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Effect of extraction method of Chia seeds Oil on its content of fatty acids and antioxidants

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Abstract-*The extracted oil from the seeds of the Salvia hispanica has been studied by different extraction methods to obtain the highest percentage of extracted oil from white and black chia seeds. Three extraction methods were used: solvent extraction (cold extraction), extraction using Soxhlet device (hot extraction), and extraction by using the screw pressing, the study concluded that solvent extraction is the best method for extraction in terms of quantity and quality of extracted oil. Fatty acids were also diagnosed in each type of oil extracted using Gas Chromatography (GC) to determine the type of fatty acids that make up the highest percentage of extracted oil, Where it found that the saturated fatty acid Arachidic and unsaturated fatty acid linoleic (omega-6) have formed the highest percentage of the extract from the white seed oil where their percentages (6.61%) and (3.54%), respectively, while the fatty acid linoleic (omiga-6) of oil extracted from black seeds was (4%). The vitamins dissolved in extracted oil from the Chia seeds were diagnosed using high performance liquid chromatography (HPLC). A qualitative examination was conducted to diagnose some of the active compounds in the extracted oil using two types of reagents for each compound, where the presence of flavonoids, terpenes and tannins was diagnosed. Extracted oil showed inhibition of protein coagulation as an indicator of anti-inflammatory action. The percentage of inhibition in oil extracted from white chia seeds was 11.8% and 38% for oil extracted from black chia seeds. The efficacy of the oil extracted as an antioxidant compared to ascorbic acid was estimated as a comparative model where it showed that the extract from black chia seeds was the highest oil efficacy, which amounted to (94.5%) at a concentration of 400 µl / ml compared with oil extracted from white chia seeds which was (88.37%) at the same concentration.*

Research paper sited from the master thesis of the first researcher.

Key words: *Salvia hispanica*, oil, Chia seeds, fatty acids, antioxidants, Extraction.

1. INTRODUCTION

Seeds of Salvia Hispanica are the seeds of the Lamiaceae and Chia seeds were used as food early in the year (3500 BC) and gained importance as a crop between 900 BC and 1500 BC in central Mexico. These seeds have been known from the medical and nutritional point of view since ancient times because of their high content of omega-3 fatty acids. Chia seeds have been described as a good source of oil, protein, dietary fiber, minerals and polyphenols [4]; [5]; [20]. Chia seed oil is a rich source of polyunsaturated fatty acids (PUFA). Chia seed oil is unique because it contains the highest percentage of omega-3 (ALA) more than any known natural source [3]; [6]. Omiga-3 plays an important role in health and is used in many foods and cosmetics. Several studies have shown that regular consumption of omega-3 has many health effects, including prevention of cardiovascular disease, high blood pressure and inflammatory diseases [1]; [8]. In addition, Chia seeds and oil extracted from Chia seeds contain a rich array of natural antioxidants such as tocopherol, phytosterol and carotene [2]; [10], as well as phenolic compounds including chlorogenic and kefic acid [5], which protect consumers from many diseases and promote human health [15].

The aim of this study was to extract the oil from the chia seeds using three different methods to determine which method yields the highest percentage of extracted oil and then to detect the fatty acids found in the extracted oil by (GC) Gas Chromatography, the diagnosis of the active compounds in the extracted oil and study the effect of extracted oil as an antagonist for oxidation.

2. Materials and method

2.1 Seeds

The commercial chia seeds used in this study were obtained from Cozmo, where the source of these seeds is Australia. The seeds are clean and homogeneous and packed in sealed plastic containers and stored at 15-20°C. The oil was then extracted from the seeds by solvents and

by soxhlet (hot extraction) and the use of solenoid screw pressing.

2.2 Solvent extraction

The oil was extracted from the chia seeds using the cold method to avoid exposure to any heat treatment using the solvent and the method used in [10] with some modifications in solvent ratios. Using the coffee grinder for 60 seconds, the crushed seeds were placed in a 500 mL glass baker and 300 mL of the hexane solvent was added (1: 3). The extraction was carried out for two consecutive times to ensure extraction of all the oil in the seed. The solvent was discarded using rotary evaporator at temperature 35°C, then collect the oil extracted in the tubes The weight of the glass tubes with the oil to determine the percentage of oil extracted from 100 g seeds, keeping the extracted oil under study at a temperature of (15-20 mm) for chemical and physical tests.

2.3 Soxhlet extraction

The percentage of oil in the Chia seeds was estimated using the Soxhlet [23] by using hexane as a solvent for extraction. A sample of 100 gm chia seeds was then grinded with a coffee mill for 60 seconds and then placed in thimble, and the weight of the beaker was recorded before and after extraction.

2.4 Screw pressing extraction

The oil was extracted from the white and black chia seeds using the solenoid method according to the method mentioned in [10], with some modifications in the extraction method. The extraction process was done at room temperature with 100 gm of chia seeds, through a small opening at the top of the device and was received oil in a container placed under the device was calculated the proportion of oil extracted through the placement in the pipe weight information and then the weight of the tubes with oil extracted.

2.5 Determination of total fatty acids

The total fatty acids in the extracted chia oil were estimated as mentioned in [14] using GC chromatography with some modifications in sample preparation rates and sample injection conditions. The sample was prepared by taking 0.5 ml of oil extract is added to 0.5 mL of methyl potassium hydroxide (Methanol+ KOH) and then 5 mL of hexane solvent is added with the stirring for a minute. This results in two layers of the upper layer hexane with the fatty acid (FAME) ester and the lower layer methyl potassium hydroxide, 1 microliter of the top layer to be injected into the device.

The conditions of the injection were as follows:

- Silica column dimensions: 60 m * 0.25 mm * 0.25 microliters
- Gas used: Hydrogen
- Gas flow rate: 1.5 ml / min
- Column temperature: 60 ° C for 2 minutes to 200 ° C at 10 ° / min and then raise to 240 ° C at 7 ° / minute and then set for 7 minutes
- Injection temperature: 270 ° C
- Detector Type: FID
- Sample size used: 1 microliter

2.6 Specific detection of some active compounds

1. Detection of terpenes

The method [9] was followed to detect turbines using the following reagents:

Drops of chloroform and concentrated sulfuric acid were added sequentially to 1 ml of extracted oil. The appearance of a brownish-red color indicates the positive detection.

Anas dehydide detector: consists of the lance dehydide detector, acetic acid and sulfuric acid. Several drops of the anhydide dehydrogen detector were added to 1 mL of oil extracted in the hour bottle.

2. Detection of tannins

The method used in [9] was followed to prepare each of the following reagents, and then added to the extracted oil to confirm the presence of tannins and as follows:

Ferric Chloride Reagent: Consists of 1 g ferric chloride in 100 ml of distilled water. Droplets of ferric chloride reagent were added to 1 ml of extracted oil, as the appearance of a bluish-green color indicates a positive detection.

Lead acetate reagent: consists of 1 g lead acetate in 100 ml distilled water. Several drops of lead acetate reagent were added to 1 mL of extracted oil, and the appearance of a white-yellow precipitate indicated a positive detection.

3. Detection of flavonoids

The method of [9] was followed to detect the presence of flavonoids in the extracted oil as follows:

Magnesium and hydrochloric acid detectors: Magnesium crystals were added to the extracted oil in an hourglass and then added to the drops of concentrated hydrochloric acid, the appearance of a red-orange color indicates a positive detection.

Sulfuric acid reagent: Add drops of concentrated sulfuric acid to the extracted oil, as the appearance of red color indicates positive detection.

2.7 Determination of antioxidant efficacy

The anti-oxidant effectiveness of extracted chia oil was tested for each type using the free stable root of the DPPH.

The measurements were made using the spectrophotometer, 517 nm wavelength and Ascorbic Acid as a positive comparison [18].
As follows:

1- A solution (0.1 mMol) of DPPH was prepared by dissolving (1.97 mg) of the substance in (50 ml) of absolute methanol.
2- Measuring anti-oxidant effectiveness:

The free radicals were extracted from the chia seed and each type (white and black) by calculating the uptake of the DPPH solution at 517 nm wavelength. The absorbance was measured by mixing 1 ml of DPPH solution at 0.1 mM (20, 40, 60, 80, 100) mg / L for the positive comparison of the ascorbic acid (25, 50, 100, 200, 400) Acid.

The samples were then measured in the incubator at 37 ° C for 30 minutes. The models were then measured in the Spectrophotometer spectroscopy at a wavelength of 517 nm and the absorbance was converted to the percentage of the electronic absorbent using the following equation:

% antioxidant activity = (absorbance for B – absorbance for A) / (absorbance for A) × 100

A = Control Comparative Absorption (Control)
B = Absorption of the model (Chia oil with DPPH solution)

3. Results and discussion

3.1 Ratio of extracted oil from Chia seeds using three methods of extraction

Table (1) shows the percentage of extracted oil from white and black chia seeds where the percentage of oil extracted by soxhlet (hot extraction) was 35.6% and 34.6% respectively. This result is similar, according to [13] where it was 35.13% and lower than that reported in [16] which is 30.22% and less than in [7] where 30.1%. The percentage of oil extracted by solvents (cold extraction) was 30% for each type of seed and this is consistent with what [21], where he pointed out that the proportion of fat was 30% while the percentage of oil extracted by screw pressing was 9% and 9.5%, respectively, and this result is less than what mentioned by [10], where he pointed out that the proportion of fat was 30%. The difference in fat ratio in Chia seeds can be explained by the use of the three extraction methods of the extracted chia seeds to the different efficiency of the extraction process. The extracted oil ratio is approximated when using the coarse extraction method and when solvent extraction is used, In order to avoid exposing the oil to any thermal treatment and thus maintain its components from damage while the method of extraction using the screw pressing gave the lowest proportion of oil and can be due to the inefficiency of the extraction device.

Table-1: Oil ratios of Chia seeds using three extraction methods

Oil from white chia seed %	Oil from white chia seed **%**	Extraction method
34.6	**35.6**	The Soxhlet Method
30	**30**	Solvent method
9.5	**9**	Screw pressing

3.2 Total fatty acid

Table (2) and Table (3) show the ratio of triglyceride fatty acids in white and black chia seed oil, where the time of appearance of the compound was compared in the model to be diagnosed with time of occurrence of the standard compound Figure (1) (2) (3) By observing fatty acids, we found that saturated arachidic acid and linoleic fatty acid (omega-6) had the highest percentage of oil extracted from white seeds, with a percentage of 6.61% and 3.54%, whereas the ratio of linoleic acid to extracted oil of black

seed 4%. In comparison of the fatty acids of the oil extracted in this study with the results of other researchers found that the arachidic fatty acid had the highest proportion while the proportion of linoleic in the extracted oil from white and black seeds has formed a low proportion, these two fatty acids are 0.15% (Arachidic) and 20.4% (Linoleic), respectively, according to [13],[19] showed that the ratio of these fatty acids was 0.3% and 18.1%, respectively, while the percentage of fatty acids for oil in this study was not consistent with what was stated in [16]. The percentage of arachidic acid was 0.29% and the proportion of linoleic acid was 18.23%, and by observation of the table we find that the proportion of saturated fatty acid meristic from the oil extracted from the white seeds is lower than in the black seeds, where the percentage was 2.82% and 3.15% respectively. By comparing the fatty acids studied with the results of the research, we found that the ratio of myristic acid is higher than in [13] where it was 0.04% while [19] for this fatty acid (nonexistent) while [16] said that the ratio of myristic acid was 0.07%, as for the unsaturated fatty acid myristoleic (omiga -5), which was diagnosed from the oil extracted from the white seed did not indicate the existence of any of the research above, as observed in table (2), we found that the percentage of saturated fatty acid (palmitic) was 3.18%, which is lower than that stated in [13] [19] and [16] with 7.47%, 7.1% and 7.07% respectively, while the ratio of olic fatty acid was 1.79%. This result is similar to that of [13], where it was 2.43%, while this result did not correspond to [19] and [16] respectively, where it was 6.4% and 7.04%, respectively, and the ratio of linoleic acid was 2.97%, where it did not correspond to what is stated in [13],[19] and [16] where it was 68.52%, 64% and 62.80%, respectively, and the ratio of fatty acid Lignoceric was 0.69%, which is higher than indicated by [16] While the percentage of Erucic fatty acid (omiga-9) was 3.3%, which was diagnosed in the oil extracted from black seeds, where not referred to Research from above.

Table-2: Fatty acids and their proportion of oil extracted from white seeds

Percentage of fatty acids in oil %	Fatty acids of white seeds
2.82	Myristic C14:0
2.11	Myristoleic C14:1n-5
6.61	Arachidic C20:0
3.54	Linoleic n-6 C18:2

Table-3: Fatty acids and their proportion of oil extracted from black seeds

Percentage of fatty acids in oil %	Fatty acids of black seeds
3.15	Myristic C14:0
3.18	Palmitic C16:0
1.79	Oleic C18:1n-9
2.97	Linolenic C18:3n-3
4	Linoleic C18:2n-6
3.3	Erucic C22:1n-9
0.69	Lignoceric C24: 0

The difference in the percentage of fatty acids obtained in comparison with other studies may be due to the difference in the type of Chia seeds and to the surrounding conditions in addition to the difference in the planting location of the seeds and the difference of climate factors, it may also be due to different extraction method and the type of solvents used and the degree of purity as well as some processes that may be performed on extracted oil such as purification factors and may also be due to the difference in the type and accuracy of the device in addition to the circumstances surrounding the preparation of the sample and the extent of experience of the person existing this Operation. All of these factors lead to divergent results.

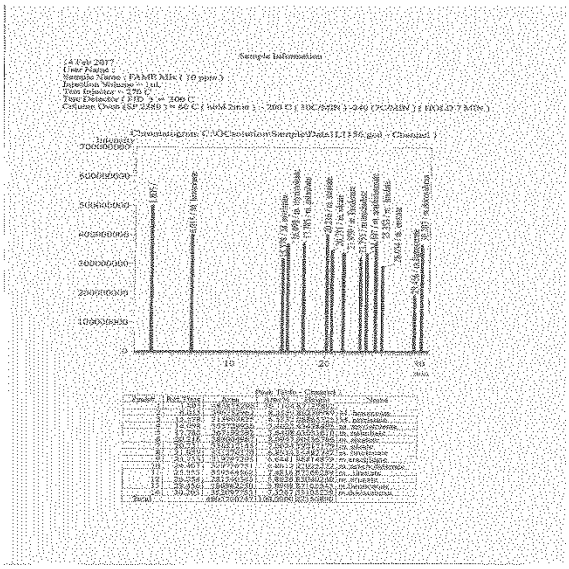

Figure -1: Standard fatty acids

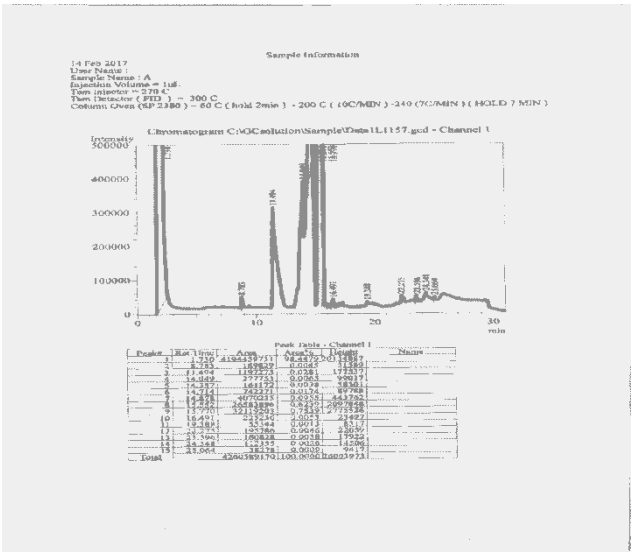

Figure -2: Fatty acids of white chia seed oil

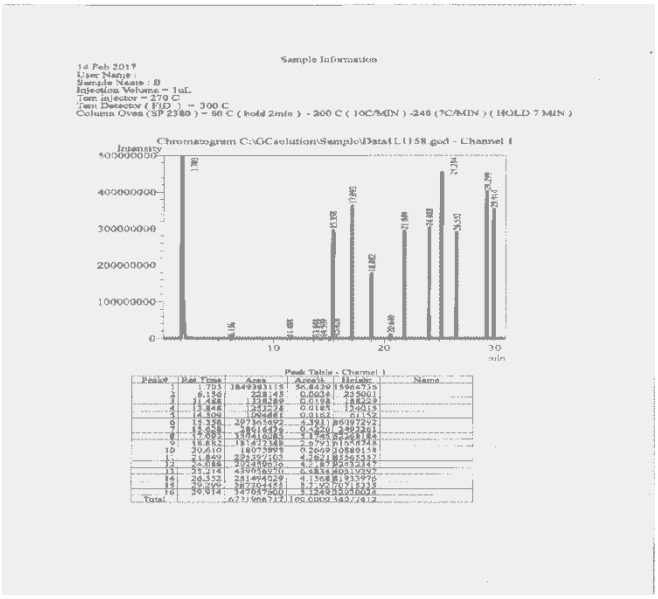

Figure -3: Fatty acids of black chia seed oil

3.3 Active compounds

The results of the qualitative detection of some active compounds in the extracted oil from white and black chia seeds showed that the oil contained the tannins, terpenes and flavonoids, as the results of the tests on these compounds were positive, as shown in Table (4). These results are consistent with the findings of [11][12][17], which refers to the presence of secondary compounds, tannins, terbines and flavonoids.

Table-4: Results of qualitative detection of active compounds in chia seed oil

black chia seed oil		White chia seed oil		Detection
Reagent B	Reagent A	Reagent B	Reagent A	Type Detector
+Brown deposit	+Reddish brown	+Brown deposit	+Reddish brown	terbines
+Yellow precipitate	+Bluish green	+ Yellow precipitate	+Bluish green	tannins
+Red	+Red Orange	+Red	+Red Orange	flavonoids

*The signal + indicates the presence of the active compound

3.4 Antioxidant efficacy

Table (5) shows the effect of the concentrations of oil extracted from white chia seeds in the percentage of antioxidant activity compared with Ascorbic Acid as a control group. The percentage of inhibition was increased to 88.37% at the concentration of 400µl/ml, 83.6%, 72.5 and 57.2 at concentrations (50, 100,200) µl/ml, respectively. The results of the present study also showed the percentage of antioxidant efficacy of oil extracted from black chia seeds compared with Ascorbic Acid as a control group. The percentage of inhibition was increased by 94.5% at 400 µl/ml, 86.38, 72.6 and 39.7 at concentrations (50) (100,200) µl/ml, respectively, and this result is consistent with what is indicated by [18]. The percentage of inhibition of oil extracted from chia seeds may reach more than 90% by using high concentration extracted oil. This may be due to the high concentration of active compounds, which may be attributed to the antioxidant effect, as the oil contains many active compounds that have been diagnosed as tanninates, turbines and flavonoids where several studies have investigated its antioxidant effectiveness, with high hydroxyl flavonoids and rutin flavonide acting as antioxidants [22]. The difference in the inhibition ratio between the two types of oil extracted from the white and black seeds may be due to the difference in concentration of these active compounds in extracted oil. In addition, the oil may contain other compounds found in the oil extracted from the black seeds and are not present in the oil extracted from the white seeds because of the different genetic makeup of the seeds.

Table-5: Comparison of extracted oil from Chia seeds as an antioxidant with ascorbic acid

Comparative model **%**	Inhibition ratio of black seed oil **%**	Inhibition ratio of white seed oil **%**	concentration l/µml
98.6	**94.5**	**88.37**	**400**
98.2	**86.38**	**83.6**	**200**
83.6	**72.6**	**72.5**	**100**
80.69	**39.7**	**57.2**	**50**
56.60	**22.06**	**22.8**	**25**

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