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Isolation of cactus oil from Palestinian ((*Opuntia ficus-indica* [L.]) Characterization comparative study and cosmetic preparation.

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Isolation of cactus oil from Palestinian ((*Opuntia ficus-indica* [L.]) Characterization comparative study &cosmetic preparation.

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Thesis Approval

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Dedication


To my beloved parents, whose unwavering love, support, and prayers have been the foundation of my journey their your sacrifices and encouragement have empowered me to pursue my dreams. To my dear professors, especially Dr. Mohammad Abu Al-Haj, thank you for your invaluable guidance, knowledge, and patience throughout this research. Your mentorship has profoundly influenced both my academic and personal growth. This work is dedicated to everyone who believed in me.

Tala Khashan

Declaration

I certify that this thesis submitted for my master's degree is my own research, except where otherwise acknowledged. Furthermore, I confirm that this thesis, or any part of it, has not been submitted for a higher degree to any other university or institution.

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Signed: 

Date : 24 / 5 / 2025

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Abstract

This study investigates the extraction, isolation, characterization, and cosmetic application of prickly pear (*Opuntia ficus-indica* L.) seed oil sourced from Palestine, comparing it to Moroccan prickly pear oil. The oil was extracted using cold-press methods to retain its natural properties. Comprehensive chemical analyses were performed, measuring acid value, peroxide value, UV-Visible Spectrophotometer absorbance, GC-MS, GC-FID, HPLC, FTIR, and refractive index. The results indicated that the Palestinian cactus oil has a low acid value and peroxide value, suggesting good oxidative stability and freshness. FTIR analysis revealed characteristic absorption peaks, confirming the presence of unsaturated fatty acids and esterified triglycerides, similar to the Moroccan sample. Fatty acid profiling showed high concentrations of linoleic acid (C18:2), oleic acid (C18:1), and palmitic acid (C16:0). HPLC analysis of tocopherols identified a higher concentration of α -tocopherol in the Palestinian oil. A cosmetic face cream was formulated with cactus oil, resulting in improved texture, skin absorption, and moisturizing performance. This research highlights the potential of Palestinian prickly pear seed oil as a high-quality, bioactive-rich ingredient for nutritional, pharmaceutical, and cosmetic applications. Future work will involve a more detailed profiling of fatty acid composition, evaluating formulation stability, and quantifying tocopherol subtypes to further establish its functional value.

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Abbreviations

- AV : Acid value .
- FFA: Free fatty acid .
- PV : Peroxide value
- M_{eq}: Oxygen in oil, measured per kilogram.
- UV : Ultraviolet ionization absorbance.
- FID : Flame detector.
- FTIR : Fourier transform infrared spectroscopy.
- IR : Infrared radiation.
- OFI: Opuntia ficus-indica.
- CLA: Conjugated linoleic acid.
- PA : Palmatic acid.
- LDL : Low density lipoprotein.
- EDTA : Edetate disodium.
- GC-MS : Gas chromatography-mass spectrometry.
- HPLC: High-performance liquid chromatography.
- FAME : Fatty acid methyl ester.

Chapter One:

Introduction:

The distinctive and uncommon characteristics of cacti (singular: cactus) enable them to adapt to extremely hot and arid or semi-arid environments. They possess a range of morphological and functional traits, such as drought tolerance and water conservation, largely attributed to their CO₂ fixation capacity through crassulacean acid metabolism (CAM). Additionally, their leaves are modified into spines, while the stem develops into a succulent, chlorophyll-containing structure, as described by (Shetty et al., 2012). Originally native to regions including North America, Venezuela, Bolivia, South Africa, Argentina, Jordan, Palestine, and the West Indies, cacti are now widely cultivated in countries such as the USA, India, Italy, Spain, and Mexico.

Although there are about 300 species in the genus *Opuntia* that produce edible fruit variations, the most significant ones include *Opuntia ficus-indica* (L.) Mill., *Opuntia robusta*, *Opuntia streptocantha*, *O. amyclaea*, *Opuntia megacantha*, and *O. hiptiacantha*. Cactus pears are cultivated in various countries for multiple purposes, such as enhancing food security, preventing soil, wind, and water erosion, serving as natural fences and forage, as well as being utilized in the food processing industry for natural coloring, juice, jam, vinegar, flour, and food supplements. They are also processed into pharmaceutical products, including essential oils, herbal extracts, and medicinal applications, and into cosmetic products such as seed oil, soap, and shampoo, as reported by (Feugang et al., 2006).



(a)



(b)

Figure 1: (a) Photograph of *Opuntia ficus-indica*; (b) Photograph of *Opuntia dillenii*

Opuntia ficus-indica (L.) Mill., also known as prickly pear or nopal cactus, belongs to the Cactaceae (dicotyledonous angiosperm) family and is considered an important cactus species in non-irrigated land agriculture due to its diverse uses in food, fodder, dye production, energy, ecosystem remediation, and soil erosion prevention, as noted by (Small et al., 2004). It is also referred to as the “fruit for the poor,” “treasure under spines,” “future plant,” “sacred plant,” and “monster tree,” terms that reflect its significant role in human life, according to (Jiménez et al., 2013).

The largest producer of the *Opuntia ficus-indica* species in Mexico, with 72000 hectares (ha) under fruit cultivation and 10,500 for nopalitos. Brazil, North African countries (Morocco, Egypt, Tunisia, Algeria, and Libya), Italy, and Chile have cultivated areas of 40000ha, 16000ha, 2500ha, and 1100ha, respectively by (Yahia EM et al., 2011), Yahia EM, Sáenz C et al., (2011). Without any technical participation, the crop is grown in two seasons every year in Chile, from February to April and July to September, with a total area of 934.4ha. Ethiopia is the greatest producer of cactus pear in Africa, with an area of 360,000ha. Other African countries that cultivate *Opuntia* species include Algeria, Morocco, South Africa, and Tunisia. Its cultivation is mostly for fence, exquisite fruit, essential oil, pharmaceutical items, food products, cosmetics, cattle feed, and forage by (Targa MG et al., 2013).

Opuntia ficus-indica contains approximately 92% water, 4–6% fiber, and 1–2% protein (Brinker et al., 2009). It has a high nutritional profile, including sugars such as glucose, fructose, and sucrose, along with protein, fat, ash, fiber, and minerals. Additionally, it is rich in free amino acids such as glutamine, proline, serine, methionine, arginine, aminobutyric acid, and histidine. Moreover, *Opuntia ficus-indica* contains several beneficial bioactive compounds, including ascorbic acid, carotenoids, and taurine (Kuti, 2004; Stintzing et al., 2005).

It contains pigments and bioactive compounds that can be used for both nutrition and medicinal purposes, making it a functional food (Saleem et al., 2006). These pigments are classified based on their chromophore structure, including conjugated systems such as carotenoids, caramel, betalains, lakes, and synthetic pigments, as well as metal-coordinated porphyrins like chlorophyll and myoglobin. The pulp of cactus pear contains varying amounts of bioactive compounds depending on the cultivar and the location of growth (Slimen et al., 2006).

Prickly pear seed oil is a triglyceride-rich natural oil primarily composed of unsaturated fatty acids, particularly linoleic acid, along with significant amounts of tocopherols (vitamin E), phytosterols, and polyphenols. As a natural glyceride, it functions not only as a source of essential fatty acids but also as an emollient that helps retain moisture and strengthen the skin barrier. Its lightweight, non-comedogenic properties make it ideal for cosmetic formulations aimed at moisturizing, anti-aging, and soothing sensitive or acne-prone skin. Due to its high antioxidant content, it also helps protect the skin against oxidative stress and environmental damage.(Meneguzzo et al.,2017)

1.1 Chemical Composition of Cactus Oils:

1.1.1 Linoleic acid:

Linoleic acid (cis, cis-9,12-octadecadienoic acid) is an unsaturated omega-6 fatty acid (Rogers et al., 2001). Major dietary sources of linoleic acid include vegetable oils, nuts, seeds, meats, and eggs (Whelan et al., 2013). It is commonly used in the production of soaps, as an emulsifier, and as a quick-drying oil (Gwas et al., 2001). When applied topically, linoleic acid from vegetable oils exhibits various biological activities related to skin health and hair growth, such as repairing the skin barrier, promoting wound healing, skin whitening, photoprotection, anti-inflammatory effects, and stimulating hair growth (Wang et al., 2024).

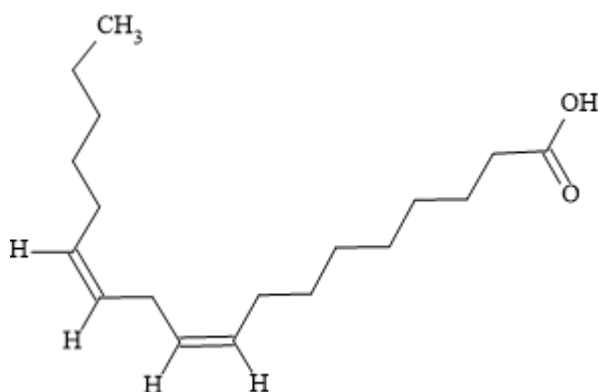


Figure 2 : Structure of linoleic acid (9Z,12Z)-Octadecadienoic acid (Z,Z).

1.1.2 Oleic acid

Oleic acid ((Z)-octadec-9-enoic acid) is a dietary monounsaturated omega-9 fatty acid found naturally in vegetable oils and animal fats. It is known for its ability to soften and moisturize the skin (Rogers et al., 2001). Topical treatment with oleic acid has been shown to have beneficial and lasting effects on skin papillomas (Lotta et al., 2004). Furthermore, oleic acid acts as a skin penetration enhancer by interacting with the lipids of the stratum corneum to form a new type of lipid domain, which reduces the skin's barrier function following treatment (Hanafi et al., 1997).

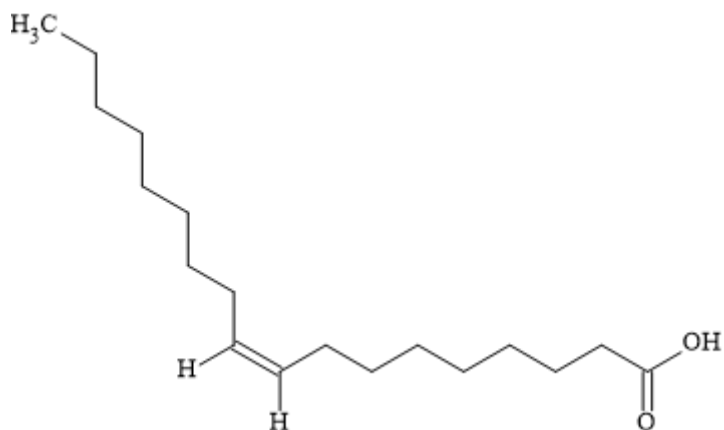


Figure 3: Structure of Oleic Acid ((9Z)-octadec-9-enoic acid).

1.1.3 Vitamin E (Tocopherols)

Vitamin E is an essential nutrient that is receiving growing attention in the skin care industry because of its antioxidant properties. The main natural sources of vitamin E are fresh vegetables, vegetable oils, cereals and nuts. Vitamin E has long been linked to skin health, including all of its possible functions in cosmetic products, to its roles in membrane integrity and even the aging process, (Thiele et al.,2007)

Vitamin E can be divided into two groups, tocopherols and tocotrienols, with four isomers (alpha, beta, gamma and delta).(Liu,et al.,2021)

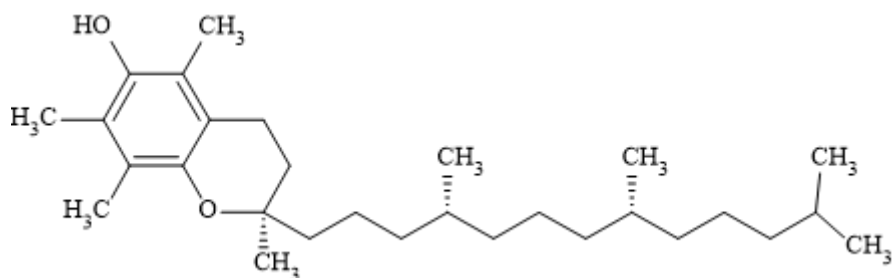


Figure 4: Structure of α -tocopherol

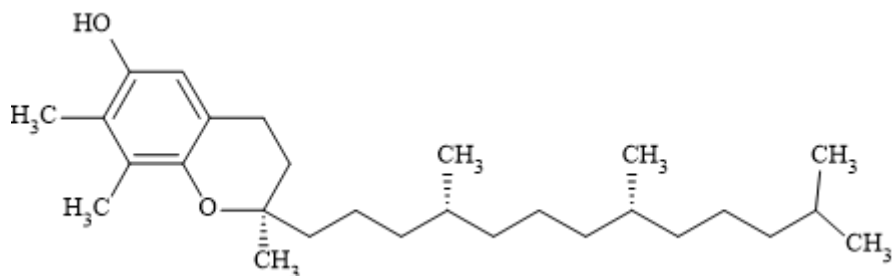


Figure 5: Structure of γ -tocopherol .

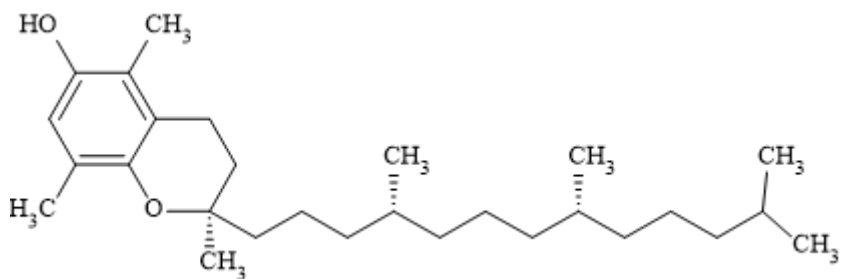


Figure 6: Structure of β -Tocopherol .

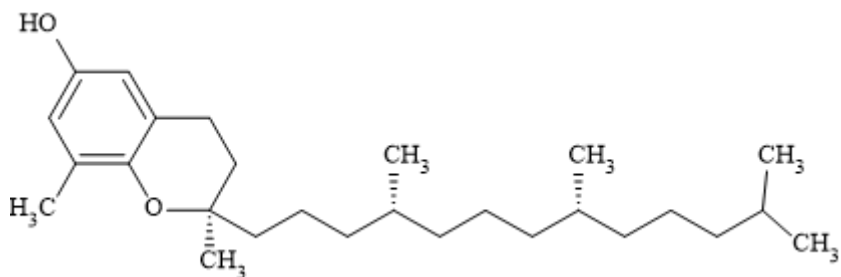


Figure 7: Structure of δ -Tocopherol.

1.1.4 Palmitic acid

Palmitic acid (hexadecanoic acid) found in animals and plants. As its name indicates, it is major component of the oil from palm trees (Rogers et al., 2001) .Palmitic showed significant enhancement in percutaneous absorption of luteinizing hormone releasing hormone.(Bhatia,et al.,1998).

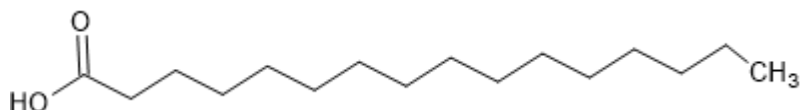


Figure 8: Structure of Palmitic acid (hexadecanoic acid)..

1.2 Problem

Despite prickly pear seed oil's rich composition of essential fatty acids and antioxidants, a cactus seed oil remains underutilized in the industrial, food, and cosmetic sectors. This situation highlights the need for comprehensive scientific research into its properties, as well as an assessment of and health potential in Palestine.

1.2 Objective

- 1.3.1 This research aims to isolate, analyze and characterize the complete oil extracted from the seeds of *Opuntia ficus-indica*.
- 1.3.2 Additionally, the study explores the oil's possible uses in cosmetics and its natural skin-boosting effects. This research is the first to be conducted in Palestine.

Chapter Two:

Literature Review:

2.1 Introduction:

This chapter deals with previous studies that are similar to the subject of the current study, which presents a study on the quality and composition of cactus seed oil, highlighting the relationship between its chemical components and oxidative stability.

2.2 Previous Studies:

1. Quality Assessment of Seed Oil from Selected Cactus Pear Cultivars (*Opuntia ficus-indica* and *Opuntia robusta*) By(De Wit,,et al. ,2017):

Method :*Opuntia ficus indica* and *O. robusta* were assessed for their seed oil content, fatty acid composition, and oil quality. The evaluation of oil quality included parameters such as refractive index, iodine value, peroxide value, free fatty acid content, p-anisidine value, and oxidative stability index,in South Africa.

Results: Cultivars exhibited oil contents ranging from 5.65% to 8.09%. The unsaturation of fatty acids varied between 55.98% and 67.62%, while saturation levels ranged from 16.59% to 20.71%. Iodine values for the oil samples fell between 110.68 and 126.24 g of iodine absorbed per 100 g of oil. Peroxide values ranged from 9.50 to 33.67 meq O₂/kg, and p-anisidine values varied between 0.02 and 3.73 mmol/kg. Overall, these oils showed a relatively low oxidative stability index, ranging from 2.16 to 4.15 hours

Conclusion: Oxidative stability was significantly correlated with oil content, as well as the levels of oleic acid, stearic acid, and monounsaturated fatty acids. Additionally, physiochemical properties, including the peroxide value and p-anisidine value, were also significantly associated with the oxidative stability index.

2. "Antioxidant activity of *Opuntia ficus-indica* oil and its application in cosmetic emulsions." By (Benattia, et al. ,2019).

Method: Prickly pear seed oil was cold-pressed and its antioxidant activity was evaluated. It was incorporated into cosmetic emulsions, which were tested for stability, pH, texture, and effects on skin hydration and elasticity in Algeria.

Result: Prickly pear seed oil demonstrated significant antioxidant activity. Emulsions containing the oil were stable and enhanced skin hydration and elasticity without causing irritation.

Conclusion: Prickly pear seed oil is effective and safe for skincare, offering antioxidant benefits and improving skin hydration and elasticity.

3. Chemical Composition of Cactus Pear Seed Oil: phenolics identification and antioxidant activity. By(Abdelkhaleq, et al .,2022).

Method: Fatty acid profiling was performed by gas chromatography coupled to an FI detector. In Moroccan.

Result: Tocopherols and phenolic compounds were analyzed by LC-FLD/UV, and the oil's phenolic-rich fraction antioxidant power was determined by phosphomolybdenum, DPPH assay and β -carotene bleaching test.

Result : Fatty acid composition was marked by a high unsaturation level ($83.22 \pm 0.34\%$) linoleic acid ($66.79 \pm 0.78\%$), oleic acid ($15.16 \pm 0.42\%$), palmitic acid ($12.70 \pm 0.03\%$). The main tocopherol was γ -tocopherol (172.59 ± 7.59 mg/kg) total antioxidant activity, scavenged DPPH up to (97.85%)and β -carotene against bleaching (97.56%).

Conclusion: The results support the potential use of cactus pear seed oil as a functional food.

4. Analytical Extraction Methods, Physicochemical Properties and Chemical Composition of Cactus (*Opuntia ficus-indica*) Seed Oil and Its Biological Activity By (Chbani,et al.,2023).

Method: Study of the chemical composition, physicochemical properties of a cactus seed oil, as well as its antimicrobial and biological effects in Morocco.

Result: Cactus seeds contain 3.4–14.4% oil, mainly rich in linoleic and oleic acids. The major triacylglycerols include LLL, LLO, LLP, and OOL. γ -Tocopherol makes up over 90% of total tocopherols, and β -sitosterol is the main phytosterol, comprising about 72–79% of the total.

Conclusion: Cactus seed oil is rich in bioactive compounds with health benefits and strong antioxidant properties. While much research has explored its composition and effects, further studies are needed to ensure its authenticity, prevent adulteration, and establish quality standards and proper storage practices

2.3 Researcher's comment:

Wit et al. (2017) studied prickly pear seed oil in South Africa, as summarized in the table, and found a moderate oil content (5.65–8.09%) with high levels of unsaturated fatty acids.

The oils showed low oxidative stability, which was linked to fatty acid composition and peroxide values.

Prickly pear seed oil is rich in healthy fatty acids and antioxidants., it has strong antioxidant effects, improves skin hydration and elasticity, and offers health benefits as a functional food.

Prickly pear seed oil is rich in healthy fatty acids and antioxidants, such as linoleic and oleic acids and γ -tocopherol, and supports health as a functional food. and further studies are needed to ensure its quality and authenticity.

Prickly pear seed oil is rich in healthy fatty acids and antioxidants, such as linoleic and oleic acids and γ -tocopherol, and supports health., and the oil needs further studies to ensure its quality, authenticity, and proper storage.

Chapter Three:

Experimental Part:

3.1 Chemical and reagents

diethyl ether, ethanol (96 %), phenolphthalein indicator (ph.ph), sodium hydroxide, chloroform, acetic acid, potassium iodide, starch, sodium thiosulfate, cyclohexane, potassium hydroxide, and hexane. Isopropyl alcohol (96%), and α -tocopherol standard, distilled water, disodium EDTA, Glycerin, xanthan gum, stearic acid, cetearyl alcohol - cetearth-20 (emulgade 1000 NI), cetearyl alcohol, caprylic/capric triglycerides (myritol 318), isooctyl myristate (crodamol IPM), cactus oil, phenoxyethanol - ethylhexylglycerin (euxy PE9010), (rosal bouquet) fragrance, were purchased from (Sigma-Aldrich), All of the solvents were of the highest analytical grade and used as supplied.

3.2 Sample collection and preparation

The ripe cactus fruits were gathered from the same location (Tulkarm, Palestine) in July and August of 2022. To remove the pulp and seeds, the ripe, prickly pears were carefully peeled. It took many days to peel the roughly one ton of prickly pears that were supplied in two batches.

3.3 Extraction of seed oil

In order to maintain the natural qualities of the ingredients, the seeds were hand-squeezed and filtered after being peeled, all without the use of chemicals or mechanical equipment. Following the separation procedure, the seeds are cleaned with water and let to dry in the

sun in order to extract the oil. In the meantime, the pulp is used for other things, like making food or doing chemical analysis. Until they were prepared for pressing, the seeds were constantly agitated while being sun-dried for three days. In order to get an oil temperature of 20–30°C, pressing was done at 180–200°C. Throughout the pressing process, the cellulose stayed heated. Since the primary goal of this study was to get prickly pear seeds, the prickly pear juice that was produced was not a major focus. Nevertheless, it can be used in food production or to extract acetic acid.

3.4 Instrumental

1. Gas chromatography–mass spectrometry GC – MS (SHIMADZU – Nexis).

Gas chromatography/mass spectrometry (GC/MS) is a precise analytical technique used in various fields such as food safety, pharmaceuticals, environmental, and forensic testing. Shimadzu offers a wide range of high-sensitivity GC-MS instruments—including triple and single quadrupole systems—designed for high performance, reliability, and minimal environmental impact, along with accessories and software to support advanced analysis.

2. Fourier transform infrared spectroscopy FTIR- (TENSOR 11 - software is opus).

The TENSOR series is a flexible FT-IR system designed for both basic quality control and advanced research. It supports various sampling methods and can be expanded with external modules for applications like micro-analysis, TGA coupling, high-throughput screening, and hyperspectral imaging.

3. HPLC (LC-40D xs).

This advanced parallel dual micro plunger pump (10 µL) offers ultra-low pulsation and high flow stability with a flow rate range of 0.1–10 µL/min, operating in multiple modes (constant flow/pressure, isocratic, and various gradient types). It supports up to 105 MPa pressure and pH range 1–14. Optional features include solvent selection valves and LPGE units. FlowPilot technology ensures safe pressure ramp-up and column protection, with automatic diagnostics and alerts. The pump provides detailed operation logs, solvent tracking, and can be controlled via keypad, browser, or HPLC/LC-MS software.

4. Gas chromatography flame ionization detector GC-FID (SHIMADZU –GC-17A).

The Shimadzu GC-17A is a digitally controlled gas chromatograph designed for high-precision applications. It offers reproducible control of all key parameters and supports multiple detectors (FID, TCD) with high sensitivity. Featuring a split/splitless injector, fast oven heating, and user-friendly software, the GC-17A is ideal for petrochemical, environmental, and pharmaceutical analysis.

5. UV-visible spectrophotometer.(V-770)

. The V-700 Series UV-Visible-NIR spectrophotometers support a wide range of accessories for biological, material science, and QA/QC applications. Some accessories are model-specific; detailed information is available in the instrument brochure.

6. Refractometer (refractive index).

Abbe refractometers are reliable instruments for measuring the refractive index of both liquids and solids, including glass and plastic films. They feature sapphire prisms, sealed optics, and often include computer interfaces, flow-through modules, and temperature control accessories—making them accurate and versatile beyond just sugar analysis.

7. Centrifuge (ROTOFIX 32 A).

The ROTOFIX 32 A is a durable and versatile benchtop centrifuge widely used in laboratories. It handles up to 6 × 94 mL or 40 blood tubes and features a simple interface. It also supports cytology rotors compatible with most slide systems and includes bio-containment lids.

3.5 Chemical analysis

3.5.1 Acidity:

Acidity, given as % of oleic acid, was determined according to (El Kharrassi et al., 2018) the method. Briefly, 1.0271 g of oil was mixed with 5 mL neutralized ethanol/diethyl ether mixture (1:1, v/v) then titrated with 0.1 M NaOH, using phenolphthalein as indicator. The acidity was calculated using the formula:

Acidity

$$\frac{V \times C \times M}{10 \times m}$$

Where V is the volume (mL) of NaOH, C is the concentration of NaOH (mol/L), M is the molecular weight of oleic acid and m is the mass of oil (g)

Table 3.1 : Weight of oil samples and concentration of NaOH used in the titration .

Sample Number	Weight of the oil sample.(g)	Volume of NaOH (mL)	Concentration of NaOH (mol/L)
1	1.0271 g	0.50 ml	0.1
2	1.0273 g	0.50 ml	0.1
3	1.0152 g	0.51 ml	0.1

3.5.2 Peroxide value:

Peroxide value was determined according to the (El Kharrassi et al., 2018) method. Briefly, 1 g of each oil sample was mixed with 2 mL chloroform, 6 mL acetic acid, and then 0.2 mL of saturated potassium iodide was added. The mixture was shaken for 5 min in darkness at room temperature, then the mixture was titrated with 0.01 M sodium thiosulfate, using starch solution as indicator. The peroxide value was calculated using the formula:

Peroxide value

$$= \frac{(V_x - V_0) \times T}{m} \times 1000$$

Where V_x is the amount (mL) of sodium thiosulfate solution used for sample titration, V_0 is the amount (mL) of sodium thiosulfate solution used for the blank, T is the normality of sodium thiosulfate solution and m is the mass of oil (g). The peroxide value was expressed as milliequivalents of active oxygen per kilogram of oil (meq active O₂/kg oil).

Table 3.2 : Weight of oil samples titrated with sodium thiosulfate used in the peroxide value experiment.

Sample Number	Weight of the oil sample.(g)	volume (in mL) of Na ₂ S ₂ O ₃ . 5H ₂ O	Concentration of Na ₂ S ₂ O ₃ . 5H ₂ O (mol/L)	Volume of blank(ml)
1	1.0046 g	0.6 ml	0.01	0.5 ml
2	1.0066 g	0.6 ml	0.01	0.5 ml
3	1.0078 g	0.6 ml	0.01	0.5 ml

3.5.3 UV-visible spectroscopy:

In fat and oil quality tests, the method of ultraviolet absorbance measures the amount of UV light absorbed by an oil sample at specific wavelengths. This technique is quick and sensitive for assessing the oxidative stability and freshness of fats and oils. Higher absorbance values indicate greater oxidation and thus lower oil quality .

UV absorbance was determined according to (El Kharrassi et al. , 2018) method using 0.25 g of oil and cyclohexane as solvent. K270 and K232 extinction coefficients were calculated from absorbance at 270 and 232 nm,

Table 3.3 : Measured UV-visible spectral

Sample Number	Weight of the oil sample.(g)	Volume of cyclohexane	Absorption at K232	Absorption at K270
1	0.25 g	100 ml	0.6231	0.2247
2	0.50 g	100 ml	1.3001	0.4571
3	1.00 g	100 ml	2.8800	0.8440

3.6 Phytochemical Analysis

3.6.1 Fatty acid analysis:

Phytochemical analysis identifies and measures the bioactive chemical compounds that occur naturally in plants, which contribute to their nutritional and therapeutic properties, as described by(El Kharrassi et al. , 2018).

Phytochemical analysis consists of several key stages. It begins with quantitative analysis, utilizing advanced techniques like high-performance liquid chromatography (HPLC) to measure the concentration of bioactive compounds. Next is the identification stage, where the chemical structures of these compounds are confirmed through sophisticated analytical methods such as Fourier-transform infrared spectroscopy (FTIR) and gas chromatography–mass spectrometry (GC-MS).

Used GC-MS: GC-MS is a powerful analytical technique known for its high accuracy in detecting various chemical compounds within complex mixtures. It effectively separates the compounds and identifies their molecular fingerprints, even in small quantities, as demonstrated by(Sparkman et al., 2011).

- GC-MS method

The gas chromatography (GC) analysis was conducted under specific conditions: the oven temperature was set to 170 °C with an equilibrium time of 2.0 minutes, and the injector temperature was maintained at 230 °C. A 30-meter long cross-bond 100% dimethyl polysiloxane column with an internal diameter of 0.25 mm and a film thickness of 0.25 μm was used. The column flow rate was 0.1 mL/min, with a linear velocity of 38.3 and a split ratio of 100, resulting in a total flow of 104.0 mL/min. Helium served as the carrier gas, and the injection volume was 1 μL. Additional settings included a scan interval of 0.5 seconds, a solvent cut-off at 0.6 minutes, a detector voltage of 1.5 kV, and an end time of 19 minutes. These parameters were established according to (USP 401 – Chapter: Fats and Fixed Oils / Composition of Fatty Acids to ensure accurate identification and quantification of the fatty acid components).

Table 3.4 : Split ratio 100

Ratio	Temp	Time
-	170	2.0
5	230	5

- GC-FID method

□ Sample Preparation: A 0.1 mL sample of oil was dissolved in 1900 mL of hexane. Next, 200 μL of 2M potassium hydroxide (KOH) in methanol was added to the solution. The mixture was shaken for 30 seconds to promote esterification, then allowed to stratify. After phase separation, the upper layer was carefully collected for analysis. This procedure followed the guidelines outlined in (USP 401, Chapter on Fats and Fixed Oils – Composition of Fatty Acids).

□ Chromatographic Conditions: The injection volume was set to 0.5 μL using a split injector maintained at 230 $^{\circ}\text{C}$ with a split ratio of 20:1. The system operated at a pressure of 0.78 bar, with a total flow rate of 32.5 mL/min, a column flow rate of 1.5 mL/min, and a linear velocity of 33.3 cm/s. Nitrogen served as the carrier gas at a flow rate of 24 mL/min, while hydrogen and air were provided as auxiliary gases at flow rates of 32 mL/min and 200 mL/min, respectively.

□ Run Conditions: The total run time was set to 22 minutes, with the flame ionization detector (FID) maintained at 230 $^{\circ}\text{C}$. Separation was achieved using a 30-meter cross-bond Carbowax polyethylene glycol column, which has an internal diameter of 0.25 mm and a film thickness of 0.25 μm . The stop time was also set at 22 minutes.

□ Analytical Process: The analytical steps were meticulously designed and executed in the laboratory with suitable reagents and conditions to guarantee the accuracy and reliability of the results. The procedures followed the standards set forth in the United States Pharmacopeia (USP 401), particularly those related to the composition of fatty acids in fats and fixed oils.

Table 3.5: Colum temperature program

Rate	Temp	Hold time
-	170	1
4	230	6

3.6.2 Tocopherol analysis

Tocopherols are a type of Vitamin E compound found in plant oils, known for their strong antioxidant properties that protect oils from oxidation (rancidity) and provide health benefits to humans. There are several types of tocopherols, including α -tocopherol, β -tocopherol, γ -tocopherol, and δ -tocopherol, each contributing differently to antioxidant activity by (Bakre et al., 2015).

- Method :
 1. Equipment

The SHIMADZU model (Organiser) high-performance liquid chromatography system is equipped with a fluorescence detector (model L-4633), configured with an excitation wavelength of 290 nm and an emission wavelength of 330 nm, as described by (Bakre et al., 2015). Material is Alpha-tocopherol (96%) standard from Sigma-Aldrich was utilized, along with methanol, acetonitrile, and isopropanol for HPLC.

2. Conditions chromatographiques

The mobile phase consisted of equal parts methanol and acetonitrile (50% each) and was delivered at a flow rate of 1 mL/min. Quantitative estimation of α -tocopherol was carried out using a reversed-phase C18 HPLC column, measuring 250 mm in length and 4.6 mm

in internal diameter, with a particle size of 5 μm . The column oven temperature was set at 40 $^{\circ}\text{C}$. Detection was achieved with an excitation wavelength of 290 nm and an emission wavelength of 330 nm, as detailed by(Bakre et al. ,2015).

3. Preparation of samples and standard

The cactus seed oil sample was diluted in a 1:10 isopropanol solution and filtered through 0.45 μm filter paper. A 5 μL aliquot of this solution was then directly injected into the C18 column of the gas chromatograph, following the protocol established by (Bakre et al. ,2015).A standard solution of 1 mg/L alpha-tocopherol was prepared in isopropanol. Following the method described by(Bakre et al., 2015), 5 μL of this solution was injected into a C18 column for analysis.

3.6.3 FTIR analysis

FTIR is a powerful analytical technique used to identify and study the chemistry of organic compounds by measuring infrared (IR) radiation. This rapid, non-destructive method effectively identifies chemical bonds and functional groups in various substances. FTIR is widely utilized in fields such as food science, oil analysis, pharmaceuticals, environmental testing, and more. Infrared spectra were obtained using a TENSOR II FTIR spectrometer, and absorption bands were identified based on their wavenumbers (cm^{-1}). Figure 19 shows the absorption bands corresponding to the carbonyl group (C=O) as reported by(Benattia et al.,2019).

3.6.4 Refractive index

The refractive index, measured at room temperature (25 $^{\circ}\text{C}$) using a refractometer, is considered a valuable parameter for quality control and detecting adulteration in oils, as reported by(Benattia et al.,2019)..

3.6.5 Developed a highly effective formula for face cream that utilizes cactus oil extract.

To create a highly stable and effective cosmetic product, we developed a meticulously crafted formula that features cactus oil (Opuntia seed extract) as the primary active ingredient. Cactus oil was selected for its well-documented moisturizing and antioxidant properties, making it a popular choice in a variety of cosmetic products, including facial moisturizers, night creams, skin serums, lip balms, and hair treatment oils. This ingredient is rich in essential fatty acids, particularly linoleic acid, and contains vitamin E (tocopherol), both of which play a crucial role in combating skin aging and enhancing skin elasticity.

3.7 Preparation of creams.

Phases A and B were measured and combined, then thoroughly stirred to form a homogeneous gel. The resulting Phase A/B mixture was heated to 75 $^{\circ}\text{C}$, while Phase C was prepared separately and heated to the same temperature. Once both phases reached 75 $^{\circ}\text{C}$, Phase C was gradually added to the Phase A/B mixture under vigorous stirring using high shear mixing until a smooth and uniform emulsion was obtained. The mixture was subsequently cooled with continuous stirring until its temperature dropped below 40 $^{\circ}\text{C}$. At this stage, Phase D was incorporated, and mixing was continued. All

components were thoroughly blended using a mechanical mixer fitted with a glass stirrer at 1,000 rpm for 5 minutes. The pH of the cream was then adjusted to the desired level, followed by an additional 10 minutes of stirring at 500 rpm. To minimize exposure to atmospheric oxygen during the mixing process, the glass stirrer was positioned at the bottom of the container and covered to prevent air entry. All creams were formulated under standardized conditions to maintain their specific physical properties, in accordance with the method described by (Benattia et al. ,2019). The formulation details and component proportions are presented in Table 3.6.

Cream formulations

Prickly pear seed oil is rich in essential fatty acids and antioxidants, making it an excellent ingredient for skincare. The oil helps moisturize the skin, improve its elasticity, and protect it from dryness and damage caused by external factors. Thanks to its nourishing and gentle properties, prickly pear seed oil is used to prepare effective natural creams for daily skin care.

Table 3.6 : (O/W) Cream Formulation of Cactus Oil.

Phase	Ingredient	Function	% W/W
Aqueous phase A	<ul style="list-style-type: none"> Water EDTA 	Continuous phase / strong emulsion stabilizer chelating agent	75.7 0.1
Phase B	<ul style="list-style-type: none"> Glycerin Xanthan gum 	Moisturizer Thickening agent	5 0.3
Oil phase C	<ul style="list-style-type: none"> Stearic Acid Cetearyl Alcohol - Cetareth-20 Cetearyl alcohol Caprylic/Capric Triglycerides(Myritol 318). Isooropyl myristate Cactus oil 	Emulsifying agent and thickener Emulsifier and surfactant Emulsifier Emollient Emollient Emollient Natural / Hydratation en profondeur	1.50 8.00 1.00 2.00 4.00 2.00
Phase D	<ul style="list-style-type: none"> Phenoxyethanol - Ethylhexylglycerin Roseral bouquet 	Preservative Fragrance	0.2 0.2

Chapter Four:

Result & Discussion:

4.1 Results

4.1.1 Acidity

Table 4.1: Acid Values for different samples analysis

Sample	Acidity Value
#1	1.45
#2	1.45
#3	1.43
Mean Standard Deviation	1.44 ± 0.01

4.1.2 Peroxide value

Table 4.2: Peroxide value (PV) for different samples analysis

Sample	Peroxide Value (meq O ₂ /Kg)
# 1	0.995
#2	0.993
#3	0.992
Mean Standard Deviation	0.993 ± 0.001

4.1.3 UV-visible spectroscopy

Table 4.3: UV-visible spectroscopy for different concentration analysis

Sample	K232 Molar absorptivity ϵ $\text{L}\cdot\text{mol}^{-1}\cdot\text{cm}^{-1}$	K270 Molar absorptivity ϵ $\text{L}\cdot\text{mol}^{-1}\cdot\text{cm}^{-1}$
#1	0.2492	0.08988
#2	0.26002	0.09142
#3	0.2880	0.0844
Mean Standard Deviation	0.2657 ± 0.02005	0.08857 ± 0.0036

4.1.4 Fatty acid analysis

GC-MS method

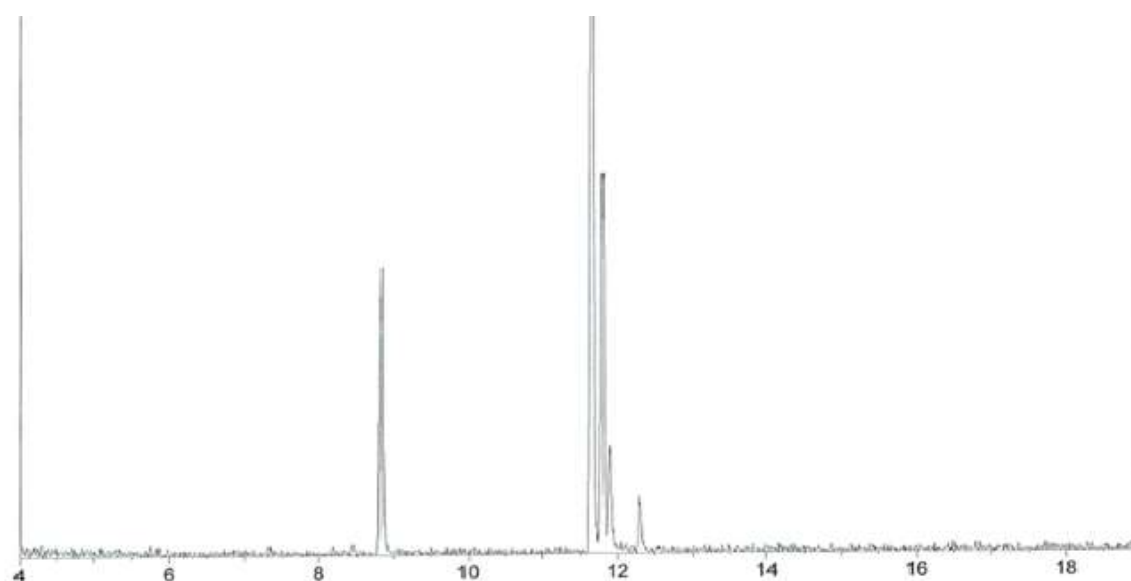


Figure 9: GC-MS Chromatogram for the attacked cactus oil at 25 C°

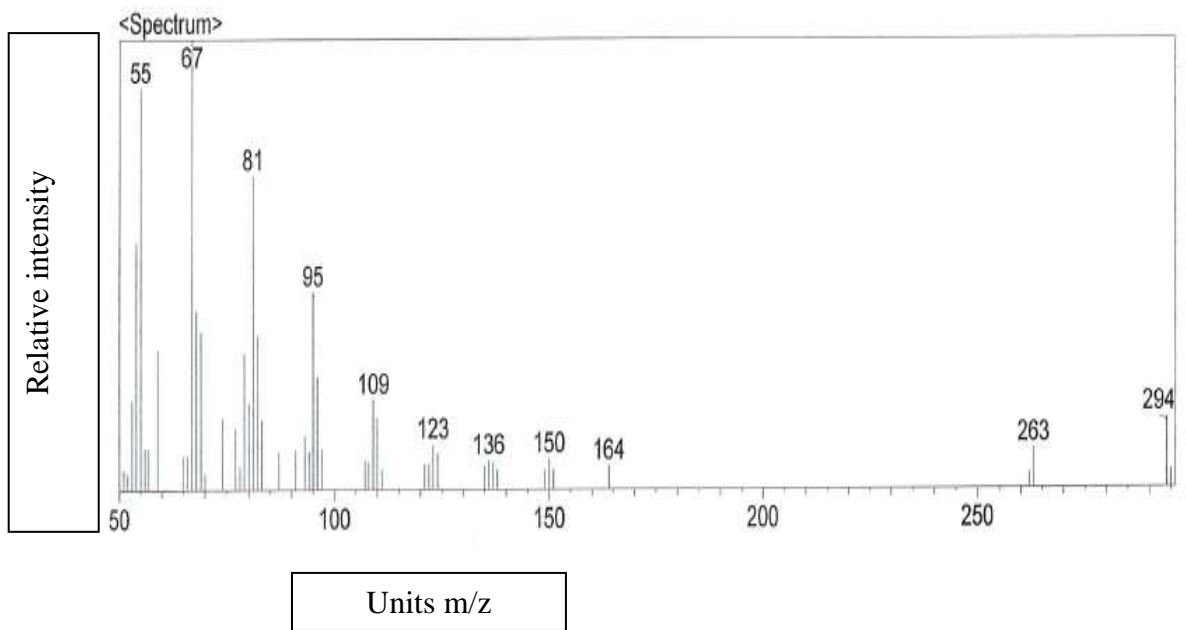


Figure 10: GC-MS of linoleic acids methyl Esters (FAMES) identified in the cactus oil.

Units m/z

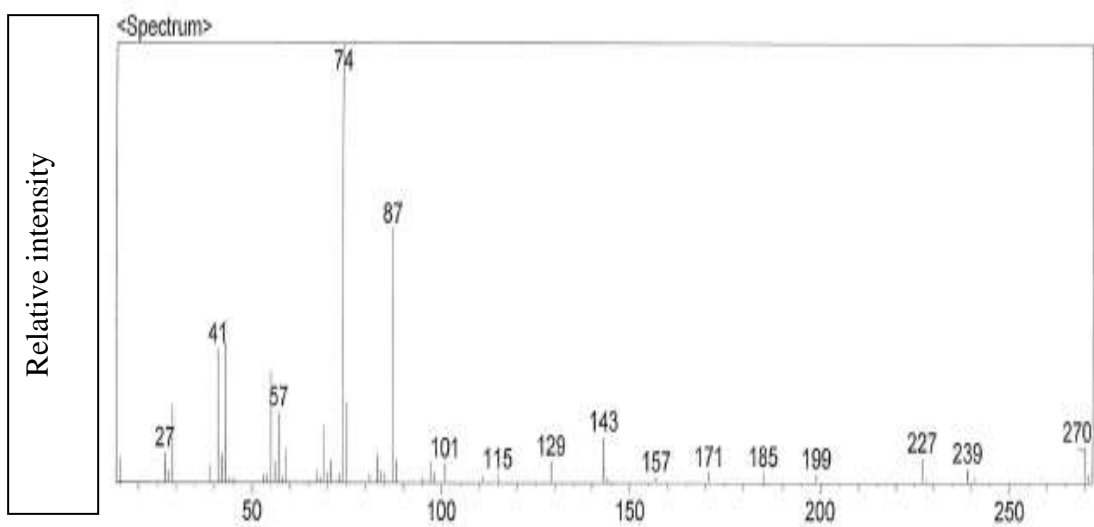


Figure 11: GC-MS of Palmitic acid methyl Esters (FAMES) acids identified in the cactus oil

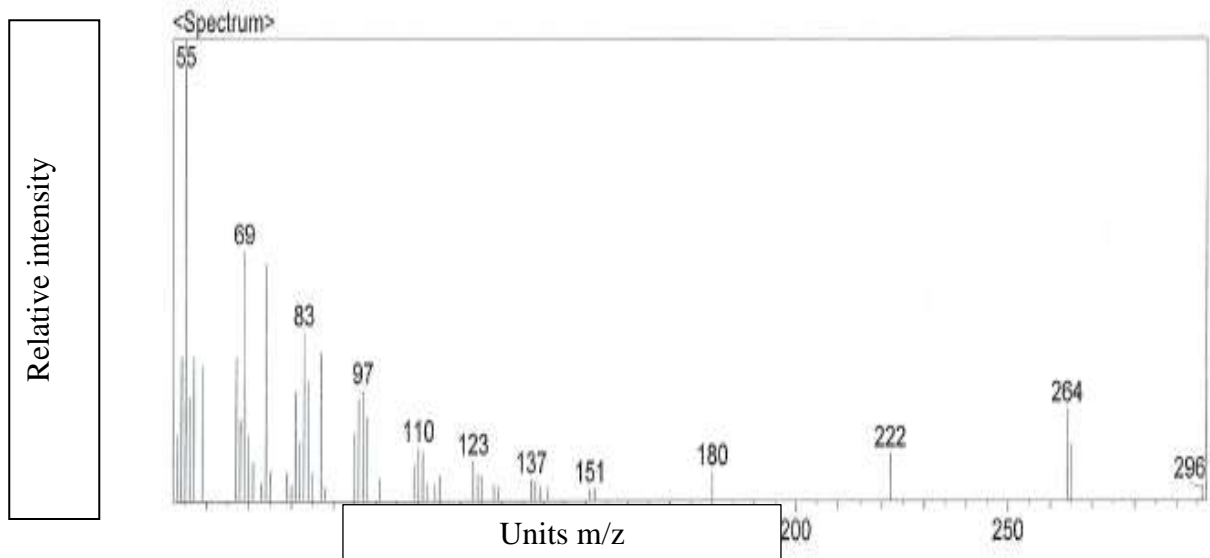


Figure 12: GC-MS of Oleic acid methyl Esters (FAMES) identified in cactus oil.

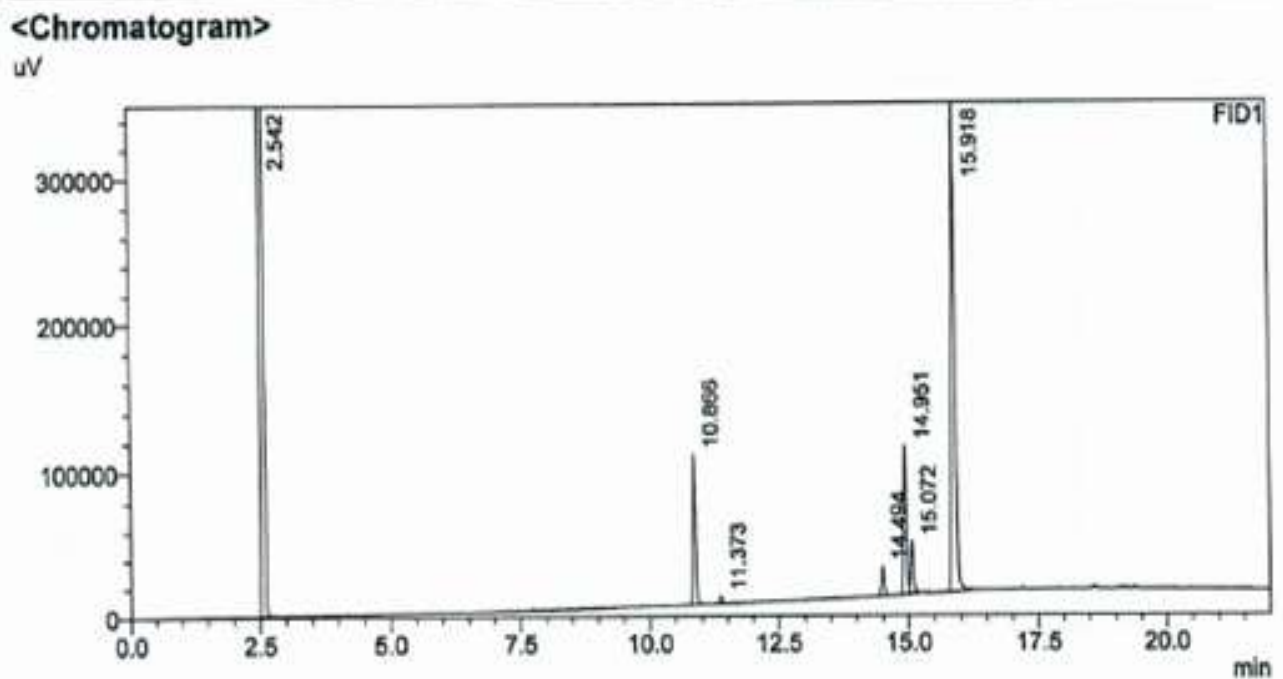


Figure 13:GC-FID Chromatogram of Fatty Acid Methyl Esters (FAMES) Identified in Cactus Oil from Palestine.

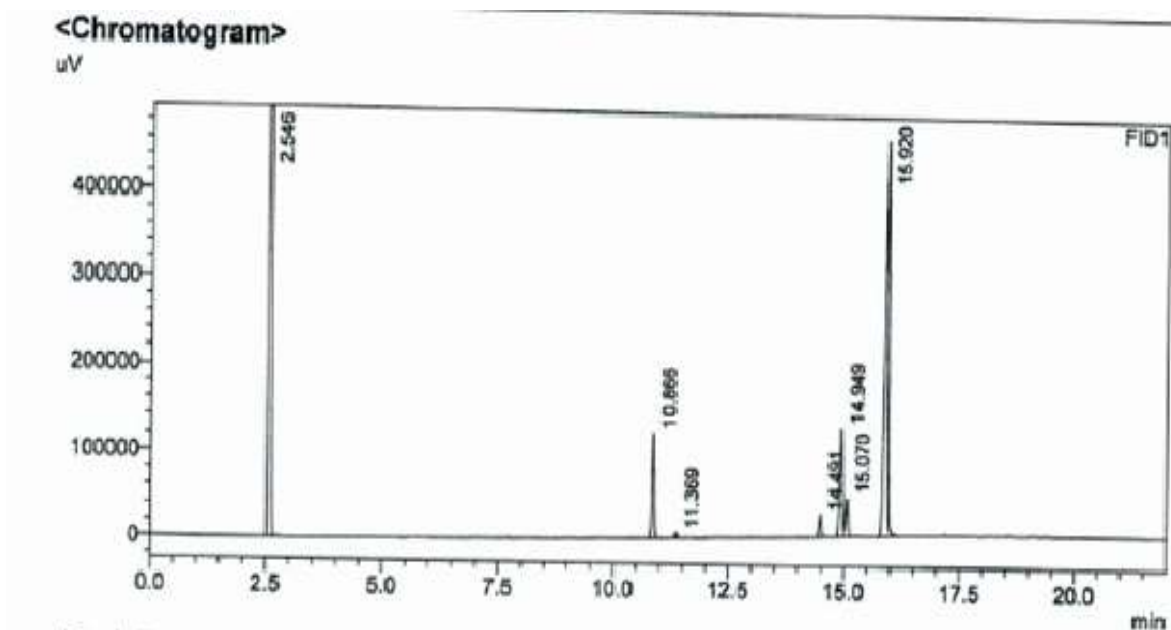


Figure 14: GC-FID Chromatogram of Fatty Acid Methyl Esters (FAMES) Identified in Cactus Oil from Morocco .

4.1.5 Tocopherol analysis

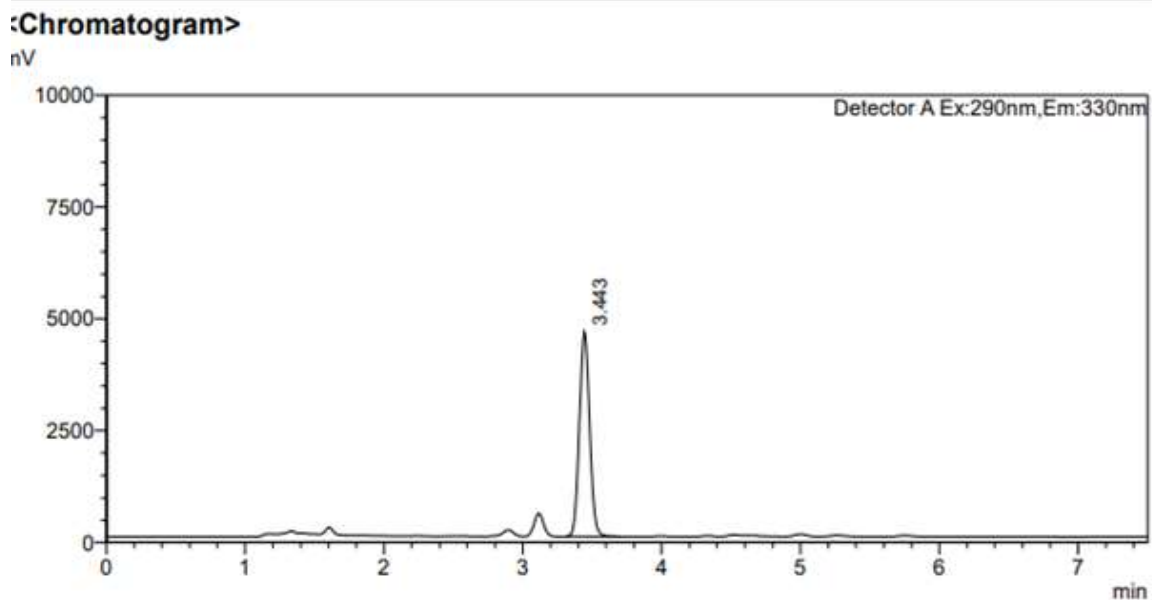
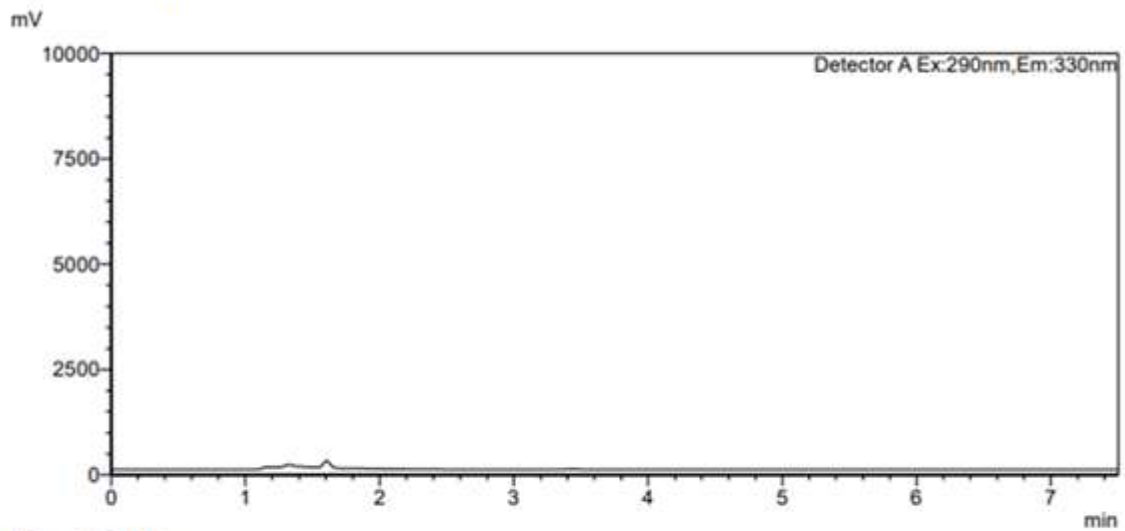


Figure 15: HPLC Chromatogram of α -Tocopherol (Standard)

<Chromatogram>



<Peak Table>

Detector A Ex:290nm, Em:330nm

Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark	Name
Total							

Figure 16: HPLC Chromatogram of Blank (Isopropanol).

<Chromatogram>

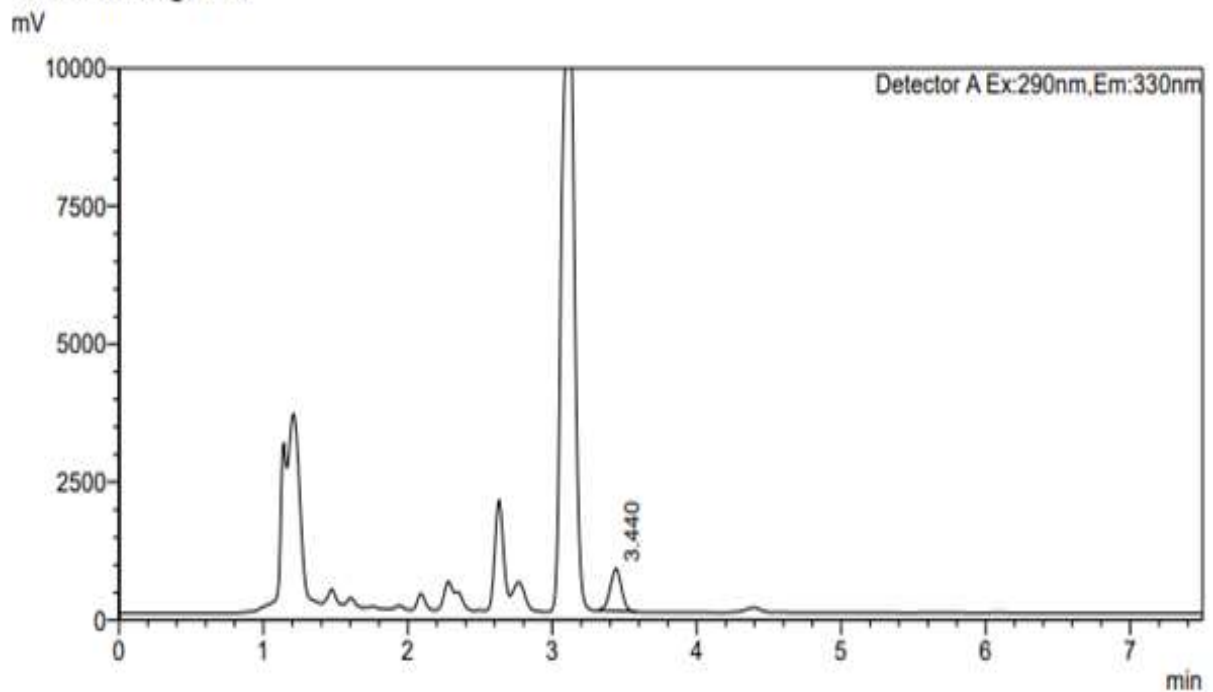


Figure 17: HPLC Chromatogram of α -Tocopherol Found in Cactus Oil from Palestine.

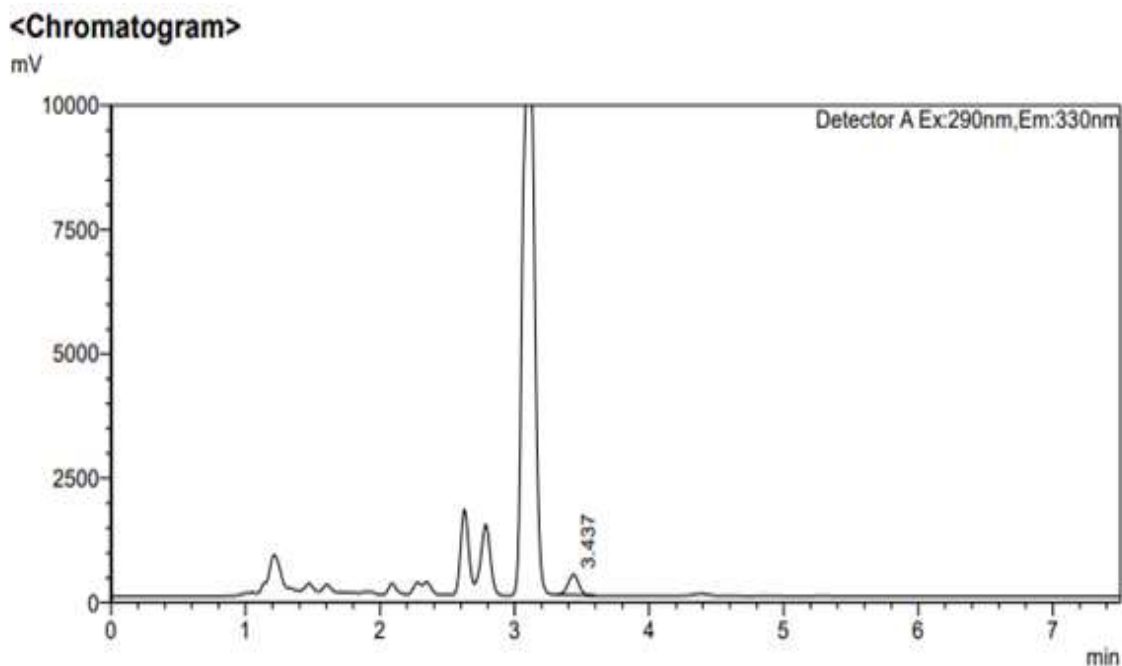


Figure 18: HPLC Chromatogram of α -Tocopherol Found in Cactus Oil from Morocco.

4.1.6 FTIR analysis

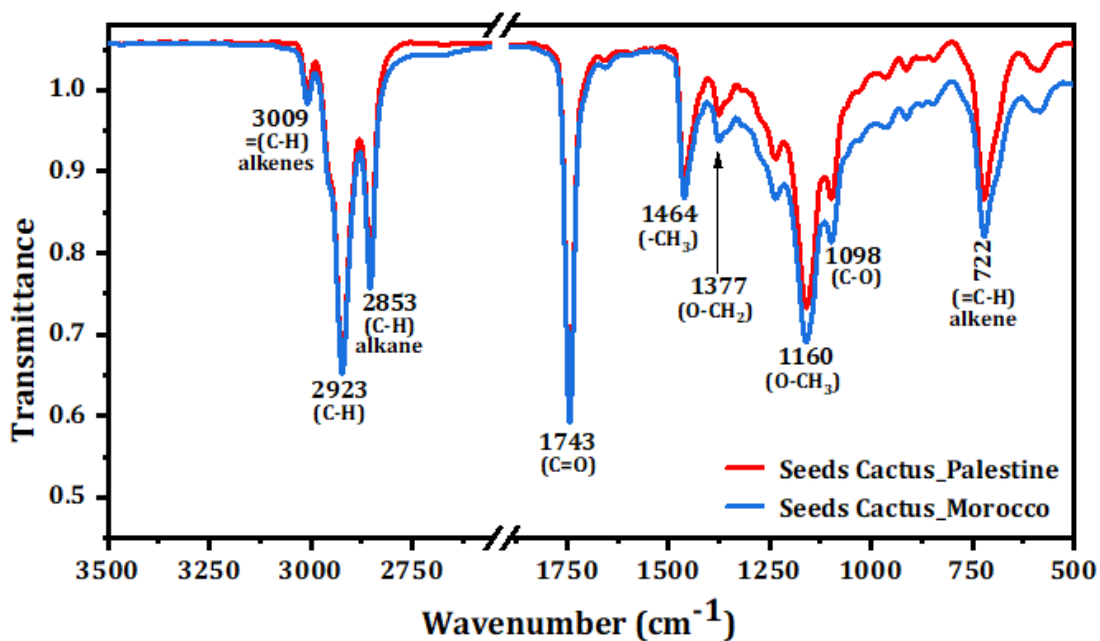


Figure 19: FTIR of cactus oil from Palestine compared to that from morocco.

Table 4.4: Expected FTIR Functional Groups in *Opuntia ficus-indica* seed Oil from Palestine and Morocco

Wave number (cm ⁻¹)	Types of vibration	Assignment
3009	Asymmetrical stretching	=C-H (alkenes)
2923	Asymmetrical stretching	C-H
2853	Symmetrical stretching	C-H Methylene (alkane)
1743	Stretching	C = O ester carbonyl in triglycerides and FAME
1464	Asymmetric stretching	-CH ₃
1377	Asymmetric stretching	O-CH ₂
1160	Stretching	O-CH ₃
1098	Stretching	C-O absorption
722	Bending and Overlapping Vibration of methylene	=C-H olefinic (alkene) group and -(CH ₂) _n methylene groups (cis disubstituted alkenes and aromatic)

4.1.7 Refractive index

Table 4.5 : Refractive index of cactus oil at 25C°

Sample (cactus oil)	Refractive index
#1	1.4724
#2	1.4724
#3	1.4723
Mean standard Deviation	1.4723.0000

4.1.8 Developed a highly effective formula that utilizes cactus oil extract:

- Created a Cream with Cactus Oil: cactus oil, especially the oil extracted from prickly pear seeds (*Opuntia ficus-indica*), is among the most valuable and beneficial oils for skin care. When incorporated into creams, it enhances the formulation with its rich content of essential fatty acids and antioxidants. When prepared a cream used

a cactus oil formula as the emulsion base, we observed a significant enhancement in both its physical and functional properties. These enhancements include: Enhanced absorption is achieved due to the unsaturated fatty acids linoleic, palmitic and oleic acid in the cream, allowed it to penetrate the skin more effectively.

4.2 Discussion

1. Acid value

"Acidity is associated with the level of free fatty acids and is considered an indicator of the shelf life and quality of edible oils. The acidity value of the OFI oil samples, as shown in Table (4.1), was found to be 1.4 mg NaOH/g, indicating a relatively good acidity level according to(ISO 660:2009), (Animal and vegetable fats and oils — Determination of acid value). This result suggests good oil quality when compared with findings from other studies (El Kharrassi et al., 2018).

2. Peroxide value (PV).

"The peroxide value (PV) is a critical indicator used to assess the initial stages of lipid oxidation in oils, reflecting their oxidative stability and freshness. In the present study, the peroxide value of the oil extract was found to be 0.993 ± 0.0015 mEq, as presented in Table (4.2), in accordance with (ISO 3960:2017). This low peroxide value suggests that the oil is relatively stable and has undergone minimal oxidation. When compared to the peroxide value reported by (El Kharrassi et al. 2018), which was 2.84 mEq, the result obtained in this study indicates superior oxidative stability, further confirming the quality and freshness of the extracted oil."

3. UV-visible spectroscopy.

The specific extinction coefficients at 232 nm (K232) and 270 nm (K270) are widely used to assess the presence of primary and secondary oxidation products, respectively, in oils. In this study, the measured values for cactus seed oil were 0.2657 for K232 and 0.08857 for K270, as shown in Table (4.3). These relatively low extinction values suggest a high oxidative stability of the oil, indicating limited formation of conjugated dienes and trienes, and thus minimal degradation. This reinforces the overall quality and suitability of cactus seed oil for cosmetic or food applications. It is important to note that, in this study, comparisons were made based on molar absorptivity due to the use of varying sample concentrations, whereas(El Kharrassi et al., 2018) conducted their analysis using direct absorbance measurements."

4. GC-MS

"Gas chromatography–mass spectrometry (GC-MS) analysis revealed the presence of three major fatty acids in the oil samples, as indicated by three distinct peaks in the chromatogram (Figure 9). These included one saturated fatty acid—methyl palmitate (C16:0), one polyunsaturated fatty acid—methyl linoleate (C18:2, omega-6), and one monounsaturated fatty acid—methyl oleate (C18:1, omega-9). Each compound was identified based on its molecular weight and retention time.

Specifically, Figure 10 shows the identification of methyl linoleate (C18:2) with a molecular weight of 294 g/mol, Figure 11 identifies methyl palmitate (C16:0) with a

molecular weight of 270 g/mol, and Figure 12 confirms the presence of methyl oleate (C18:1) with a molecular weight of 296 g/mol. The presence of these dominant fatty acids aligns with the typical composition reported for cactus seed oil and supports its high nutritional and functional value, particularly due to the high content of unsaturated fatty acids known for their beneficial roles in skin health and oxidative stability .

5. GC- FID

Gas chromatography with flame ionization detection (GC-FID) was employed to compare the fatty acid profiles of prickly pear seed oil samples from Palestine and Morocco, as illustrated in Figures 13 and 14, respectively. While both oils exhibited generally similar chromatographic patterns, the primary distinction lay in the concentrations of individual fatty acids. This analysis aimed to identify the specific fatty acids present in each sample. By comparing the results to the standards outlined in USP 401 (Chapter: Fats and Fixed Oils / Composition of Fatty Acids), the quality and profile of the fatty acids in the prickly pear seed oils were effectively evaluated, confirming the compositional differences and overall quality of the oils.

6. Tocopherol analysis

The chromatographic profile of cactus seed oil shown in Figure 17 demonstrates that α -tocopherol is clearly resolved on the C18 column and identified by comparing its retention time with that of a known α -tocopherol standard. The α -tocopherol peak observed in the Palestinian oil sample appeared at a retention time of 3.440 minutes (Figure 17), whereas the Moroccan oil sample exhibited a peak at 3.437 minutes (Figure 18). The slightly higher retention value and peak intensity in the Palestinian sample suggest a comparatively higher α -tocopherol content. These findings are consistent with the method described by(Bakre et al., 2015), and they highlight the potential antioxidant richness and quality of the Palestinian cactus seed oil .

7. FTIR analysis

Fourier Transform Infrared (FTIR) spectroscopy is a powerful analytical tool used for the structural characterization of plant-derived oils, particularly for identifying functional groups and assessing chemical quality (Muchtaridi et al., 2019). In this study, FTIR analysis was applied to prickly pear seed oil extracted from two samples sourced from Palestine and Morocco, as presented in Table (4.4) and Figure (19)

The results showed distinctive absorption peaks in both samples. A prominent peak was observed at 3009 cm^{-1} , corresponding to the presence of unsaturated (C=C-H) bonds, indicating a significant content of unsaturated fatty acids. This was followed by strong peaks at 2921.75 cm^{-1} and 2853 cm^{-1} , attributed to the asymmetric and symmetric stretching vibrations of saturated aliphatic $-\text{CH}_2$ groups, respectively, reflecting a balanced composition of saturated and unsaturated components.

A sharp absorption band around 1743 cm^{-1} , characteristic of ester carbonyl (C=O) groups found in triglycerides, confirmed the esterified nature of the oil. Additional peaks at 1464 cm^{-1} and 1377 cm^{-1} were associated with the asymmetric bending of $-\text{CH}_3$ and the vibrations of the O- CH_2 groups, respectively—these are typically found in mono-, di-, and triglycerides, and their presence suggests a refined oil profile rather than a pure FAME (Fatty Acid Methyl Ester) spectrum. The peak at 722 cm^{-1} corresponds to in-plane bending

of cis double bonds, indicating the presence of naturally unsaturated fatty acids. Furthermore, the absorption at 1098 cm^{-1} , related to the stretching of the C–O ester group, is particularly sensitive to oils rich in oleic acid chains. As oxidation progresses, the intensity of this band generally decreases, reflecting structural changes such as the formation of dimerized carboxylic acids and alteration of double bonds.

When comparing the spectra of the Palestinian and Moroccan oils (Figure 19), there was a high degree of similarity in peak positions, indicating a comparable chemical structure. However, some differences in absorption intensities were noted, which may be attributed to variations in the relative concentrations of components due to environmental or agricultural factors. These findings affirm the chemical quality and structural consistency of prickly pear seed oil from both regions.

8. Refractive index

The refractive index (RI) of cactus seed oil was measured to be 1.47237 ± 0.00000 , as presented in Table (4.5). This value falls within the standard range for pure and unadulterated vegetable oils, indicating the absence of impurities or contaminants that could influence the optical properties of the sample (ISO 6320:2017). The consistency and precision of this RI reading suggest a high level of purity and quality in the extracted oil. Moreover, this result is in close agreement with previously reported values in the literature. For instance, (Mouna et al., 2023) reported a similar refractive index for cactus seed oil, reinforcing the reliability of the current findings and validating the analytical methods used. The refractive index is a key indicator of oil identity and quality, and the alignment with published data further supports the chemical integrity of the Palestinian cactus seed oil sample.

9. Cream with Cactus Oil

The incorporation of cactus seed oil into the cream base formulation significantly enhanced the overall performance of the final product. Notably, the presence of cactus oil improved key functional skin benefits, including increased hydration, improved dermal absorption, and enhanced penetration. These physiological effects are attributed to the oil's rich content of essential fatty acids—particularly linoleic and oleic acids—as well as natural antioxidants such as tocopherols and phytosterols, which are known for their ability to support skin barrier function and retain moisture. In addition to these bioactive properties, the cactus oil also contributed to the sensory quality of the formulation. Observations showed improved texture, smoother application, and more uniform diffusion across the skin surface. These sensory enhancements are particularly important for consumer acceptance and product differentiation in premium cosmetic products. Collectively, these results validate the use of cactus oil as an effective active ingredient in high-quality skincare formulations, supporting both its functional and aesthetic contributions.

Chapter Five:

Conclusion and future work:

5.1 Conclusion

In conclusion, Palestinian prickly pear seed oil stands out as a high-value natural resource, abundant in essential fatty acids and potent antioxidants. Its distinctive biochemical composition not only contributes to skin nourishment and protection against oxidative stress, but also underscores its broad therapeutic and cosmetic potential. This makes it an exceptional candidate for integration into diverse applications—including functional foods, pharmaceutical preparations, and advanced skincare formulations. With its multifaceted benefits and premium quality, this oil holds great promise as a versatile and sustainable ingredient across multiple industries.

5.2 Future work

1. **Conduct Stability Testing:** It is recommended to perform comprehensive physical and chemical stability assessments of the formulated prickly pear seed oil cream. Parameters such as color, texture, odor, and pH should be regularly monitored to ensure product consistency, safety, and efficacy during storage.
2. **Explore Full Utilization of Prickly Pear Components**
Further research is advised to investigate the potential uses of all parts of the prickly pear fruit. For instance, peels may serve as animal feed, fibers as an alternative to oats, and juice in the production of vinegar or confectionery. However, detailed studies are needed to validate their safety, nutritional value, and commercial feasibility.
3. **Analyze Bioactive Compound Profiles**
It is recommended to carry out detailed quantitative analysis of fatty acids, along with alpha- and gamma-tocopherol concentrations, in Palestinian cactus seed oil. This would help in standardizing quality, understanding nutritional profiles, and supporting potential applications in the food and cosmetics industries.

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استخلاص زيت الصبار من التين الشوكي الفلسطيني (*Opuntia ficus-indica* [L]) -
دراسة مقارنة للتوصيف والتحضير التجميلي.

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الملخص:

تتناول هذه الدراسة عملية استخلاص وعزل وتوصيف زيت بذور التين الشوكي (*Opuntia ficus-indica* L) المستخرج من فلسطين، ومقارنته بزيت التين الشوكي المغربي. تم استخدام طريقة العصر البارد لاستخلاص الزيت بهدف الحفاظ على خصائصه الطبيعية. أُجريت تحاليل كيميائية وآلية شاملة شملت قياس قيمة الحموضة، وقيمة البيروكسيد، وامتصاص الأشعة فوق البنفسجية، والتحليل الطيفي الكتلي باستخدام الكروماتوغرافيا الغازية (GC-MS)، والتحليل الكمي باستخدام كاشف اللهب (GC-FID)، والكروماتوغرافيا السائلة عالية الأداء (HPLC)، والتحليل الطيفي بالأشعة تحت الحمراء (FTIR)، ومعامل الانكسار.

أظهرت النتائج أن زيت الصبار الفلسطيني يتميز بانخفاض قيمة الحموضة وقيمة البيروكسيد، مما يشير إلى استقرار أكسدي جيد وكشفت تحاليل FTIR عن قمم امتصاص مميزة تؤكد وجود الأحماض الدهنية غير المشبعة والتريغليسريدات، بما يتشابه مع العينة المغربية. كما أظهرت نتائج تحليل الأحماض الدهنية تركيزات عالية من حمض اللينوليك (C18:2)، وحمض الأوليك (C18:1)، وحمض البالمتيك (C16:0). وكشف تحليل HPLC لمركبات التوكوفيرول عن تركيز أعلى من الفا-توكوفيرول في الزيت الفلسطيني.

تم تحضير كريم تجميلي باستخدام زيت الصبار، وقد أظهر تحسناً في القوام وسرعة امتصاصه عبر الجلد وفعالية الترطيب. تبرز هذه الدراسة إمكانات زيت بذور التين الشوكي الفلسطيني كمكون عالي الجودة وغني بالمواد النشطة بيولوجياً، لاستخدامه في التطبيقات الغذائية والدوائية والتجميلية. وسيضمن العمل المستقبلي تحليلاً أكثر تفصيلاً لتكوين الأحماض الدهنية، وتقييم استقرار التركيبات، وتحديد كميات أنواع التوكوفيرول المختلفة لتعزيز إثبات قيمته الوظيفية.