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Determination of chemical composition and biological activity of flaxseed (*Linum usitatissimum*) essential oil

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Abstract

The essential oil was obtained from flaxseed (*Linum usitatissimum*) through cold press oil machine of ripe seeds. The chemical composition of the flaxseed essential oils was analyzed by GC-MS. The results revealed that the chemical composition of flaxseed essential oil was found as 9,12-Octadecadienoic acid (Z, Z)-(33.16%), Tributyl acetylcitrate (15.31%), 9,12,15-Octadecatrienoic acid (Z, Z, Z)-(15.28%), 9,12,15-Octadecatrienoic acid, 2,3-dihydroxypropyl ester (Z, Z, Z)-(12.72%), and Ethanol, 2-(9,12-octadecadienyloxy)-(Z, Z)-(9.54%) were found as major compounds followed by Ethyl Linoleolate (3.64%), Tricyclo [6.4.0.0 (3,7) and dodecane (2.04%)].These chemical compounds identified has general biological activities (Antioxidant, Antimicrobial Activity ,anti-inflammatory, Nematicide, Antihistaminic Antieczemic, Insectifuge). As a result of this study, it can be suggested that flaxseed essential oil in the biological application.

Keywords: Flaxseed (*Linum usitatissimum*), Essential oil, GC-MS, Biological activity, Chemical composition

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1. Introduction

One of the earliest crops to be farmed, flaxseed (*Linum usitatissimum* L.) is still widely planted for food, oil, and fiber (Jhala, & Hall, 2010). In 2022, the average global production of flaxseed was 3,973,931.78 tons (FAO, 2023). Because of its quick polymerization, flaxseed oil is a great source of linolenic acid, an omega-3 fatty acid, with normal levels of 55% of the oil (Oomah, 2001). This makes it perfect for paints, varnishes, and inks. Flaxseed is being used as a functional food due to the growing desire for edible oil sources that contain notable percentages of omega-3 fatty acids. In order to enhance the health and reproductive performance of animals, flaxseed is also added to their feed (Salem, 2022; Turner et al., 2014).

Flaxseed is consumed in a variety of forms, such as whole seeds, ground whole seeds, flaxseed oil, partially and fully defatted flaxseed meal (usually from solvent extraction), flaxseed hulls, flaxseed extracts, and partially defatted flaxseed meal (usually from expeller pressing) (Ganorkar & Jain, 2013, Kaur et al., 2018). These goods are all linked to particular positive health outcomes.

The main purpose of the current study is to determine the chemical composition and biological activities of flaxseed (*Linum usitatissimum*) essential oil.

2. Material and Methods

2.1. Plant material and extraction of essential oil

Flaxseeds (*Linum usitatissimum*) were obtained from a local supermarket in Kastamonu, Türkiye then the seed has been cold-pressed. To this end, flaxseeds were dried at a temperature lower than 40°C and then grounded. After grinding, the oils were collected in oil collecting chutes by pressing them without any exposure to heat. After the oil was squeezed, the pulp was discarded and the oil was left to rest for 5 days. The solid layer in the rest oil is sent to the paper or cloth filters without moving. At this stage, the

filtered oils get clarity and kept at room temperature until used.

2.2. Determination of chemical components by GC-MS Analyze

To identification of chemical components, oil Sample was analyzed by GCMS QP 2010 Ultra (Shimadzu) equipped with a Rtx-5MS capillary column (30m·0.25 mm; coating thickness 0.25 μm).

The essential oil components were identified by comparing their relative retention times and mass spectra with those of authentic samples (analytical standards from Aldrich, Acros and Fluka; purity ≥ 97%). Sample solutions were prepared in n-hexane (GC grade, Merck) at 1.0% (w/w).

A summary of the working conditions of the gas chromatography-mass spectrophotometer device are given in Table 1.

Table 1. The effects of root tip meristem application method, different doses (%) and soaking durations of colchicine

Feature	Conditions
Column	RTX-5MS Capillary column (30 m; 0,25 mm; 0,25 μm)
Carrier gas	Helium
Column oven temperature	90˚C
Injection temperature	250˚C
Pressure	90 kPa
Injection mode	Split
Split ratio	10
Injection volume	1µL
Oven temperature program	5 minutes at 90 C, 4 C min-1 increments from 90 C to 250 C, 5 min at 250 C
Interface temperature	250˚C
Ion source temperature	200˚C

3. Results and Discussion

3.1. Chemical composition

Table 2 and Figures 1 shows the results pertaining to the GC-MS chromatogram analyses of flaxseed oil. The compounds are listed in order of their elution time on the column. 31 compounds were detected in flaxseed oil.

The results revealed that 9,12-Octadecadienoic acid (Z, Z)-(33.16%), Tributyl acetylcitrate (15.31%), 9,12,15-Octadecatrienoic acid (Z,Z,Z)-(15.28%), 9,12,15-Octadecatrienoic acid, 2,3-dihydroxypropyl ester (Z, Z, Z)-(12.72%), and Ethanol, 2-(9,12-octadecadienyloxy) (Z, Z)-(9.54%) were found as major compounds followed by Ethyl Linoleolate (3.64%), Tricyclo [6.4.0.0(3,7) and dodecane (2.04%)].

3.2. Biological activities

Previous studies have demonstrated that 20 of 31 of flaxseed oil compounds has a variety of pharmacological and biological functions (Table 3). 9,12-Octadecadienoic acid is the predominant ingredient and essential bioactive ingredient in flaxseed oil, and its health benefits and pharmacological activities have been confirmed according to a large number of animal and clinical experiments (Zheng et al., 2020, Gharibzahedi & Smith, 2021; Shenoy, et al., 2022; Nasr et al., 2024; Attia et al., 2024).

Dietary fiber, protein, and fat are all abundant in flaxseed. According to chemical analysis, flaxseed typically had 30-40% oil, 20-25% protein, 20-28% total dietary fiber, 4-8% moisture, and 3-4% ash. The inclusion of physiologically active food components in the oil provides vitamins A, B, D, and E, as well as minerals and amino acids, which may have health advantages beyond basic nutrition. In recognition of flaxseed's high concentration of dietary fiber, natural phenolic antioxidants, alpha-linolenic acid (ALA), and important omega-3 fatty acids, its use in food and food products has been growing daily. One of the main sources of phytochemicals is now flaxseed (Shahzad et al., 2006). These chemical compounds (phenolic acids, cinnamic acids, flavonoids and lignins) are antioxidants as well as affect the cell growth and viability (Salem et al., 2023; Lakwani & Salem, 2024). According to Amin and Thakur (2014), flaxseed has significant potential as an alternative source of phenolic compounds and is a vital supply of high-quality protein and soluble fiber.

The most abundant source of lignans in diet is flaxseed, where secoisolariciresinol diglucoside (SDG) predominates. Plant lignans are a biologically significant class of phenolic chemicals (Salem & Moammer, (2024). Few studies on the stability of lignans during the food process have demonstrated that SDG levels did not change when flaxseed-containing breads and cookies were made (Cardoso et al., 2012). One of the components, dietary fibers, reduces serum cholesterol and flattens the blood glucose profile, similar to guar gum, oat gum, and other viscous fibers (Jenkins, 1995). Flavonoids have antibacterial, anticancer, anti-inflammatory, and mildly hypersensitive effects of oxidative cell damage in linseed (Pruthi, 2007; Taştan & Salem, (2021).

The potential bioactivities and health benefits of flaxseed oil are summarized and discussed in detail below Table 3.

Table 2. Results of chemical composition analysis of flaxseed oil

No	R.Time	Peak Area (%)	Name of the compound
1	10.027	0.80	Hydroperoxide, 1-ethylbutyl (CAS)
2	10.405	0.89	Pentane, 3-ethyl-2,4-dimethyl- (CAS)
3	10.791	0.15	3-Hexen-2-ONE
4	11.450	0.09	Myrcene
5	11.742	0.39	Heptandienal <2,4-trans,trans->
6	12.285	0.08	Heptandienal <2,4-trans,trans->
7	12.875	0.32	dl-Limonene
8	22.983	0.21	7-Methylene-9-oxabicyclo [6.1.0]non-2-ENE
9	23.094	0.09	2,4-Decadienal, (E, E)- (CAS)
10	23.197	0.10	Tridecane
11	23.913	0.40	endo-Dicyclopentadiene dioxide
12	43.170	0.22	Palmitic acid
13	46.216	0.09	10,13-Octadecadienoic acid, methyl ester
14	46.374	0.31	9,12,15-Octadecatrienoic acid, methyl ester, (Z, Z, Z)-
15	47.049	1.40	2-Ethylhexyl methyl isophthalate
16	47.295	1.40	Tetradecalactone <delta->
17	47.717	22.85	9,12-Octadecadienoic acid (Z, Z)-
18	47.948	7.88	9,12,15-Octadecatrienoic acid, 2,3-dihydroxypropyl ester, (Z, Z, Z)-
19	48.215	9.54	Ethanol, 2-(9,12-octadecadienyloxy)-, (Z, Z)-
20	48.295	10.31	9,12-Octadecadienoic acid (Z, Z)-
21	48.620	15.28	9,12,15-Octadecatrienoic acid, (Z, Z, Z)-
22	50.192	15.31	Tributyl acetylcitrate
23	50.845	4.84	9,12,15-Octadecatrienoic acid, 2,3-dihydroxypropyl ester, (Z, Z ,Z)-
24	51.135	2.04	Tricyclo [6.4.0.0 (3,7)] dodecane
25	51.322	3.57	Ethyl Linoleolate
26	52.055	0.14	2H-Pyran-2-one, tetrahydro-6-tridecyl-
27	54.637	0.14	Bicyclo[10.1.0]tridec-1-ene
28	54.745	0.24	DI-(9-Octadecenoyl)-Glycerol
29	54.854	0.54	9,12,15-Octadecatrien-1-ol, (Z, Z, Z)-
30	55.175	0.21	2-Bromotetradecane
31	56.893	0.17	Ethyl Linoleolate

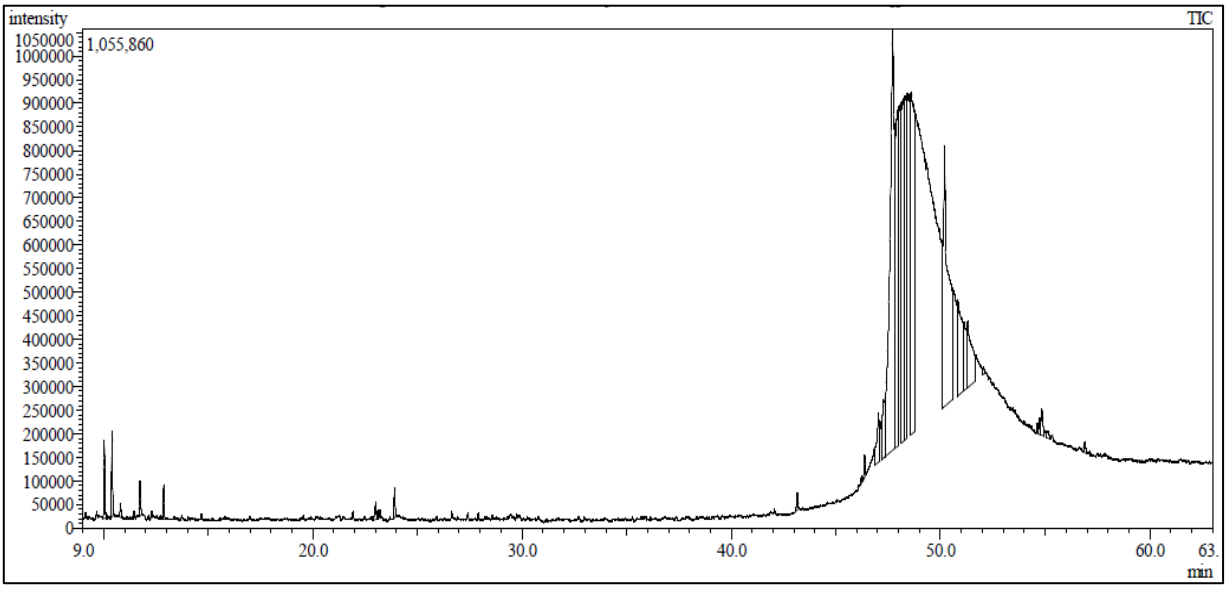

Figure 1.The chromatogram from the GC-MS analysis of the flaxseed oil

Table 3. The chemical compounds identified from the flaxseed oil and their general biological activities

No	Name of the compound	Reported Biological Properties	References
1	Myrcene	Antimicrobial	Inoue et al. (2004)
2	Heptandienal <2,4-trans,trans->	Antimicrobial	Wang et al. (2018)
3	dl-Limonene	Antioxidant, Antimicrobial, Anti-inflammatory, Anticancer	Erasto & Viljoen (2008)
4	2,4-Decadienal, (E,E)- (CAS)	Has negative effects on marine invertebrate larval survival	Caldwell et al. (2005)
5	Tridecane	Antibacterial	Sreedharan et al. (2019)
6	Palmitic acid	Anti-inflammatory	de Souza et al. (2018)
7	9,12,15-Octadecatrienoic acid, methyl ester, (Z, Z, Z)-	Anti-inflammatory, Hypocholesterolemic, Antihistaminic	Srinivasan et al. (2013)
8	9,12-Octadecadienoic acid (Z, Z)-	Anti-inflammatory, Anti-arthritic	Lalitharani et al. (2009)
9	9,12,15-Octadecatrienoic acid, 2,3-dihydroxypropyl ester, (Z, Z, Z)-	Antioxidant, Antimicrobial, Activity, Anti-inflammatory, Nematicide, Antihistaminic, Antieczemic, Insectifuge	Al-Gara et al. (2019)
10	Ethanol, 2-(9,12-octadecadienyloxy) -, (Z, Z)-	Antibacterial, Anti-inflammatory	Hase et al. (2017)
11	9,12,15-Octadecatrienoic acid, (Z, Z, Z)-	Antimicrobial	Al-Gara et al. (2019)
12	Tributyl acetylcitrate	Anti-bacterial, Antioxidant, Anti-inflammatory	Al-Rubaye et al. (2017a, 2017b)
13	Ethyl Linoleolate	Antioxidant	Masuda et al. (2006)
14	2H-Pyran-2-one, tetrahydro-6-tridecyl-	Antidiabetic, Gastro intestinal, Antibacterial, Antioxidant, Mitogenic, Anticancer	Ramya et al. (2015)
15	9,12,15-Octadecatrien-1-ol, (Z, Z, Z)-	Antioxidant, Antimicrobial	Fatema et al. (2019)
16	2-Bromotetradecane	Antioxidant, Antimicrobial	Sasikumar et al. (2020)

4. Conclusion and Future Perspectives

Finally, it can be suggested that the essential oils of flaxseed have strong biological activities such as Antioxidant, Antimicrobial Activity, anti-inflammatory, Antihistaminic Antieczemic, Insectifuge Antibacterial, anti-inflammatory. The flaxseed essential oils may find industrial applications as natural preservatives and antimicrobial agents in cosmetics and food industries.

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Conflict of interest

The authors declare that there is no conflict of interest.

Ethical Approval

For this type of study, formal consent is not required.

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