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<u>Abstract</u>

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Keywords

Physicochemical properties Metal composition Lepidium sativum Fatty acids Proximate analyses GC-MS The present study has been carried out to determine the proximate composition, elemental analysis, physicochemical properties, and fatty acid composition of Lepidium sativum seed oil collected from Eastern Ethiopia. The seed of the plant was extracted with n-hexane to yield 23.72% oil. The oil was characterized by GC-MS showed the presence of docosatrienoic acid (C22:3; 47.66%), linoleic acid (C18:2; 11.51%), eicosenoic acid (C20:1; 10.63%), and palmitic acid (C16:0; 10.13%). The total saturated and unsaturated fatty acids found in the oil were 18.31% and 81.67%, respectively. The proximate analysis the oil contains 24.60% protein, 30.94% carbohydrate, 4.15% w/w moisture and volatilities, 6.49% crude fiber, 28.75% lipid, and 4.35% ash. The metal compositions the oil revealed that 937.26 mg/100 g potassium, 575.487 mg/100 g phosphorous, 470.55 mg/100 g magnesium, 249.88 mg/100g calcium, 14.27 mg/100g sodium, 11.30 mg/100 g iron, 8.61 mg/100 g zinc, 6.42 mg/100 g copper, 1.85 mg/100 g manganese, 1.523 mg/100 g nickel, and 0.0723 mg/100 g lead. The physicochemical characteristics of the oil also measured: 198.05 mgKOH/gm saponification value, 185.3 gL/100g iodine value, 3.42 MeqO₂/g peroxide value, 6.59mg KOH/100 g acid value, 1.465 refractive index, 0.9246 specific gravity, and 0.53%w/w unsaponifiable matter. Linoleic acid constituent of the seed oil extract from Lepidium sativum could be served as a good source of essential fatty acid in food formulations.

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Introduction

Lepidium sativum belongs to family Brassicaceae is a fast growing annual herb and originated primarily in the highland region of Ethiopia and Eritrea (Sharma *et al.*, 2000). Its young leaves are eaten raw or cooked; while its seeds are used, fresh or dried, as a seasoning with a peppery flavor (Facciola and Cornucopia, 1990). In Ethiopia the seed and its oil are primarily used medicinally, but also as condiment and in baking. The main character of *L. sativum* is that it can grow in any type of climate and soil condition (Wadhwa *et al.*, 2012).

The traditional aerial part of the plant is used in the treatment of asthma, cough, and bleeding piles. In Europe the herb is used to treat cough and constipation, as a diuretic and to improve the immune system (Bigoniya *et al.*, 2011). The primary fatty acids found in *L. sativium* seed oil were oleic (30.6%) and linolenic acids (29.3%) (Moser *et al.*, 2009). Unsaturated fatty acids especially linoleic acid exhibited the best skin permeation enhancing effect, while amongst the saturated fatty acids; palmitic acid and stearic acids had the most potent skin permeation enhancing effect (Kim *et al.*, 2008). The determination of metals in vegetable oils and fats has been given a considerable attention by researchers in the past and in recent times because metal causes deleterious effects on the quality and stability of the oils and fats (Olabanji *et al.*, 2013). The composition of the oil varies widely with the variety, geographical location, climate condition, method of extraction, and season of collection.

Even though some work on metal and proximate analysis of the oil was conducted previously. However, there is still insufficient report on physicochemical parameters, elemental analysis, and nutritional values of *L. sativium* seed oil up to date. Therefore, this study was aimed at characterizing the fatty acid composition, nutritional value, determining metal composition, and physicochemical parameters of *L. sativium* seed oil.

Materials and Methods

Collection and identification of the plant material

Dry seeds of *L. sativum* were collected from Eastern Ethiopia in November, 2012. The Botanical

specimens of the plant were identified by Mr. Abeduruzak Abdulhahi and the voucher specimen was deposited at the Herbarium of the Department of Plant Science, Haramaya University. After collection, the seeds were washed repetitively and air dried in the shade to make it easily grindable and kept in a refrigerator at 4°C.

Extraction of seed oil

Air dried seeds of *L. sativum* were grounded by blander and its powder grounded (100 g) was extracted with n-hexane for 6 hrs in Soxhlet apparatus. Anhydrous sodium sulphate was added to remove any trace of moisture from the extracted solution and filtered using whatman No.1 filter paper and concentrated at 40°C to yield pale yellow oil (23.72%).

Digestion of L. sativium seed oil

L. sativium seed oil was digested separately for mineral analysis by the wet digestion method. 0.8 gof the sample was weighed and transferred into 75 mL micro digestion tubes and 4 mL concentrated H_2SO_4 and 2 mL H_2O_2 were added carefully. The tubes were heated in a block digester preheated to 270°C for 30 min and taken out then allowed to cool. Additionally 2 mL of H₂O₂ was added and heated continuously until the digestion was completed. The mineral content such as calcium, manganese, magnesium, zinc, iron, copper, nickel, lead, and sodium, potassium were determined using Atomic Absorption Spectrophotometer and flame photometry, respectively, at Haramaya University, Soil Science Research Laboratory. The equipment had been calibrated using 100 mg/L of the standard solution of each element to be determined. Phosphorus was determined by the phosphovanado-molybdate (yellow) method (Zia-Ul-Haq, 2007).

Physicochemical and proximate analysis of L. sativium *seed oil*

The physicochemical characterizations from *L. sativium* seed oil were conducted for specific gravity, acid value, saponification value, iodine value, peroxide value, and refractive index according the standard procedure (AOAC, 1990). The proximate analysis of the seed oil such as protein, moisture content, lipids, ash, crude fibre, and carbohydrates were determined according to the standard procedure (AOAC, 1990)

Results and Discussion

The nutritional status of L. sativium seed oil

Table 1.	Physicochemical characterization of
	L. sativium seed oils*

Analysis	Value			
State at room temperature	slightly viscous liquid			
Taste	bitter			
Odour	disagreeable			
Color	pale yellow			
Specific gravity (at 25°C)	0.9246±0.013			
Refractive index (at 20°C)	1.465 ± 0.067			
Peroxide value (MeqKOH /g)	3.42±0.04			
Acid value (mgKOH/100 g)	4.5±0.265			
Iodine value (g $I_2/100$ g)	185.30±1.53			
Saponification value (mgKOH/gm)	198.05±1.68			
Unsaponification matter (% w/w)	0.53±0.03			

*Mean (n=3) ±SD

was investigate; oil content (23.72%) was revealed a higher yield to that of previous reported study (22.70%) by Moser et al. (2009). Protein content (24.60%) of the oil were higher than to that of reported (24.19%) by Al-Jasass and Al-Jasser (2012) and (24.18%) by Muhammad Zia-Ul-Haq (2012). Carbohydrate content (30.94%) of the plant also lies in the range of other oils which are used for food purposes and other applications. The moisture content is 4.15%, lies in the range of 3.0-9.9% of other used oils; this suggests the low percentage of moisture in L. sativium as compared to the others may increase the shelf life of these spices during packaging and storage. Moreover, the oil of the plant in this study was also investigate and reported for ash (4.35%), crude fiber (6.49%), and total lipids (28.75%).

The physicochemical parameters of oil extracted from L. sativium seed was presented in (Table 1) had pale yellow and agreeable odour. Acid value (4.50) is used as an indication of edibility of oil and suitability to be used in the paint industry and that are within range of (4.05 to 6.59), falls within the recommended codex of 0.6 and 10 for virgin and non-virgin edible oils and fats (Dawodu, 2009). The refractive index values (at 20°C) obtained from L. sativium seed oil were (1.465) in close agreement with values reported for conventional oils from soybean (1.466-1.470). The high refractive index of this oil seems to confirm the high number of carbon atoms in their fatty acids (Falade et al., 2008). The oil had specific gravity of 0.9246 (at 25°C); this is closer to 0.9256 specific gravity reported for Bischofia javanica seed (Indra et al., 2013). The iodine value of the oil is 185.30; that lie in drying oil. The oil shows a high iodine value due to its high content of unsaturated fatty acids and could be used to quantify the amount of double bonds present in the oil which reflects the susceptibility of oil to oxidation. Saponification value of L. sativium seed oil was 198.05, which was revealed a similar

		-	•	-
Peak#	R.Time	Area	Area%	Name of the components
1	11.987	1851331	0.07	Heptadecnoic acid methyl ester
2	16.443	191693	0.08	Cyclopropaneoctanoic acid, methyl ester
3	16.566	331182	0.13	Hexadecenoic acid, methyl ester
4	17.105	25091321	10.13	Palmitic acid methyl ester
5	21.103	28497429	11.51	9,12-octade cadienoic acid (Z,Z) , methyl ester
6	21.300	118045105	47.66	8,11,14-Docosatrieonoic acid, methyl ester
7	21.829	8264864	3.34	Octadecanoic acid, methyl ester
8	25.502	704815	0.28	11,13-Eicosadienoic acid, methyl ester
9	25.624	26332577	10.63	11-Eicosenoic acid, methyl ester
10	25.748	375658	0.15	Cyclopropaneoctanoic acid,2-hexyl-,ME
11	26.165	8011205	3.23	Eicosanoic acid, methyl ester
12	29.233	11651316	4.70	Metyl (Z)-5,11,14,17-eicosateraenoate
13	29.667	10901268	4.40	13-Docosenoic acid, methyl ester
14	30.162	1948440	0.79	Docosanoic acid, methyl ester
15	33.660	2981139	1.20	9- Octadecenoic acid, methyl ester
16	34.328	2873065	1.16	15-Tetracosenoic acid, methyl ester
17	35.066	1289177	0.52	Tetracosanoic acid, methyl ester
	Total	247675385	99.98	

Table 2. GC-MS analysis fatty acid methyl esters of L. sativium seed oil

saponification value with *Prunus armeniaca, Prunus persica, Datura metel,* and *Hyptis suaveolens* (Indra *et al.,* 2013). Unsaponifiable matter of oils is 0.53% that lies in the range of (0.48-0.71) which showed less impurity in oils, so it could be used in soap industry (Kirsehenbauer, 1965; Amoo, 2004). The peroxide value of *L. sativium* seed oil is low (3.42 meqKOH/g) compared to the maximum acceptable value of 10 meqKOH/g set by the Codex Alimentarius Commission for groundnut seed oils (Abayeh *et al.,* 1998).

The metal compositions of the seed oils were studied and investigated for the level of potassium (937.26 mg/100 g), phosphorous (575.487 mg/100 g), magnesium (470.55 mg/100 g), calcium (249.88 mg/100g), sodium (14.27 mg/100g), and iron (11.30 mg/100 g) were quite high in comparison with other conventional seed oils. Zinc (8.61 mg/100 g), copper (6.42 mg/100 g), manganese (1.85 mg/100 g), nickel (1.523 mg/100 g) and lead (0.0723 mg/100 g) were found in moderate level. The higher amount of calcium, potassium, and magnesium in seed oil indicates for good food formulation and mainly used for repair of worn out cells, strong bones and teeth in humans, building of red blood cells and for body mechanisms (WHO, 1996). The high amount of iron in this plant was very important for maintaining the good health has been recognized (Vaughan and Judd, 2003) and plays an important role in biology, forming complexes with molecular oxygen in haemoglobin and myoglobin.

The sample of *L. sativium* seeds oil was esterified to bring them into volatile components by transforming the fatty acid from *L. sativium* into fatty acid methyl esters. The GC-MS result of the oil revealed that total 17 fatty acid methyl esters were determined in the esterified oil. As shown in (Table 2 and Figure 1) the major component of *L. sativium* seed oil was docosatrienoic acid (C22:3; 47.66%)

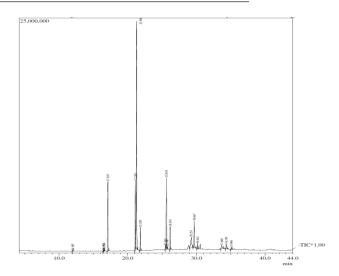


Figure 1. GC chromatogram of L. sativium seed oil

followed by linoleic acid (C18:2; 11.51%), eicosenoic acid (C20:1; 10.63%), palmitic acid (C16:0; 10.13%), arachidonoic acid (C20:4; 4.70%), eruic acid (C22:1; 4.40%), stearic acid (C18:0; 3.34%), and arachidic acid (C20:0; 3.23%). L. sativium seed oil was composed of total mono-unsaturated fatty acids (16.32%), poly-unsaturated fatty acids (65.35%), and saturated fatty acid (18.31%). This study was supported by Al-Jasass and Al-Jasser (2012) research works in which the percentages of total saturated and unsaturated fatty acids was reported as 16.76% and 83.24%, respectively. The slight differences in the results obtained might be due to environmental factors, cultivars used, and different methods used for extraction. Fatty acid profile of L. sativium seed oil provide an important source of Omega 6 and Omega 9 fatty acids for food supplement and medicinal purposes on commercial scale. Linoleic acid is one of the most important polyunsaturated fatty acids in human food because of its prevention of distinct heart and vascular diseases (Matos et al., 2009).

Conclusion

The present study shows that the elemental and proximate analysis of seed oils of L. sativium could be used as food supplement in human diet as it contains considerable amount of phosphorous, potassium, magnesium, calcium, sodium, iron, carbohydrates, proteins, lipids, and crude fibers. L. sativium seed oil contain appreciable amounts of nutrients which may serve as beneficial health sources if consumed regularly and can be used as food supplements for edible oils, besides its uses as a condiments in home. This plant can be utilized to cure number of diseases that are mainly caused due to the deficiency of these minerals. The oil can be classified as unsaturated oil due to the presence of sufficient amounts of docosatrienoic acid and linoleic acids. Therefore, it is amiable to have more research on L. sativium seed oil in the future to explore its potentials for industrial oilseeds crop. Ultimately, the physical and chemical properties of L. sativium seed oil as enumerated in this work make it a great potentials as a nutritionally promising oil and for a variety of industrial applications such as cosmetics and soap making.

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