

Article

## The Detection of Bioactive Compounds in Hemp Seed Oil Using GC-MS Techniques

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**Abstract:** The study was conducted in the Laboratories of the College of Agriculture, University of Basrah, Iraq in 2023 to detect the bioactive compounds in hemp seed oil using GC-MS technology. The detection device by GC-MS showed 33 bioactive compounds in hemp seed oil. The results indicate that hemp seed oil contained 16 unsaturated fatty acids. It was found that the content of hemp seed oil was high in the two unsaturated fatty acids, Linoleic acid methyl ester and Oleic acid methyl ester. The peak area percentage of these two unsaturated fatty acids was about 44.15% and 37.45%, respectively. The two unsaturated fatty acids, Palmitic acid methyl ester, and Stearic acid methyl ester recorded a lower peak area percentage, reaching 10.05% and 5.98%, respectively. The other fatty acids detected in the hemp seed oil sample recorded very lower peak area percentage ranging within the range (0.003% - 0.730%). The unsaturated fatty acid  $\alpha$ -Linolenic acid, methyl ester in hemp seed oil recorded the lowest peak area percentage of all the compounds detected by GC-MS, which was 0.003%. The data indicated the presence of six unsaturated fatty acids with high molecular weights, namely Erucic acid, 11-Eicosenoic acid, methyl ester, n-Pentacosanoic acid, n-Tricosanoic acid, n-Heneicosanoic acid, and Heptacosanoic acid. The results revealed that hemp seed oil contains Dioxaphetyl butyrate (0.03%), Naloxone (0.03%), Odorine (0.10%), and Pretazettine (0.01%) for medical and therapeutic uses. Hemp seed oil contains the two most important bioactive compounds of the phytosterols, namely Beta-Sitosterol and Campesterol (0.13% and 0.02%).

**Keywords:** Alkaloid, Fatty Acid, Linoleic Acid, Oleic Acid, Peak Area, Phytosterol

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### 1. Introduction

Hemp (*Cannabis sativa* L.) is an important industrial crop for its stem fibers. This plant belongs to the Cannabaceae family and is a dioecious and unisexual summer crop. Central Asia is the original home of this plant. Its cultivation is currently widespread in many world countries such as India, China, Japan, Russia, Korea, America, Iran, Turkey, and other European countries [1, 2]. Its seeds have nutritional and medicinal uses and are called a superfood, commercially known as Hemp seed due to their rich content of antioxidants and vitamins E and B12 [3]. Its seeds also contain very small or no amounts, reaching less than 0.3%, of the psychoactive drug and the feeling of euphoria due to its content of the compound tetrahydrocannabinol (THC). Therefore, it is different from the narcotic plant marijuana or Indian hemp *Cannabis ruderalis*, [4]. Hemp seeds are small, brown seeds that are rich in proteins, minerals, and healthy fatty acids, including omega-6, which has antioxidant effects. The seeds treat symptoms of many diseases, including scalp and skin diseases, regulate high blood pressure, relieve pain, fight inflammation, and promote digestive health, and support cell and blood vessel health. Its seeds also contain gamma-

linolenic acid (GLA), which has high medical benefits, is anti-inflammatory and helps maintain hormonal balance in the body [4, 5]. Hemp seed oil also contains Cannabidiol (CBD), a non-narcotic chemical compound that does not cause noticeable psychological effects and has many medical uses, including relieving chronic pain and joint inflammation, treating anxiety and psychological stress, and helping to relieve epileptic seizures and pain associated with cancer and Alzheimer's treatments [6]. It also helps reduce the need for nicotine when used as a spray treatment to quit smoking. In addition, the seeds contain large amounts of arginine acid, which produces nitric oxide in the body [7]. This is used to dilate blood vessels, which leads to lower blood pressure and reduces the risk of heart disease [8]. Hemp oil is also used in cosmetics and soaps because it penetrates the skin layer and promotes the growth of healthy cells, which facilitates obtaining soft and smooth skin. In addition to its benefits in treating skin disorders such as psoriasis, eczema, and itchy skin [9]. This is due to its role in improving the levels of essential fatty acids in the blood. The seeds also contain terpenes that contribute to protecting the brain and preventing the formation of tumors [10]. GC-MS analysis showed that hemp oil contains 33 bioactive compounds, including Cannabidiol (CBD), which ranked first, followed by Gamma-gnaiene, alpha-endesmol, aromadendrene oxide, and beta-mealseine [11]. Jokic [12] noted that the most bioactive substances in the inflorescences of the hemp plant are cannabidiol (CBD), alpha-pinene, beta-pinene, beta-myrcene, and limonene. 13. Motiejauskaitė et al. [13] indicated that the inflorescences of the hemp plant contain many bioactive compounds, the most important of which are linoleic acid, cannabidiolic acid, palmitic acid, alpha-linolenic acid, and stearic acid. The research aims to detect the bioactive components in hemp seed oil by extracting the oil from the seeds using the cold method and estimating its components using GC-MS technology.

## 2. Materials and Methods

The bioactive compounds in hemp seed oil by extracting the oil from the seeds using the cold method in the Laboratories of the College of Agriculture, University of Basrah, Iraq. GC-MS investigation was by gas chromatography device that contacted a Mass Spectrometer Model: GC MS QP210 Ultra, Shimadzu, APAN supplied with capillary column DB-MS5 (95% methyl polysiloxane, 5% phenyl) as stationary phase in addition to use helium gas (99.9%) utilizing. The subsequent conditions: Injection Mode: Split, Column Oven Temperature: 50°C, Injection Temperature: 250°C, Column flow: 1.53 ml min<sup>-1</sup>, Pressure: 90.0 kPa, Purge flow: 6.0ml min<sup>-1</sup>, Split ratio: 46.9, Total flow: 79.2 ml min<sup>-1</sup>, Linear velocity: 44.8 cm sec<sup>-1</sup>, Interface Temperature: 250°C, The mass spectrometer: Ion Source Temperature: 200°C, Cut time of solvent Cut: 4.00 min, Detector Gain: 0.84 kV +0.40 kV, Start Time: 4.00min, End Time:41.71min, ACQ Mode: Scan, Event Time: 0.40 sec, Scan Speed: 2000, Start m/z: 35.00, End m/z: 800.

## 3. Results and Discussion

Table 1 shows that the detection device by GC-MS showed 33 bioactive compounds in hemp seed oil. The results in this table also indicate that hemp seed oil contained 16 unsaturated fatty acids. The results of 33 bioactive compounds were consistent with those found by Palmieri et al. [11] but differed qualitatively from them. It was found that the content of hemp seed oil was high in the two unsaturated fatty acids, Linoleic acid methyl ester and Oleic acid methyl ester. This result is consistent with the result found by Kiralan et al [14]. The peak area percentage of these two unsaturated fatty acids was about 44.15% and 37.45%, respectively. The two unsaturated fatty acids, Palmitic acid methyl ester, and Stearic acid methyl ester recorded a lower peak area percentage than the two fatty acids mentioned above, reaching 10.05% and 5.98%, respectively. The rest of the fatty acids

detected in the hemp seed oil sample recorded a lower peak area percentage ranging within the range (0.003% - 0.730%). The unsaturated fatty acid  $\alpha$ -Linolenic acid, methyl ester in hemp seed oil recorded the lowest peak area percentage of all the compounds detected by GC-MS, which was 0.003%. The results of the GC-MS analysis indicated the presence of six unsaturated fatty acids with high molecular weights, namely Erucic acid, 11-Eicosenoic acid, methyl ester, n-Pentacosanoic acid, n-Tricosanoic acid, n-Heneicosanoic acid, and Heptacosanoic acid. It is noted from the data in the table that Heptacosanoic acid had the highest molecular weight among the other fatty acids present in hemp seed oil, which amounted to 410.70 g mol<sup>-1</sup>. The results of the GC-MS analysis revealed that hemp seed oil contains the bioactive compound Dioxyphenyl butyrate, which is used as an opioid analgesic in the pharmaceutical industry [15]. The percentage of the peak area of this active compound was 0.03%. The results of the analysis also revealed that hemp seed oil contains the biologically active anti-opioid compound Naloxone. The percentage of the peak area of this biologically active compound was about 0.03%. Naloxone is an anti-opioid drug used to treat people who are addicted to opiates [16]. Hemp seed oil was also found to contain the bioactive compound Odorine, which is used as an anti-cancer agent [17]. The peak area percentage of this bioactive compound was about 0.10%. Hemp seed oil contains the two most important bioactive compounds of the phytosterols, namely Beta-Sitosterol and Campesterol. The peak area percentage of these phytosterols was about 0.13% and 0.02%, respectively (Table 1). Beta-sitosterol reduces harmful cholesterol in the body and Campesterol is used as a preparation that improves blood flow in the skin [18, 19]. Hemp seed oil was also found to contain the biologically active alkaloid compound Pretazettine, which acts as an effective anti-cancer agent against some types of leukemia by inhibiting protein synthesis and preventing the formation of peptide bonds [20, 21]. The peak area percentage of this alkaloid was about 0.01%. The results of the GC-MS examination confirmed the presence of bioactive compounds such as organic acids Chrysanthemumic acid, Phthalic acid, 4-bromophenyl ethyl ester, monomethyl succinate (monoterpenoid), Trichloroethyl- Trichlorotoluene, Tetrachloro-m-nitro anisole, Methyl triphenyl acetate, Cyclooctyne, Flurochloridone, Guaiphenesin, and Sesamin in a lower percentages of peak area ranging from 0.02% - 0.25%.

**Table 1.** The content of hemp seed oil from bioactive compounds using GC-MS technique

No.	Bioactive compound	Formula	Mw (g mol <sup>-1</sup> )	RT	Area	Peak area (%)
1	Dioxyphenyl butyrate	C <sub>22</sub> H <sub>27</sub> NO <sub>3</sub>	353.5	5.808	11230	0.03
2	$\alpha$ -Linolenic acid, methyl ester	C <sub>19</sub> H <sub>32</sub> O <sub>2</sub>	292.5	6.992	1057	0.003
3	Trichloroethyl- Trichlorotoluene	C <sub>9</sub> H <sub>6</sub> Cl <sub>6</sub>	326.9	8.750	9110	0.03
4	Neovasipyrindone A	C <sub>21</sub> H <sub>35</sub> NO <sub>3</sub>	349.5	9.250	8145	0.02
5	Alpha-Linolenic acid	C <sub>18</sub> H <sub>30</sub> O <sub>2</sub>	278.4	13.242	7259	0.02
6	Tetrachloro-m-nitroanisole	C <sub>7</sub> H <sub>3</sub> C <sub>4</sub> NO <sub>3</sub>	290.9	20.267	16343	0.05
7	Methyl triphenylacetate	C <sub>21</sub> H <sub>18</sub> O <sub>2</sub>	302.4	21.125	9424	0.03
8	Chrysanthemumic acid	C <sub>10</sub> H <sub>16</sub> O <sub>2</sub>	168.23	21.692	6400	0.02
9	Monomethyl succinate	C <sub>14</sub> H <sub>24</sub> O <sub>4</sub>	256.34	23.400	6977	0.02
10	Erucic acid	C <sub>22</sub> H <sub>42</sub> O <sub>2</sub>	338.6	23.392	4648	0.01
11	Cyclooctyne	C <sub>8</sub> H <sub>12</sub>	108.18	24.475	15873	0.05
12	Flurochloridone	C <sub>12</sub> H <sub>10</sub> C <sub>2</sub> F <sub>3</sub> NO	312.11	26.683	6065	0.02
13	Guaiphenesin	C <sub>10</sub> H <sub>14</sub> O <sub>4</sub>	198.22	27.042	7662	0.02
14	Methyl palmitoleate	C <sub>17</sub> H <sub>32</sub> O <sub>2</sub>	268.4	27.483	9262	0.03

15	Paullinic acid	C <sub>20</sub> H <sub>38</sub> O <sub>2</sub>	310.5	27.600	54083	0.16
16	Palmitic acid methyl ester	C <sub>17</sub> H <sub>34</sub> O <sub>2</sub>	270.5	28.125	3377674	10.05
17	Phthalic acid, 4-bromophenyl ethyl ester	C <sub>16</sub> H <sub>13</sub> BrO <sub>4</sub>	349.17	28.723	7638	0.02
18	Pentadecanoic acid	C <sub>15</sub> H <sub>30</sub> O <sub>2</sub>	242.40	30.008	17325	0.05
19	Naloxone	C <sub>19</sub> H <sub>21</sub> NO <sub>4</sub>	327.4	30.775	9523	0.03
20	Pretazettine	C <sub>18</sub> H <sub>21</sub> NO <sub>5</sub>	331.4	31.000	2934	0.01
21	Linoleic acid methyl ester	C <sub>19</sub> H <sub>34</sub> O <sub>2</sub>	294.5	31.392	14844242	44.15
22	Oleic acid methyl ester	C <sub>19</sub> H <sub>36</sub> O <sub>2</sub>	296.5	31.550	12590873	37.45
23	Oleic acid methyl ester	C <sub>19</sub> H <sub>36</sub> O <sub>2</sub>	296.5	31.700	11976	0.04
24	Stearic acid methyl ester	C <sub>19</sub> H <sub>38</sub> O <sub>2</sub>	298.5	31.917	2011587	5.98
25	11-Eicosenoic acid, methyl ester	C <sub>21</sub> H <sub>40</sub> O <sub>2</sub>	324.5	34.842	67364	0.20
26	n-Pentacosanoic acid	C <sub>25</sub> H <sub>50</sub> O <sub>2</sub>	382.7	35.325	245571	0.73
27	n-Tricosanoic acid	C <sub>23</sub> H <sub>46</sub> O <sub>2</sub>	354.6	38.533	48956	0.15
28	n-Heneicosanoic acid	C <sub>21</sub> H <sub>42</sub> O <sub>2</sub>	326.6	41.517	35473	0.11
29	Heptacosanoic acid	C <sub>27</sub> H <sub>54</sub> O <sub>2</sub>	410.7	44.300	11181	0.03
30	Sesamin	C <sub>20</sub> H <sub>18</sub> O <sub>6</sub>	354.4	47.225	84825	0.25
31	Campesterol	C <sub>28</sub> H <sub>48</sub> O	400.7	47.800	6155	0.02
32	Odorine	C <sub>18</sub> H <sub>24</sub> N <sub>2</sub> O <sub>2</sub>	300.4	48.033	33743	0.10
33	Beta-Sitosterol	C <sub>29</sub> H <sub>50</sub> O	414.7	49.125	42333	0.13

#### 4. Conclusion

The GC-MS detected 33 bioactive compounds in hemp seed oil. The hemp seed oil was recorded to have a high content of the two unsaturated fatty acids, Linoleic acid methyl ester, and Oleic acid methyl ester, among the 16 unsaturated fatty acids. Palmitic acid methyl ester and Stearic acid methyl ester were the second most abundant in the hemp seed oil by peak area percentage. The GC-MS device detected some bioactive compounds that can be used in the pharmaceutical industry, including Odorine, Dioxaphetyl butyrate, Naloxone, and Pretazettine, Beta-Sitosterol, and Campestral.

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