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EXTRACTION AND CHARACTERIZATION OF FIXED OIL FROM *SPINACIA OLERACEA* SEEDS: PHYSICOCHEMICAL PROPERTIES AND BIOACTIVE COMPOUNDS

Madiha Batool[a], Uzman Khan[a, 1], Sehar Ashraf[b], Razia Batool[a], Majid Nazir[a], Abdul Shakoor[a], Ejaz Ahmad[a], Hafiz Muhammad Faizan Haider[a], Syed Ahmad Raza Bokhari[c], Hamad Ahmad[d], Nasir Khan[e], Najeeb Ullah[f], Asim Ali[g], Ume Kalsoom[h], Fareed Ahmad[i], Ayesha Aslam[i], Syed Sibt-E-Hassan Kazmi[j]

[a] *Department of chemistry,* Government College University, Lahore, *Pakistan,*
[b] Government Islamia college cooper road Lahore, *Pakistan,*
[c] George Brown College, M5T239, Toronto, Canada
[d] *Department of Chemistry, University of Management and Technology, Lahore 54000, Pakistan*
[e] *Lahore Garrison University, Lahore, Pakistan*
[f] *University of the Punjab, Lahore, Pakistan*
[g] *Comsat University, Lahore, Pakistan*
[h] *University of the Central Punjab, Lahore, Pakistan*
[i] *Minhaj University Lahore, Pakistan*
[j] International School Lahore Pine Avenue

[1] Corresponding Author: Dr. Uzman Khan, E-mail: *uzmanuk@gmail.com*
Tel: (+92)-305-6505302

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ABSTRACT:

Spinacia oleracea, commonly known as spinach, is a green, leafy vegetable belonging to the family Amaranthaceae. Spinach seed oil is rich in essential nutrients, making it beneficial for skin and hair health, particularly in addressing damage, aging, and brittleness. This oil contains a diverse range of vitamins and antioxidants crucial for overall well-being. Given its nutritional significance, extensive research has been conducted on this plant.

The present study focuses on the extraction and characterization of fixed oil from Spinacia oleracea seeds. The extraction process using n-hexane yielded 4.15% oil. The obtained oil was further analyzed for various physicochemical properties, including refractive index, color, saponification value, iodine value, free fatty acid content, and peroxide value.

Analysis on GCMS revealed the presence of multiple bioactive compounds, such as Cedren-13-ol, 2-butyl-1-octanol, octadecanoic acid (2-oxo-, methyl ester), hexadecenoic acid (methyl ester), 1-hexadecanol (2-methyl-), 9-octadecenoic acid (Z, methyl ester), 10-octadecenoic acid (methyl

ester), heptadecanoic acid (16-methyl-, methyl ester), 4-octadecenal, 13-methyltetradec-9-enoic acid (methyl ester), phthalic acid (di(6-methylhept-2-yl) ester), and 2,6,10-dodecatrienoic acid (3,7,11-trimethyl-methyl ester).

The high degree of unsaturation in the extracted oil highlights its potential for edible applications and nutritional benefits.

KEYWORDS: Spinacia oleracea seed oil**,** Bioactive compounds**,** Physicochemical properties**,** Oil extraction**,** Nutritional benefits

INTRODUCTION:

The botanical name for spinach is *Spinacia oleracea* (Figure 1). *Spinacia* comes from the Latin word for spine and refers to the prickly seed coat. The species name, *oleracea*, refers to a plant that is edible. **Spinach** (*Spinacia oleracea*) is an edible flowering plant in the family of Amaranthaceae [1].

Figure 1: Spinach Leaves

Spinach is annual for leaf production and biennial for seed production, which grows to a height of up to 30 cm. Common spinach, *S. oleracea*, was long considered to be in the family Chenopodiaceae, but in 2003, that family was merged into the family Amaranthaceae in the order Caryophyllales (table 1).

Table 1: Scientific classification of Spinach

Kingdom	Plantae
Order	Caryophyllales
Family	Amaranthaceae, formerly Chenopodiaceae
Subfamily	Chenopodioideae
Genus	Spinacia
Species	S. oleracea
Botanical name	*Spinacia oleracea L.*

OCCURRENCE:

Spinach (*Spinacia oleracea*) is a hardy annual related to beets and Swiss chard that has been used by humans for a long time. Native to Southwest Asia, it was first cultivated in Persia (Iran) over 2000 years ago and used by the Chinese in the 6th century. It grown in northern Europe. It may be found in new England state (Connecticut, Maine, Massachusetts, New Hampshire, Rhode Island). It was originated from Iran and first declared as an herb of Persia by the Chinese. Turkey, United States, Japan, China, Turkey, Indonesia is considered the largest producers of spinach (figure 2).

Figure 2: Spinach crop

POTENTIAL BENEFITS OF SPINACH:

1. **Potential Benefits of Spinach for men:**
   Spinach offers numerous health benefits for men. It is a powerhouse of essential nutrients like vitamins A, C, and K, as we well as minerals like iron and magnesium [2]. These nutrients support overall well-being and can help boost energy levels, enhance muscle function, and improve cardiovascular health.
2. **Potential Benefits of Spinach for women:**
   Spinach offers a plethora of benefits for women's health. It's rich in vital nutrients, including iron, calcium, and folate, which are particular important for women.

Produce seeds on spinach:

Spinach (*Spinacia Oleracea*) is a cool-season crop that produces seeds through a process called "bolting". As the seed pods mature, the plant's energy is redirected from leaf growth to seed production, causing the plant to "bolt" or stretch upward, often producing a tall stem with seed pods (figure 3) [3]. There are following steps to produce spinach seeds:

- Flower formation,
- Pollination,
- Seed development,
- Bolting,
- Seed maturation,
- Harvesting seeds

Figure 3: Seed pods on spinach

SPINACH SEEDS:

Spinach seeds are small, round, and slightly oval shape. They are usually hard in texture and are typically about 3-5 millimeters in diameter. Spinach seeds are typically sown directly into the soil in early spring or late summer, depending on your climate. They should be planted about half an inch deep and spaced 6-12 inches apart in rows. Spinach prefers well-drained soil with plenty of organic matter and full sun to partial shade. Keep the soil consistently moist, and thin the seedlings to allow proper spacing as they grow (figure 4).

Figure 4: Spinach seeds

NUTRITIONAL CONTENTS IN SPINACH SEEDS:

Spinach seeds are rich in protein, which is essential for muscle growth, repair, and overall body function (table 2) [4].

Table 2: Nutritional Contents in Spinach seeds

Components	Amount
Protein	3.2 g
Fiber	0.6 g
Ascorbic acid	51 mg
Fat	0.3 g
Carbohydrates	4.3 g
Iron	3.1 mg

Potassium	470 mg
Calcium	93 mg
Vitamin A	141 mcg
Folate	58 mcg
Vitamin K	483 mcg

FIXED OIL:

The term oil applies to a number of bodies not miscible with water, from both the vegetable and animal kingdom, which are fluid at ordinary or slightly elevated temperature [5]. When placed on paper they render it translucent, or impart to it a greasy stain. Oils may be divided conveniently with reference to the volatility, into two groups: Fixed or fatty acid and fats, and volatile or essential oils. Fixed oil derived the name from not being volatilized by the vapors of boiling water. They do not evaporate like essential oil and are often called the Carries oils. In the plant kingdom fixed oil are mostly derived from the seeds of dicotyledonous plants, although some monocotyledonous plants, such as the palm trees, furnish several of the technically important fixed oil. They are basically base oil used for the making soaps and lotions [6].

EXTRACTION OF OIL:

MECHANICAL EXTRACTION:

Oils can be removed via mechanical extraction, termed "crushing" or "pressing." This method is typically used to produce the more traditional oils (e.g., olive, coconut etc.), and it is preferred by most "health-food" customers in the United States and in Europe. There are several different types of mechanical extraction. Expeller-pressing extraction is common, though the screw press, ram press, and Ghani (powered mortar and pestle) are also used [7]. Oilseed presses are commonly used in developing countries, among people for whom other extraction methods would be prohibitively expensive; shown as Ghani (20-30 %), Expeller (34-37 %) and solvent (40-43 %) (figure 5).

Figure 5: Mechanical extraction

SOLVENT EXTRACTION:

The processing of vegetable oil in commercial applications is commonly done by chemical extraction, using solvent extracts, which produces higher yields and is quicker and less expensive.

The most common solvent is petroleum-derived hexane. This technique is used for most of the "newer" industrial oils such as soybean and corn oils. Supercritical carbon dioxide can be used as a non-toxic alternative to other solvents [8]. Soxhlet apparatus follow a solvent extraction method (figure 6).

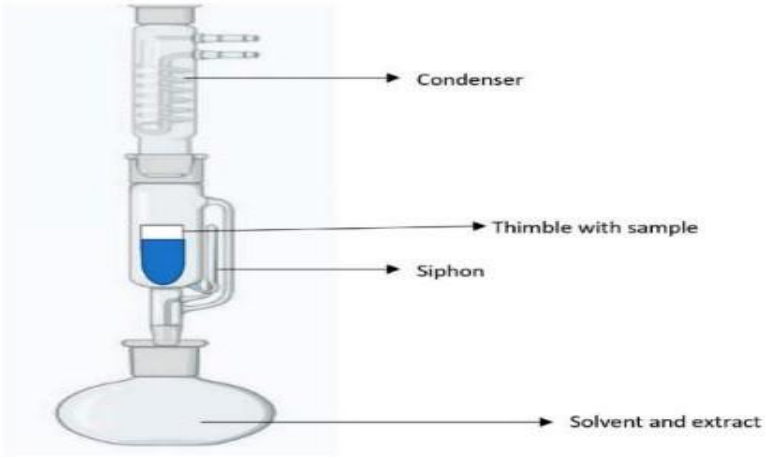

Figure 6: Solvent extraction

SPINACH SEEDS OIL:

Spinach seeds oil is typically dark green in color and has a mild, nutty aroma (figure 7).
Spinach seeds oil have following characteristics:
Seeds of spinach also provide extremely healthy attributes which assist the skin and hair [9].
The Spinach oil contains an extraordinary range of vitamins and antioxidants which are essential to the body for optimal health [10].
Spinach seed oil is abundant with copious amounts of nutrients and is ideal for damaged, aging skin and brittle lackluster hair [11].

Figure 7: Spinach seeds oil

PROPERTIES OF SPINACH SEEDS OIL:

MOISTURIZING:

It has excellent moisturizing properties, making it suitable for hydrating dry skin and hair.

Nutrient-rich:
Rich in essential fatty acids, particularly linoleic acid and alpha-linoleic acid, which are crucial for maintaining skin health and overall well-being [12].

Antioxidant:
Spinach seeds oil contain vitamin E, a powerful antioxidant that helps protect the skin from damage caused by free radicals, thus slowing down the aging process.

Anti-inflammatory:
Spinach seeds oil has anti-inflammatory properties that can help reduce redness, irritation, and inflammation on skin [13].

Absorbent:
It absorbs quickly into the skin without leaving a heavy residue, allowing for comfortable application and fast absorption.

Nutritional:
When consumed as a dietary supplement, spinach seed oil provides essential nutrients like fatty acids and vitamins, promoting overall health and well-being [14].

Health benefits of Spinach seeds oil:
Spinach seeds oil has many important roles to play in maintaining a healthy body; various health benefits of spinach seeds oil include the following:

Prevent Cancer:
Spinach oil has a high source of zeaxanthin and carotenoids that can flush out the free radicals from your body. These free radicals make your body prone to many diseases including cancer and as a result, spinach is said to prevent cancer [15]. So, all need to consume spinach and prevent from stomach cancer, mouth cancer, and esophagus cancer.

Reduce Blood Sugar:
Spinach oil, rich in antioxidants and fiber, plays a vital role in regulating blood sugar levels [16]. Its high magnesium content helps enhance insulin sensitivity, aiding in glucose metabolism. Incorporating spinach in diet can contribute to better glycemic control, making it a valuable addition for individuals managing blood sugar levels [17].

Aids in Good Bone Health:
Spinach contains vitamin K that aids in good bone health and this mean adequate consumption of vitamins can do good to health [18]. It also improves calcium absorption by body. Spinach seeds oil contains 250 milligrams of calcium per cup and this is much required by bones and teeth. Calcium is a strengthening agent for bones and keeps bones healthy.

Good for Eyes:
Spinach oil benefits for eyes are evident through the antioxidants found in it, lutein and zeaxanthin, which contribute to good eyesight and protect against issues like cataracts, age-related macular degeneration, and other eye problems [19]. Additionally, the vitamin A found in spinach, benefits in maintaining mucus membranes that are essential for normal eyesight [20].

Reduce Hypertension:
Hypertension is also known as high blood pressure is responsible for causing many heart diseases, kidney diseases and strokes [21]. Consuming at least once a day can reduce anxiety and stress [22].

Has Anti- inflammatory Properties:
Spinach seeds oil contained neoxanthin and violaxanthin are two anti-inflammatory properties that regulate inflammation [23]. Its high source of anti-inflammatory properties can help to prevent osteoporosis, migraine, asthma, arthritis and headaches [24].

Prevents Anemia:
Spinach seed oils have a high source of iron content in it that can prevent from the risks of developing anemia. Iron is also good for women who are menstruating, children and adults [25].
Natural Anti-ageing Properties Premature ageing is one of the most common problems [26]. However, spinach oil loaded with antioxidants that have the tendency to destroy and prevent free radicals that cause premature ageing. Spinach leaves have a long-standing reputation in folklore medicine for their diverse medicinal uses.

Material and Method:

Methods:
Following methods were applied for extraction and characterizations of spinach seed oil.

- Extraction of Spinach seeds oil,
- Determination of Oil yield,
- Determination of refractive Index,
- Determination of saponification value,
- Determination of free fatty acid value,
- Determination of peroxide value,
- Determination of specific gravity,
- Determination of moisture content,
- Determination of ester value.,
- Identification of fatty acids by GC

Extraction of Spinach seeds oil:
The seeds of Spinacia oleracea was crushed in a pestle mortar to a maximum possible finesse and weighted accurately. The crushed material was placed in a filter paper thimble and covered at the top (figure 8).
The thimble was placed in Soxhlet apparatus. Fitted with water cooled condenser at the top and round bottom flask at the lower end and n-hexane was used as solvent for extraction of oil and was put into round bottom flask. The apparatus is placed on water bath and then heated. The volatile solvent vaporized on heating which passed up the side arm of Soxhlet and When sufficient solvent has been transferred to extracting tube to fill the siphon arm, it siphons back into the round bottom flask.
This process was continued for 12 hours until the extraction of oil was complete. The solvent was distilled off and the residual oil was heated until the last traces of the solvent were removed.

Figure 8: Soxhlet apparatus

OIL YIELD:

The oil yield refers to the amount of oil extracted from a particular source, such as seeds, or plants usually measured in term of weight. The yield can vary depending on factors like the type of source, extraction method (figure 9).

Figure 9: Oil yield

The condensed solvent falls drop by drop into thimble. water cooled condensation method was utilized. Dry spinach seeds were 130 grams. Therefore, the oil yield obtained from the spinach seeds using the Soxhlet apparatus was approximately 4.15%.

Percentage Oil yield = Weight of oil/ Weight of dry seeds x 100

Weight of dry seeds = 130 g Weight of extracted oil = 5.39 g

Oil Yield [%] = 5.39/ 130 x 100

REFRACTIVE INDEX VALUE:

The refractive index of a medium was the ratio of the speed of light at a definite wave length in vacuum to its speed in the medium. The refractive index of a given substance varies with the wave length of the light used and with temperature. Abbe's refractometer (Japan) was used for the determination of RI (figure 10).

Figure 10: Refractometer

The refractive index of spinach seeds oil is 1.4394. This value indicates how much light bends when passing through the oil. The refractive index of a given substance varies with the wave length of the light used and with temperature.

BUTYRO VALUE:

The butyro value is a significant parameter used to assess the purity and composition of fats and oils. The butyro value of spinach seeds oil is 59.9. This value represents the degree of unsaturation in the oil.

The initial weight of the spinach oil extracted is 5.39 grams. After the extraction process, the weight of the Higher butyro values indicate a greater proportion of unsaturated fatty acids.

Butyro value of Spinach seeds oil is 59.9.

SAPONIFICATION VALUE:

The saponification value of oil is the number of milligrams of potassium hydroxide required to saponify completely one gram of the oil i.e., to neutralize the free fatty acids and fatty acids combined with glycerol (figure 11).

Figure 11: Saponification value test

An alcoholic solution of Potassium hydroxide (KOH) and 2 g spinach seeds oil is mixed. The mixture is boiled. Onedrop of phenolphthalein indicator is added. 21 mL of 0.5 N HCl solution is used for titration. The pink color disappears during titration. Blank test also performed same as it is without sample. The saponification value obtained is 210.375 mg of KOH per gram of the spinach seeds oil.

Saponification Value $= (B-A) \times 0.02805/W \times 100$

A = Volume used for titration for oil sample = 21 mL

B = Volume used for titration for blank sample = 39mL

W = Weight of sample = 2 g

Saponification Value $= (21-39) \times 0.02805/2 \times 100$

= 210.375 mg of KOH / g

ACID VALUE:

The acid value is the number of mg of potassium hydroxide required to neutralize the free acid in 1 g of the oil. The acid value of a fat or oil gives the information about the amount of free fatty acids presents in it.

Figure 12: Acid value test

Spinach seeds oil and 10 mL distilled water was mixed. The mixture placed on a hot plate and use a stirrer for mixing. One drop of phenolphthalein indicator is added. 0.025 mL of 0.074 N KOH solution is used for titration. Blank test also performed same as it is without sample. The pink color appears during titration. The acid value obtained is 0.10 mg of KOH / g of the spinach seeds oil (figure 12).

Acid Value = V × N × Eq.wt W
N = Normality of KOH = 0.074 N
V = Volume of KOH = 0.025 mL
W = Weight of oil = 1 g
Eq. wt = Equivalent weight of KOH = 56.1g/mol
So, Acid Value = 0.025 × 0.074 × 56.1/ 1
= 0.10 mg of KOH / g

PEROXIDE VALUE:

Peroxide value is the number of micrograms of active oxygen present in 1 g of fat or oil or mill equivalent of peroxide oxygen per kilogram of sample (figure 13).

Figure 13: Peroxide value test

Spinach seeds oil and 30 mL solvent mixture (3:2 ratio of acetic acid and chloroform) and 1 mL of saturated KI solution is mixed. Add 30 mL of distilled water into it. Use 1 mL starch solution as an indicator. 7 mL volume of 0.01 N sodium thiosulphate is used for titration. The blue color disappears during titration. The peroxide value obtained is 3.5 mq / kg of the spinach seeds oil
Peroxide Value = V × N ×100/W
N = Normality used for titration = 0.01 N
V = Volume of sodium thiosulphate =7 mL
W = Weight of oil = 2 g
So, Peroxide Value = 7 × 0.01 ×100/2 = 3.5 mq / kg

SPECIFIC GRAVITY:
The specific gravity of spinach seed oil refers to the ratio of the density of spinach seed oil to the density of water at a specific temperature. The unit of specific gravity is dimensionless because it is a ratio pf the density of a substance to the density of a reference substance.

Specific gravity of Spinach seeds oil is 0.8 and it shows that oil is less dense than water, meaning it would float on the surface of water (figure 14).

Specific gravity of Spinach seeds oil = 8/10 Specific gravity of Spinach seeds oil = 0.8 Moisture content.

Moisture content refers to the amount of water present in a substance, usually expressed as a percentage of the substance's total weight. Spinach seed oil typically has a very low moisture content. This is because oils are typically extracted from seeds using processes that remove moisture to ensure the stability and quality of oil.

The moisture content represents the percentage of water present in the spinach seed oil. Spinach seed oil has a 0.02 % moisture content. Lower moisture content indicates better stability and longer shelf life. Moisture content in Spinach seeds oil = 0.02 %.

Figure 14: Specific gravity test

Table 3: The Physicochemical Characteristics of Spinach seeds oil

Sr. No.	Parameters	Results
1.	Oil Yield [%]	4.15
2.	Refractive Index [20°C]	1.4394
3.	Butyro value	59.9
4.	Saponification Value [mg KOH/g]	210.375
5.	Acid Value [mg KOH/g]	0.10
6.	Peroxide Value [meq/Kg]	3.5
7.	Specific Gravity	0.8
8.	Moisture Content [%]	0.02

The oil yield was 4.15, refractive index 1.4394 at 20°C, acid value 0.10 mg KOH/g, peroxide value of spinach seeds oil is 3.5 meq/Kg, specific gravity 0.8, moisture content of the spinach seeds oil is 0.02% (table 3).

DISCUSSION:

The physicochemical characterization of the fixed oil extracted from *Spinacia oleracea* (spinach) seeds was conducted in the present study. The extraction yielded 4.15% oil, which is comparable to previous studies, though slight variations exist. The refractive index of the extracted oil was measured at 1.4394 at 20°C, while in previous research, it was reported as 1.4296 at the same temperature. This variation could be attributed to differences in extraction methods, environmental factors, or genetic variations in spinach seeds.

The acid value of the extracted spinach seed oil was determined to be 0.10 mg KOH/g, indicating a low level of free fatty acids, which suggests good oil stability and resistance to rancidity. In contrast, previous studies reported an acid value of 0.19 mg KOH/g, which is slightly higher than the current findings. The peroxide value, an important indicator of oil oxidation, was found to be 3.5 meq/Kg in this study, whereas previous research documented a significantly lower value of 0.19 meq/Kg. This difference may be due to variations in storage conditions, seed quality, or oil extraction methods. The specific gravity of the extracted oil was determined to be 0.8, and its moisture content was found to be 0.02%, which is slightly lower than the previously reported moisture content of 0.03%. The low moisture content suggests better stability and shelf life of the extracted oil.

Gas Chromatography-Mass Spectrometry (GC-MS) analysis revealed the presence of multiple bioactive compounds in spinach seed oil. The identified compounds include Cedren-13-ol, 2-butyl-1-octanol, octadecanoic acid (2-oxo-, methyl ester), hexadecanoic acid (methyl ester), 1-hexadecanol (2-methyl-), 9-octadecenoic acid (Z, methyl ester), 10-octadecenoic acid (methyl ester), heptadecanoic acid (16-methyl-, methyl ester), 4-octadecenal, 13-methyltetradec-9-enoic acid (methyl ester), phthalic acid (di(6-methylhept-2-yl) ester), and 2,6,10-dodecatrienoic acid (3,7,11-trimethyl-, methyl ester). These compounds contribute to the nutritional and functional properties of the oil and may have potential health benefits. The presence of high levels of unsaturated fatty acids in the extracted oil further highlights its suitability for edible applications and potential nutritional advantages (figure 15 & table 4).

Overall, the findings of this study suggest that the physicochemical properties of *Spinacia oleracea* seed oil exhibit variations when compared to previous research. However, the presence of bioactive compounds underscores its potential applications in food, cosmetic, and pharmaceutical industries. Further studies are recommended to explore its stability, shelf life, and bioactive properties in different formulations.

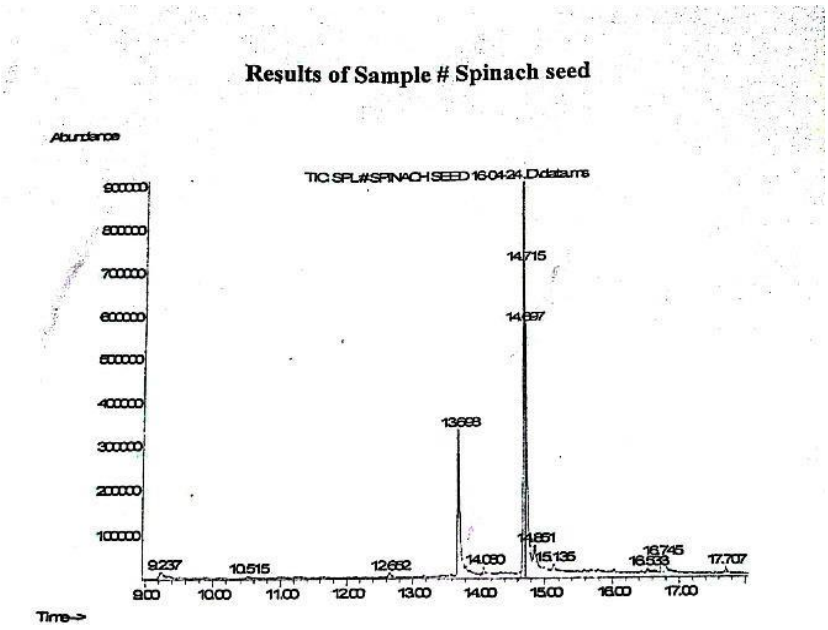

Figure 15: GCMS chromatogram of Spinach seeds oil analysis

Table 4: GCMS Characterization of Spinach seeds oil.

Sr. No.	Retention Time	Compound Found	Area
1.	9.237	Cedren-13-ol	1.859
2.	10.515	2-butyl-1-Octanol	0.561
3.	12.652	Octadecanoic acid, 2-oxo-, methyl ester	0.390
4.	13.698	Hexadecanoic acid, methyl ester	24.906
5.	14.080	1-Hexadecanol, 2-methyl-	1.206
6.	14.697	9-Octadecenoic acid (Z)-, methyl ester	24.137
7.	14.715	10-Octadecenoic acid, methyl ester	34.612
8.	14.851	Heptadecanoic acid, 16-methyl-, methyl ester	3.270
9.	15.135	4-Octadecenal	1.436
10.	16.533	13-Methyltetradec-9-enoic acid methyl ester	0.618
11.	16.745	Phthalic acid, di (6- methylhept-2-yl) ester	4.426
12.	17.707	2,6,10-Dodecatrienoic acid, 3,7,11-trimethyl-methylester	0.941

CONCLUSION:

The global consumption of edible oil has significantly surpassed its production, with annual demand reaching approximately 2.3 million tonnes. Only 20% of this demand is met by domestic production, while the remaining quantity is imported. Given the increasing reliance on edible oils, exploring alternative sources has become crucial. Traditionally, oilseeds have been cultivated for centuries and classified into conventional and non-conventional sources. When conventional resources fall short of meeting demand, non-conventional sources such as spinach seed oil offer a viable alternative.

Spinach seed oil, a non-conventional oil source, has gained attention due to its high unsaturated fatty acid content (83.3%), making it a potential substitute for widely used edible oils such as corn oil, soybean oil, and sunflower oil. The quality of edible oil is primarily determined by its fatty acid composition. A higher proportion of unsaturated fatty acids enhances the oil's nutritional profile, contributing to better health benefits, including improved heart health and reduced cholesterol levels.

The physicochemical analysis conducted in this study confirms that spinach seed oil possesses favorable properties, including a low acid value and high oxidative stability, making it suitable for edible and industrial applications. The presence of bioactive compounds identified through GC-MS analysis further supports its potential in food and cosmetic industries. Additionally, its moisture content and peroxide value indicate a good shelf-life, ensuring its viability as an alternative oil source.

Given the increasing demand for healthier and more sustainable edible oils, further research is recommended to explore large-scale extraction, refining techniques, and commercial applications of spinach seed oil. Its potential use in nutraceuticals and functional foods should also be investigated to maximize its health benefits. With proper processing and optimization, spinach seed oil can emerge as a valuable non-conventional source in the edible oil industry.

In conclusion, the findings of this study emphasize the significance of spinach seed oil as a sustainable and nutritionally beneficial alternative to conventional oils. With its high unsaturated fatty acid content and bioactive compounds, it holds promise for future applications in various industries. Continued research and development can further enhance its commercial viability, making it a valuable addition to the edible oil market.

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DISCLOSURE STATEMENT:

No potential conflict of interest.

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