Study of Chemical Composition and Evaluation of Anti-Hypertensive Effect of A Fixed Oil Extracted From Linum usitatissimum Grains

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Abstract: Globally grown flaxseed is regarded as an oilseed with numerous medicinal and health benefits such as: lowering blood pressure, blood sugar, decreasing the risk of neurological and obesity-related illnesses. The objective of this study is to extract the flaxseed oil and to characterize it chemically and physically; by pressing the seeds into two different temperatures: 67°C and 90°C oil yields; 24% and 29% were obtained from flaxseed. The followings are the outcomes of the physico-chemical analyses: pH value: 0.476; saponification index: 168.3; KOH/g: 28.05; peroxide index: 174; density: 0.951; refractive index: 1.482 at 18.1°C and 1.485 at 18.2°C; peroxide index: 1.70 at 0.96, 1.70 mg/100; all are related to the peroxide index. In this study we tried to test the “anti-hypertension” activity of this oil by simulating a blood cycle in the laboratory which allowed us to determine the variation in blood pressure as a function of the volume of oil injected into the blood cycle from 17.4 to 14.10.

Keywords: Linum usitatissimum ; linaceae ; oil ; HPLC ; Hypertension.

1. INTRODUCTION

Since antiquity medicinal plants have been the most fertile source of leads for medication development, global studies have been conducted to validate their efficacy, and some of the findings have been resulted in the manufacture of plant-based medications[1], [2]. Flaxseed is the seed from the flax plant (Linum usitatissimum L.), which is a member of the Linaceae family [3], [4]. The generic name “Linum” comes from Celtic word Lin means ‘thread’ and the species name “usitatissimum” given by Carl Linnaeus, which means very useful [5,6]. Many Linum species are related to flaxseed but only Linum usitatissimum L. is grown for commercial production of oil[7]. Flaxseed is among the oldest crop plants cultivated for the purpose of oil and fiber, medicines and textiles; therefore, it is been of great importance for human culture and development for more than 8,000 years[6], [8]. Flax seed lignans have shown promising results for treatment and prevention of several types of cancer[9]. The short-stemmed flaxseed bears seeds of high oil [10].The oilseed flax (linseed) (Linum usitatissimum L.) is predominantly the source of valuable oil, in which the most appreciated are omega-3 fatty acids where it contains cyclic hydrophobic peptides known as “cyclolinopeptides” which influence blood pressure and which are composed of eight or nine amino acid residues[11], [12]. Flaxseed is a promising candidate for improving various aspects of
health markers such as cholesterol levels, inflammation, blood pressure, cardiovascular diseases remain a leading cause of mortality worldwide, prompting researchers to investigate dietary interventions that could help reduce the risk factors associated with heart disease. The lignans found in flaxseed are known for their antioxidant properties\cite{11}, \cite{12}, which could potentially reduce inflammation and oxidative stress in the body. Overall, it is expected that regular consumption of flaxseed may have a positive impact on cardiovascular health. The objective behind this investigation was studying the chemical composition of flaxseed oil extracted by two methods (cold and hot), and comparing the quality of the oil plant based on international criteria. A preliminary in vitro study was carried out on the antihypertensive effect of flaxseed oil using an experimental device developed in our laboratory (Lab. MBSC).

2. MATERIALS AND METHODS

Flaxseed is grown in various regions around the world due to its versatility and nutritional benefits, thrives in cooler regions with temperate climates such as Canada, Russia, China, particularly the northern states. These regions provide the ideal conditions for flaxseed cultivation, including well-drained soil and moderate temperatures.

2.1. Extraction of the oil flax seed

Flaxseed oil was extracted in an amount of 2 kg using a cold or hot pressing device at a temperature of 67°C and 90 °C; 580 ml and 480 ml of oil were obtained respectively. Finally the filtration process was carried out to separate the oil from impurities.

2.2. Chemical analyses

2.2.1. Acid Index

We weigh 2 g of flaxseed oil, add 5 ml of 95% ethanol and 5 drops of 0.2% phenolphthalein (PP), then neutralize it with an ethanol solution of KOH (0.1 mol/l) until a pink color is obtained. The index acid is calculated by the following formula.

\[
\text{AI} = \frac{M(\text{KOH}) \times V \times C}{m}
\]

AI: Acid Index
M (KOH): Molar mass of potassium hydroxide.
V: Volume of potassium hydroxide.
C: Concentration of potassium hydroxide.
m: The mass

2.2.2. Saponification Index (SI)

The saponification index corresponds to the number of milligrams of potash necessary to saponify the fatty acids contained in a gram of fat. This involves treating the ester with sufficient concentrated and hot potash, which regenerates a following reaction. Total alcohol and potassium salt of the acid, 2g of flaxseed oil, then 25 ml of KOH added with a concentration of 0.5 mol/L, we heat it for one hour and add 0.5 ml of 0.2% phenolphthalein. Finally, the excess KOH is titrated with the hydrochloric acid (HCl) solution at 0.5 mol/l until the phenolphthalein turns colorless. The saponification index (SI) is determined as follows:

\[
\text{SI} = \frac{(V_0 - V_1) \times C \times 56.1}{m}
\]

SI: Saponification index
V0: Volume of hydrochloric acid necessary to titrate the blank.
V1: Volume of hydrochloric acid necessary to titrate the test.
C: Concentration of the standard hydrochloric acid solution.
56.1: The molecular weight of KOH
m: The mass

2.2.3. Peroxide Index (PI)

The peroxide index is the number of micrograms of active oxygen content in a large body of water and susceptible to potassium iodide. It is the first microgram of a gram or more of a millimeter of equivalent oxygen activity per kilogram, and this solution is suitable for a solution in a mixture of acetic acid (CHOOH) and chloroform, treated with one of the solutions iodure of potassium (KI). The iodine released by a solution of sodium thiosulfate (Na₂S₂O₃) is titrated in the presence of starch (colored indicator).

Two grams flaxseed oil was used, 10 ml of chloroform and 15 mL of acetic acid are added, then 1mL of saturated potassium iodide (KI) solution for 5 min away from light. 75 ml of distilled water and few drops of starch was added and the iodine library with the solution of sodium thiosulfate Na₂S₂O₃ (0.002N) is agitated with the total decoloration of the solution. The index of peroxyde (IP) causes the main effect:

\[ PI = \frac{N(V1 - Vn)}{m} \times 1000 \]

PI : Peroxide Index
Vn: The indicator volume which equals 0.
V1: The volume of sodium thiosulfate necessary for the determination.
N: The exact concentration of the standard solution of sodium thiosulfate.
m: The mass

2.2.4. Iodine Index (II)

The index on the iodide is the mass of the iodogrammes fixed on the doubles placed on 100 g of flesh. Addition to a solution of monochlorous iodine in a mixture of acetic acid and tetrachlorure of carbon. Titratre with the sodium thiosulfate (Na₂S₂O₃) solution (0.0992 N) until a yellow color is obtained. A few drops of starch and continue the titration until the blue color disappears after vigorously shaking the contents and a blank test in the same way was prepared. This device is worn as a suit:

\[ II = \frac{(12.69C(V1 - V2))}{M} \]

II: Iodine Index
V1: The volume of sodium thiosulfate required for the blank.
V2: The volume of sodium thiosulfate required for the sample.
C: The normality of sodium thiosulfate solution.
M: The weight of the sample in grams

2.2.5. Indication of reaction

The index of the reaction is the rapport into the sinus of accidental angles and the reaction of the long rayon on the day, allowing the air in the air to be maintained at a constant temperature. To the
fractomètre, at 20°C for the oils. On the other hand, the shell of the drop line on the blade fractometer, the reflection index value directly.

2.3. Polarimeter and rotary power

The rotational power of a substance is measured using a polarimeter. Biot’s law \( (\alpha = [\alpha] + l.c) \) makes it possible to link the specific rotating power of a molecule to the angle of deviation measured under given conditions.

2.4. UV-Vis spectroscopy analysis

The device used is callityimovation ANALYTIC JANA type with an interval ranging from 190 nm to 800 nm controlled by the software. We put a quantity of flaxseed oil with a solvent of Butanol-2 in a quartz tank with 5ml; start the final analysis in order to obtain the specific spectrogram for the sample.

2.5. IR spectroscopic analysis

The device used is Spectro of 50 DO249Tj Part N°: 56070646. Fourier transform infrared spectroscopy (FTIR) is a measurement technique for the acquisition of infrared spectra; a drop of the sample is placed on on IR-transparent material, Launches the analysis from the software and can directly obtain our infrared results.

2.6. High-Performance Liquid Chromatography (HPLC)

Chromatography in liquid phase at high performance is a powerful analytical technique used to separate, identify, and quantify components in a mixture. It is a form of liquid chromatography that is widely used in various fields such as pharmaceuticals, food and beverage analysis, environmental monitoring, forensic science. The preparation of the sample for HPLC analysis is a crucial step to ensure accurate and reliable results. The HPLC system used was a SHIMADZU Scientific Instruments’ system LC-20A (Shimadzu, Kyoto, Japan) with an injector of 20 μl Rheodyne 1907 sample loop, a pump LC-20A, a vacuum degasser DGU-20A5, and a Shimadzu SPD-20 with a variable wavelength ultraviolet (UV) detector. Chromatographic data were collected, stored, and analyzed using the LC Lab solution software (Shimadzu, Tokyo, Japan) [11], [12].

Chromatographic condition: We diluted a few drops of flaxseed oil in an acetonitrile solution; then placed the sample in special HPLC tubes. The mobile phase used was acetonitrile with a volume injection of 20 μL at a flow rate of 0.5 mL/min, Chromatographic separations were conducted at ambient temperature and UV detector was set at 254 nm.

2.7. Miniature model of blood circulation system

To better discover the impact of this flaxseed oil on a possible reduction in blood pressure, we have developed a small device in the laboratory of bioactive molecules and chiral separation (LMBSC) which is used to simulate the blood circulation of an adult individual. The combination of these principles of suction and discharge results in a powerful self-priming positive displacement action. This pump is the subject of a perfect simulation of the functioning of a human heart. By activating the pump, the mixture begins to pump (150ml physiological serum + flaxseed oil) with a temperature maintained at 37°C, the mixture then passes through a pipe connected to an electronic tensiometer in order to measure the tension of the mixture in the system. Since the pump is equipped with a control that is used to adjust the flow rate, the flow rate is increased to simulate high blood pressure.
3. RESULTS AND DISCUSSION

3.1. Flaxseed Oil Extraction

In our work we practiced the two processes at 67°C and 90°C in order to make a comparison in terms of quality of flaxseed oil and extraction yield. Flaxseed oil was extracted by the extraction method using an oil expeller with filter machine and the following results were obtained:

<table>
<thead>
<tr>
<th>Extraction temperature</th>
<th>Mass (g)</th>
<th>Color of HF</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>67°C</td>
<td>551.58</td>
<td>Light yellow</td>
<td>24 %</td>
</tr>
<tr>
<td>90°C</td>
<td>456.48</td>
<td>Brown</td>
<td>29 %</td>
</tr>
</tbody>
</table>

From the results obtained we note that the extraction process at 90°C of flaxseed oil has a higher yield than the extraction process at 67°C (Table 1), but with different quality; initially we base ourselves on the color of the oil which changes from yellow to brown which can be explained by the oxidation of the heated oil.

3.2. Analysis characterization and chemical composition of fixed oils

3.2.1. Physico-chemical analysis of flaxseed oil

The evaluation of the physicochemical parameters of flaxseed oil according to AFNOR standards made it possible to find good results presented in Table 2.

<table>
<thead>
<tr>
<th>T=67°C</th>
<th>T=90°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acid index (mg de KOH/g oil )</td>
<td>0.2805</td>
</tr>
<tr>
<td>Saponification index (mg of KOH /g of oil)</td>
<td>168.3</td>
</tr>
<tr>
<td>Peroxide index meqD2/kg of oil</td>
<td>0.96</td>
</tr>
<tr>
<td>Iodine index (g/100 g oil)</td>
<td>174.23</td>
</tr>
<tr>
<td>Refractive index</td>
<td>1.482(18.1°C)</td>
</tr>
<tr>
<td>Density (g.cm⁻³)</td>
<td>0.951</td>
</tr>
</tbody>
</table>

According to the results obtained, we notice a similarity in the density and in the refractive index since they are almost the same physical properties of this liquid, while there is an average difference in the others parameters (acid index, saponification index, peroxide index and iodine index). It can be deduced that there is a slight difference in the physicochemical properties between its two products.

a. Acid Index

Knowing the acid index of a fatty substance is a good way to determine its alteration by hydrolysis. It is a criterion for the purity of the oil flaxseed oil standards.

b. Saponification Index

The saponification index is related to the length of the fatty acids constituting the oil. Knowledge of the saponification index of a fatty substance tells us about the length of the carbon chain of the acids constituting this fatty substance. The saponification index of a fatty substance is higher when the carbon chain of the fatty acids is short [13]. The saponification index of our flaxseed oil (168.3 and 28.05) is close to the range of the codex alimentarius standard which set them between 187 and 197 [14].
c. Peroxide Index

The peroxide index is a quality criterion; it allows you to see the oxidation state of the oils and to control the first stages of oxidative alteration. The peroxide index is linked to the conservation conditions and methods of extraction. The value of the peroxide index found in this study is of the order of 0.96 and 1.7 meqO/Kg of oil.

d. Iodine Index

In the analysis of fats, it is the iodine index which represents the most useful constant because it is in relation to the values of this index that the important division of vegetable oils into drying oils, semi-drying and non-drying. Indeed, the iodine index tells us about the degree of unsaturation of the fatty acids contained in given oil. It is directly related to the degree of oxidation of oil. Thus, the more unsaturated an oil is, the higher its sodium index, and we can base ourselves on this quantity to evaluate the ease of processing given that the more unsaturations it contains, the more sensitive it will be to oxygen. The value found in this study is of the order of 174.23 and 210 (g/100g of oil). The index value iodine in our oil shows that it complies with the standard established by the Food Codex which set them between 170 and 213.

e. Refractive Index

The refractive index represents a criterion of oil purity. It depends on the chemical composition of the oils and the temperature. Generally, the index increases with unsaturation or the presence of secondary product. The refractive index value of our oil is 1.482 and 1.485.

f. Relative Density

Determining the density of oil tells us about its purity, it depends on the chemical composition of the oils and the temperature. Flaxseed oil has a density (0.951) almost similar to that found in marketed products (0.949), and it is between 0.925-0.955 according to the standard given by the Codex Alimentarius.

3.3. Optical Purity

Optical activity occurs in the solid state, in the liquid state or for dissolved substances. In what follows we focus on the last case. When we carry out the experiment to demonstrate the optical activity of an active compound between crossed polarizers, with non-monochromatic light, we see that it is not possible to obtain complete extinction by rotating the analyzer. This comes from the fact that the specific rotating power of the substance depends on the wavelength. In the case of our samples the polarimeter result was zero (0); so, we can say that the absorbance of polarized light for all the molecules that exist in the sample is equal on both sides and this is due to two major phenomena; the mixture is either racemic or completely achiral (absence of chiral molecules), or acids, saponosides.
3.4. Flaxseed oil analysis by UV-VIS spectroscopy

Figure 1. The UV-VIS spectrum of *linum usitatissimum* oil.

Table 3. The characteristic UV-vis bands of fixed flaxseed oil at 90°C

<table>
<thead>
<tr>
<th>Band I</th>
<th>Band II</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \lambda_{\text{max}} )</td>
<td>190 nm</td>
</tr>
<tr>
<td>Absorbance</td>
<td>1.3</td>
</tr>
<tr>
<td>( \Delta E ) (Kcal/mol)</td>
<td>152.06</td>
</tr>
</tbody>
</table>

Table 4. The characteristic UV-vis bands of fixed flaxseed oil at 67°C

<table>
<thead>
<tr>
<th>Band I</th>
<th>Band II</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \lambda_{\text{max}} )</td>
<td>195 nm</td>
</tr>
<tr>
<td>Absorbance</td>
<td>0.75</td>
</tr>
<tr>
<td>( \Delta E ) (Kcal/mol)</td>
<td>146.75</td>
</tr>
</tbody>
</table>

The UV-vis spectrum of *linum usitatissimum* oil at 90°C recorded in 2-butanol presents two characteristic absorption bands:

Figure 2. Energy diagram of electronic transitions (UV-Vis spectrum for flaxseed oil extracted at 90°C).
Band I: Band I is located at 190 nm with an absorbance of 1.3 and with an electronic transition energy of 152.06 kcal/mol (Table 3), this band is attributed to the electronic transition of type $\pi \rightarrow \pi^*$, corresponds to the C=C double bond of a ketone or an aldehyde and that of carbonyl C=O.

Band II: Band II is located at 315 nm with an absorbance of 1.2 and with the electronic transition energy of the order of 90.84 kcal/mol (Table 3), this band is attributed to the $n \rightarrow \pi^*$ type electronic transition corresponds to the free C=O doublet of carboxylic acids.

The UV-vis spectrum of *Linum usitatissimum* oil at 67°C recorded in 2-butanol presents two characteristic absorption bands.

![Energy Diagram](image)

**Figure 3.** Energy diagram of electronic transitions. (UV-Vis spectrum for flaxseed oil extracted at 67°C.

We notice that there is a similarity in the UV-Visible spectra for the two products (T= 67°C and T= 90°C) indicating that there is almost the same chemical composition of the two products.

Band I: Band I is located at 190 nm with an absorbance of 0.75 and with an electronic transition energy of 146.75 kcal/mol (Table 4), This band is attributed to the electronic transition of type $\pi \rightarrow \pi^*$, corresponds to the C=C double bond of a ketone or an aldehyde and that of carbonyl C=O.

Band II: Band II is located at 320 nm with an absorbance of 1.1 and with the electronic transition energy of the order of 89.42 kcal/mol (Table 4), this band is attributed to the $n \rightarrow \pi^*$ type electronic transition corresponds to the free C=O doublet of carboxylic acids.

By comparison between the two UV-Vis spectra recorded from the extracted flaxseed oil (at 67°C and 90°C), we notice a bathochromic shift in the $2_{\text{max}}$ values of band II of the flaxseed oil. Extracted at 67°C compared to flaxseed oil extracted at 90°C and a hypochromic shift in the band intensity values of flaxseed oil extracted at 67°C compared to flaxseed oil extracted at 90°C.

3.5. Analysis of flaxseed oil by infrared spectroscopy

The IR spectrum recorded by Fourier transform IR-TF spectrophotometer shows nine characteristic absorption bands:

A thin, moderately intense band appears at 3009 cm$^{-1}$ corresponding to the elongation or valence vibrations of ethylene C-H bonds which are found in the structures of unsaturated fatty
acids such as: oleic acid (C18: 1ω9), linoleic acid (C18: 2ω6) and α-linoleic acid (C18:3ω3), this is confirmed by the presence of an intense and thin band around 721 cm⁻¹ attributed to vibrations deformation outside the ethylenic C-H bond plane (≡C-H) of these fatty acids which are part of the chemical composition of flaxseed oil (Table 5).

Table 5. The characteristic IR spectrum bands of linumusitatissimum oil extracted at 67°C and 90°C

<table>
<thead>
<tr>
<th>No</th>
<th>Group</th>
<th>HL à 67°C</th>
<th>Frequency (Cm⁻¹)</th>
<th>HL à 90°C</th>
<th>Frequency (Cm⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>δ C-H (Aromatic)</td>
<td></td>
<td>721</td>
<td></td>
<td>721</td>
</tr>
<tr>
<td>2</td>
<td>ν C-O (aliphatic ketone)</td>
<td></td>
<td>1160</td>
<td></td>
<td>1159</td>
</tr>
<tr>
<td>3</td>
<td>ν C-O(aromatic and α,β-unsaturated)</td>
<td></td>
<td>1235</td>
<td></td>
<td>1235</td>
</tr>
<tr>
<td>4</td>
<td>δ O-H (Alcohol, Carboxylic acid)</td>
<td></td>
<td>1377</td>
<td></td>
<td>1377</td>
</tr>
<tr>
<td>5</td>
<td>δ CH2</td>
<td></td>
<td>1459</td>
<td></td>
<td>1459</td>
</tr>
<tr>
<td>6</td>
<td>ν C=O (Carboxylic acid)</td>
<td></td>
<td>1742</td>
<td></td>
<td>1742</td>
</tr>
<tr>
<td>7</td>
<td>ν C=Ω</td>
<td></td>
<td>1620</td>
<td></td>
<td>1620</td>
</tr>
<tr>
<td>8</td>
<td>δ C-H (Cetone or Aldehyde)</td>
<td></td>
<td>2853, 2922</td>
<td></td>
<td>2853</td>
</tr>
<tr>
<td>9</td>
<td>ν -OH (Carboxylic acid)</td>
<td></td>
<td>2922</td>
<td></td>
<td>2922</td>
</tr>
<tr>
<td>10</td>
<td>ν C-H (Aromatic)</td>
<td></td>
<td>3008</td>
<td></td>
<td>3009</td>
</tr>
</tbody>
</table>

![Figure 4. IR spectra of flaxseed oil used (at 67°C and 90°C)](image)

A thin and very intense band located at 1742 cm⁻¹ attributed to the valence vibrations of the double carbonyl bond (C=O) which characterize the fatty acids and aldehydes which enter into the chemical composition of flaxseed oil.
Two other bands matched respectively at: 1160 cm\(^{-1}\) and 1235 cm\(^{-1}\) correspond to the valence vibrations of the (C-O) \(\alpha\ \beta\)-unsaturated bond by the C=O double bond of the function acid; this allows confirmation of the presence of an acid function.

Two other intense and fine bands appear in the area: 2800-2990 cm\(^{-1}\), more precisely located respectively at 2853 cm\(^{-1}\) and 2922 cm\(^{-1}\), correspond to the symmetric and asymmetric valence vibrations of C-H bonds of CH\(_2\) and CH\(_3\), which confirms by the significant intensity of these bands, the presence of a slow hydrocarbon chain of acids and esters which enters into the chemical composition of flaxseed oil.

The thin and intense band at 1459 cm\(^{-1}\) attributed to deformation vibrations in the same C-H bond plane of CH\(_2\) and CH\(_3\).

A thin and intense band appears at 1377 cm\(^{-1}\) attributed to deformation vibrations in the same plane of a hydroxy group (O-H) of the acid function.

3.6. Analyzes of *Linum usitatissimum* oils by HPLC

From the chromatogram we can deduce that this oil contains several chemical compounds which absorb around this wavelength, in particular by way of designation; we can distinguish between 9 and 15 compounds with selectivity factors.

![HPLC chromatogram of linum usitatissimum oil T=90°C](image)

**Figure 5.** The HPLC chromatogram of *Linum usitatissimum* oil T=90°C

**Table 6.** Results of analysis by HPLC of *Linum usitatissimum* oil T=90°C; Vial: 1-5, 10 uL; Column: C 18, Mobile Phase: ACN, \(\lambda=254\) nm, Flow Rate: 0.5 mL/min.

<table>
<thead>
<tr>
<th>Peak</th>
<th>Rt (min)</th>
<th>A</th>
<th>(k')</th>
<th>Rs</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.52</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>15.34</td>
</tr>
<tr>
<td>2</td>
<td>2.04</td>
<td>--</td>
<td>0.343</td>
<td>3.397</td>
<td>4.17</td>
</tr>
<tr>
<td>3</td>
<td>3.99</td>
<td>4.743</td>
<td>1.629</td>
<td>4.348</td>
<td>10.84</td>
</tr>
<tr>
<td>4</td>
<td>5.80</td>
<td>1.731</td>
<td>2.820</td>
<td>0.594</td>
<td>21.09</td>
</tr>
<tr>
<td>5</td>
<td>6.01</td>
<td>1.048</td>
<td>2.956</td>
<td>3.718</td>
<td>8.82</td>
</tr>
<tr>
<td>6</td>
<td>7.36</td>
<td>1.299</td>
<td>3.848</td>
<td>3.718</td>
<td>7.34</td>
</tr>
<tr>
<td>7</td>
<td>7.69</td>
<td>1.057</td>
<td>4.059</td>
<td>0.980</td>
<td>3.26</td>
</tr>
<tr>
<td>8</td>
<td>7.91</td>
<td>1.036</td>
<td>4.207</td>
<td>0.968</td>
<td>0.08</td>
</tr>
<tr>
<td>9</td>
<td>8.19</td>
<td>1.043</td>
<td>4.390</td>
<td>4.305</td>
<td>2.38</td>
</tr>
<tr>
<td>10</td>
<td>9.87</td>
<td>1.252</td>
<td>5.497</td>
<td>1.031</td>
<td>7.97</td>
</tr>
<tr>
<td>11</td>
<td>10.34</td>
<td>1.056</td>
<td>5.804</td>
<td>4.165</td>
<td>3.14</td>
</tr>
<tr>
<td>12</td>
<td>13.95</td>
<td>1.410</td>
<td>8.183</td>
<td>3.511</td>
<td>7.35</td>
</tr>
<tr>
<td>13</td>
<td>18.95</td>
<td>1.346</td>
<td>11.015</td>
<td>8.22</td>
<td></td>
</tr>
</tbody>
</table>

Rt: retention time, \(\alpha\): selectivity factor, \(K\): capacity factor, Rs: resolution.
3.7. Chemical composition of flaxseed oil

3.7.1. Fatty acids and triglycerides

The fatty acid composition is presented as it is found in the literature bringing together different origins (Europe, Canada), which has the effect of widening the ranges of values, and by comparison with the chromatographic results, we note that flaxseed oil extracted at 67°C contains 3.8% saturated fatty acids and flaxseed oil extracted at 90°C contains 7.39% saturated fatty acids. We note the absence of palmitic acid in flaxseed oil at 67°C, and the absence of Behenic acid in both flaxseed oils at 67°C and 90°C. The results obtained show that the monounsaturated fatty acid content of the two flaxseed oils is around 21%, a content very close to international standards (between 11% and 23%). We also note that polyunsaturated fatty acids retain their presence in the two flaxseed oils according to international standards at around 67%. Given the significant proportion of alphalinolenic acid in flaxseed oil, the composition of its triglycerides shows a preponderant amount of trilinolenin (LnLnLn: 22.8% and 21.09%). In terms of the structure of triglycerides.

3.7.2. Tocopherols, sterols

Flaxseed oil contains 40 to 60 mg/100 g of tocopherols, a quantity similar to that of walnut oil, but lower than that of rapeseed and soybean oils; the gamma tocopherol form with high antioxidant protective potential is practically the only form present (96-98%); the alpha and delta-tocopherol forms are therefore present in very small quantities. The sterol contents and composition of flaxseed oil are comparable to those of most vegetable oils with beta-sitosterol in the majority.

Table 7. Results of HPLC analysis of *linum usitatissimum* oil T=67°C

<table>
<thead>
<tr>
<th>Peak</th>
<th>Rt</th>
<th>A</th>
<th>k’</th>
<th>Rs</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.517</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>7.754</td>
</tr>
<tr>
<td>2</td>
<td>1.634</td>
<td>--</td>
<td>0.077</td>
<td>0.141</td>
<td>1.082</td>
</tr>
<tr>
<td>3</td>
<td>1.696</td>
<td>1.535</td>
<td>0.118</td>
<td>0.063</td>
<td>1.758</td>
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<tr>
<td>4</td>
<td>5.678</td>
<td>23.195</td>
<td>2.742</td>
<td>9.934</td>
<td>3.371</td>
</tr>
<tr>
<td>5</td>
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<td>1.418</td>
<td>3.888</td>
<td>1.529</td>
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<tr>
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<td>5.322</td>
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<tr>
<td>7</td>
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<td>7.249</td>
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<tr>
<td>8</td>
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<tr>
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<td>1.197</td>
<td>10.234</td>
<td>9.823</td>
<td>0.429</td>
</tr>
</tbody>
</table>

Rt: retention time, α: selectivity factor, K: capacity factor, Rs: resolution.
3.8 Blood pressure and anti-hypertension activity

In this part of the study, the main objective is to evaluate the antihypertensive effects of fixed flaxseed oils (*Linum Usitatissimum*) by in vitro tests; using an experimental device that simulates the blood cycle in the human body. To achieve this objective, it was more specifically:

a. To evaluate with preliminary in vitro tests the effects of flaxseed oil (used in traditional medicine) on blood pressure.

b. Determine the dose of flaxseed oil which allows the regulation of blood circulation or the favorable dose for essential anti-hypertension activity.

3.8.1. Effect of flaxseed oil on blood pressure

The addition of flaxseed oil to the mixture is done gradually from 0.1 mL to 1mL; we generally notice that the tension measured by the tensiometer decreases with the progressive addition of the oil compared to the measurement taken by the serum alone; this means that this oil affects high blood pressure simulated by the system described previously and schematized in Figure 7.

![Figure 7](image)

*Figure 7.* Presentation of the constituents of an experimental device for the in vitro evaluation of the anti-hypertension activity of flaxseed oil.

We can easily notice from the two graphs the effect of flaxseed oil on the remarkable reduction in high blood pressure; we also notice that the reduction in diastolic blood pressure is more important and more effective than systolic blood pressure. It can be interpreted by the fact that since the diastolic blood pressure indicates the residual pressure at the time of the relaxation phase of the heart, its reduction is very remarkable because the heart will be in a passive position and the arteries are not at their maximum dilation.
4. CONCLUSION

Flaxseed oil is considered one of the richest nutritional supplements because it contains fiber, vitamins and minerals that help strengthen the immune system. We were able to achieve a satisfactory yield of 24% to 29% in our work by applying two distinct methods of pressing at temperatures of 67°C and 90°C. The physical and chemical characteristics (density, refractive index, acid index, saponification index, iodine index, peroxide index) were determined, allowing the Food Codex to define criteria to evaluate the quality of the extracted flaxseed oil. The work’s findings indicate that the two flaxseed oils’ monounsaturated fatty acid content is roughly 21%, which is extremely close to the 11%–23% range that is considered worldwide. Additionally, we see that the two flaxseed oils still contain roughly 67% of the globally recognized polyunsaturated fatty acids. Furthermore, we tried to test the anti-hypertension activity of this oil by simulating a blood cycle in the laboratory which allowed us to determine the variation in blood pressure as a function of the volume of oil injected into the blood cycle from 17.4 to 14.10.

References

[5] H. Kumar et al., “Effect of Foliar Application of Plant Growth Regulators on Growth and Yield Attributes of Linseed (Linum usitatissimum L.).”


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