%, indicating that they could be stored for a longer time without deterioration. Higher moisture content can cause food spoilage due to microbial and enzymatic action [28]. The crude fiber content was 4.00%, which is relatively similar to sesame (4.6%) and sunflower (3.4%), but lower than cotton seeds (10.8%). This may be attributed to the use of de-hulled seeds [28]. Determining the ash content is important for assessing the quality of feeding materials used for poultry and cattle feed [36]. The ash content of the seeds was 6.65%, significantly higher ($P<0.05$) than the 3.33% reported by [28], indicating a higher mineral content in this study. The crude protein content was 33.81%, in agreement with a study conducted by [33]. The oil content, an important nutritional factor, was 37.96%, slightly higher than the 36.90% reported by Jafari et al. (2012). The carbohydrate level was 13.25%, lower than the 19.32% reported by [28].

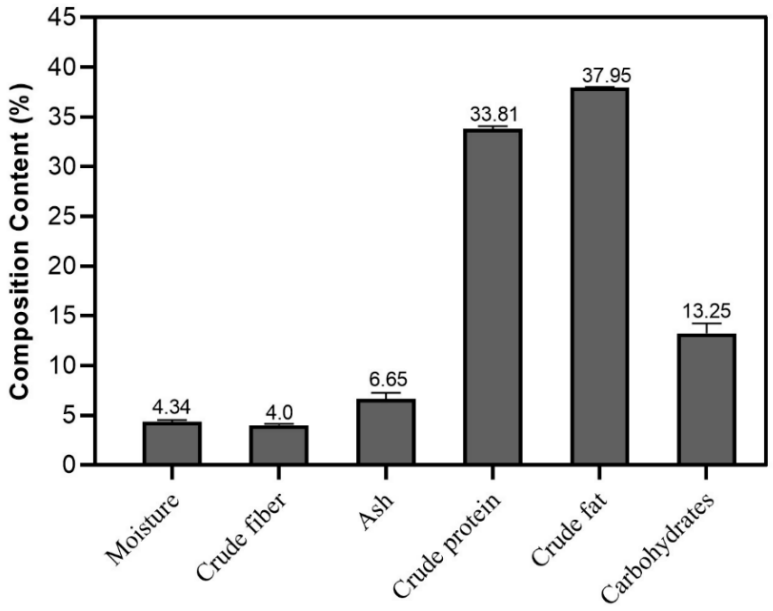

Fig 2. The chemical compositions of pumpkin seeds.

3.3. Mineral content of pumpkin seeds

The mineral content of pumpkin seed kernel was found to be 0.27% calcium, 0.08% magnesium, 0.017% sodium, 0.205% potassium, 0.215% phosphorus, 0.043 mg/L iron, 0.150 mg/L zinc, and 0.244 mg/L copper (**Fig. 3**). Devi et al. [20] also reported that pumpkin seeds are a rich source of minerals, including zinc, phosphorus, manganese, potassium, magnesium, copper, calcium, iron, sodium, and cobalt. Calcium helps ease insomnia and regulate the passage of nutrients through cell walls. Without calcium, muscles cannot contract correctly, blood clotting is affected, and nerves cannot transmit messages. If the body does not receive enough calcium from food, it automatically draws from the bones. If this calcium depletion continues over

time, bones become weak and prone to fractures. Calcium ions are also essential for the normal functioning of nerves and muscles [37]. Calcium values are crucial in preventing rickets, osteoporosis, and tachycardia [3]. Magnesium is also essential for humans due to its impact on health, such as the regulation of blood sugar levels, involvement in energy metabolism, and protein synthesis [19]. Sodium is necessary for the body to regulate blood pressure, blood volume, fluid balance, and the proper functioning of muscles and nerves [37]. Potassium is required to increase iron utilization and is beneficial for people taking diuretics to control hypertension and experiencing excessive potassium excretion. Potassium is an important element in carbohydrate biosynthesis and energy transfer reactions [37]. Phosphates play important roles as buffers that prevent changes in the acidity of body fluids due to their ability to combine with additional hydrogen ions. Their combination with phosphorus enables nutrients to cross the cell membrane [37,38]. Iron serves multiple functions in the body. It aids in blood formation and facilitates the transfer of oxygen and carbon dioxide between tissues. Iron deficiency leads to anemia, which hampers muscle metabolism. In children, iron deficiency can cause learning difficulties and behavioral issues [37]. This suggests that pumpkin seeds could be used as a food supplement. Zinc plays an important role in the structure of proteins and cell membranes and protects against damage. It also plays important roles in growth, development of the immune response, neurological function, and reproduction. Zinc blocks the essential rate-limiting enzyme, Delta-6-desaturase, in the transformation of essential fatty acids into important prostaglandins [39].

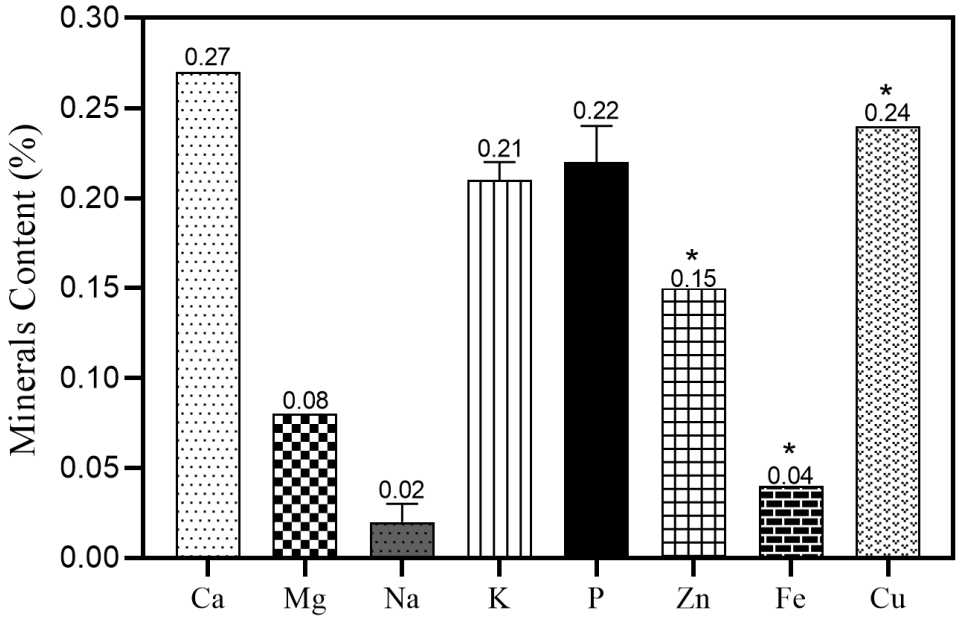

Fig. 3. Minerals compositions of pumpkin seed kernel (%, *). Means were taken in triplicate, (*= mg/L)

3.4. Physicochemical properties of PSO

Physicochemical properties of PSO were shown in **Fig. 4**. The refractive index (RI) of PSO is an optical parameter that analyzes the light rays passing through the material. The RI value increases with the formation of conjugated fatty acids due to thermal degradation of oil during frying or auto-oxidation of oil [40]. The RI value measured was 1.62, which is higher than the value of 1.46 reported by [40]. The density (Dn) of PSO provides information about the weight of the fat and the solid content at a specific temperature. Our findings showed a Dn of 0.97 g/mL at 30 ºC, which matches the density of 0.903 g/mL reported by [41]. The viscosity (VS) of PSO is its resistance to flow. It increases with the molecular weight of the oil and decreases with increasing unsaturation and high temperature. The viscosity of PSO was measured to be 57.0 poise. Color is an important parameter that affects consumers' perception of the quality of edible oils [42]. The color values of PSO were measured using a lovibond tintometer, which showed significant differences in the optical properties of PSO [40]. The yellowness value of PSO was recorded as 10.0, which was not significantly different from the standard color recorded. The acid value (AV) of PSO indicates the rancidity of the oil by measuring the presence of free fatty acids. The AV is a measure of the amount of KOH (mg) needed to neutralize the free acids in 1.0g of fat [41]. The AV of PSO was 2.09 mg KOH/g oil, which is lower than the values reported previously by [41]. The saponification value (SV) of PSO indicates the chain length and average molecular weight of fatty acids. The SV measured in this study was 189.7 mg KOH/g, which was higher than the value of 172.61 mg KOH/g reported by [40]. The iodine value of PSO was 116.5 g of I2/100g, indicating a higher proportion of unsaturated fatty acids in the oil. This value was higher than the value of 99.76 g of I2/100g reported by [42]. The peroxide value (PV) of PSO reflects the status of primary oxidation in oils and is an important quality control parameter for edible oils [43]. The peroxide value measured was 8.82 meq O2/kg oil, which is lower than the value reported by [40,42]. The unsaponifiable matter (USM) in PSO refers to substances dissolved in the fat that are insoluble in aqueous solution but soluble in organic solvent after saponification [41]. The percentage of USM in PSO was 2.40%, which is higher than the value reported by [40]. This could be due to a longer extraction time in the presence of solvent, which extracted more of such matter from the seeds and transferred it into the oil.

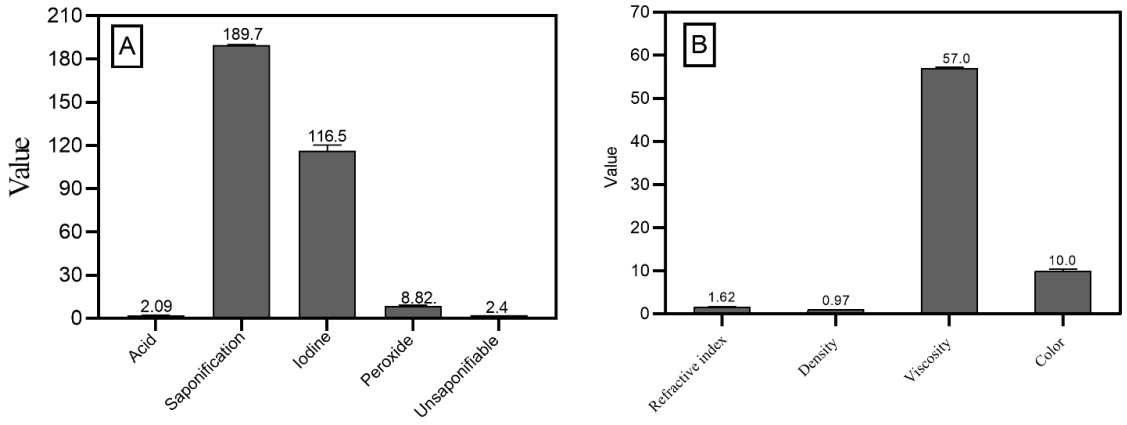

Fig. 4, A, B. Physicochemical properties of pumpkin seed oil (Mean ± SD). Means were taken in triplicate.

3.5. Fatty acid profile of PSO

The fatty acid composition data of pumpkin seed oils can be used to assess their stability and nutritional quality. A higher degree of oil unsaturation makes it more susceptible to oxidative deterioration [44]. Gas chromatography (GC-MS) analysis revealed that PSO contains 78.90% oleic acid, 14.80% palmitic acid, and 1.0% isostearic acid among other minor components (**Table 1**). The fatty acid profile of PSO, particularly its high oleic acid content, aligns with its classification as a nutritionally beneficial oil [45]. Oleic acid is known for its cardiovascular health benefits, making PSO a valuable dietary component [46]. The presence of palmitic acid, while less favorable due to its association with increased cholesterol levels, is balanced by the high unsaturation levels provided by oleic acid [47]. This profile supports the use of PSO in health-oriented food products and potentially in managing conditions like high blood pressure and cardiovascular diseases.

Table 1. Fatty acid profile of pumpkin seed oil by GC-MS.

Peak	Name	R.T. (min)	Area	Area (%
1	Butylated Hydroxytoluene	15.865	4,493,630	0.24
2	Methyl tetradecanoate	20.029	1,511,008	0.08
3	9-hexa-decanoic acid, methyl ester(z)	23.278	3,524,063	0.18
4	Hexa-decanoic acid, methyl ester	23.728	282,974,633	14.80
5	Methyl 9-heptadecanoic or 9-17:1	24.944	2,616,933	0.14
6	Heptadecanoic acid, methyl ester	25.319	5,288,791	0.28
7	9-Octadecenoic acid, methyl ester (E)-	26.798	150,855,492	78.90
8	Heptadecanoic acid, 16-methyl-, methyl ester	27.080	8,045,440	0.42
9	Methyl 10 trans, 12-cis-octadecanoic	27.411	2,582,458	0.14
10	Ethyl oleate	27.600	1,468,066	0.08
11	Methyl9-cis, 11-trans-octadecadienoate	27.938	2,996,507	0.16

12	Cis-10-Nonadecenoic acid, methyl ester	28.082	1,825,621	0.10
13	Cyclopropaneoctanoic acid, 2-[[2-[(2-thyley)]	29.245	11,233,235	0.59
14	9,12-Octadecadienoyl chloride, (Z,Z)-	29.311	726,236	0.04
15	Methyl 12-hydroxy-9-octdecenoate	29.534	13,216,778	0.69
16	cis-11-Eicosenoic acid methyl ester	29.597	14,318,312	0.75
17	Cis-11-Eicosenoic acid methyl ester	29.684	3,510,926	0.18
18	Methyl 18-methylnonadecanoate	29.961	19,140,296	1.00
19	E-8-Methyl-9-tetradecen-1-olacetate	31.094	801,154	0.04
20	1,3-Dipalmitin trimethylsilyl ether	32.075	1,124,567	0.06
21	13-Docosenoic acid (methyl ester) (Z)	32.420	5,079,094	0.27
22	Docosanoic acid, methyl ester	32.741	5,671,494	0.30
23	15-tetracosanoic acid, methyl ester	35.048	864,234	0.05
24	Tetracosanoic acid methyl ester	35.330	3,002,904	0.16
25	13-Docosenoic acid (Z)-	36.129	1,847,093	0.10
26	Squalene	36.543	5,458,887	0.29

4. Conclusion

The study evaluated the antimicrobial efficacy, nutritional value, and physicochemical characteristics of pumpkin seed oil (PSO). The results showed that PSO has significant antimicrobial activity, especially at higher concentrations, making it a promising natural agent for health and food preservation. Nutritional analysis confirmed the richness of pumpkin seeds in essential fatty acids, proteins, and minerals, highlighting their potential as a nutritious food source. Physicochemical assessments of PSO indicated excellent stability and high unsaturation, with a substantial presence of beneficial fatty acids like linoleic and palmitic acids. This research provides valuable insights for the food and health industries, suggesting PSO as a multifunctional ingredient with potential applications in combating antibiotic-resistant bacteria and enhancing food safety. Future studies should focus on clinical trials to validate these health benefits and explore the scalability of PSO extraction and its economic viability in the nutraceutical and pharmaceutical sectors.

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