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Physiochemical Properties, Lipid Breakdown, β-Carotenoids, Tocopherols, Vitamins, Amino and Fatty Acid Profiles of Soxhlet Extracted Oil from Different Garden Cress Seed (*Lepidium sativum* L.) Genotypes in Ethiopia

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Abstract: Physiochemical properties, lipid breakdown, β-carotenoids, tocopherols, and vitamins as well as amino and fatty acid profiles of Soxhlet-extracted oil from five different garden cress (*Lepidium sativum* L.) seed genotypes (namely: CG8, CG7, CG17, CG4, and 207910) across Ethiopia regions were investigated. Results showed that despite the seeds' proximate peak and least values, the extraction yield, viscosity, specific gravity, refractive index, lipid breakdown, and boiling point of garden cress seed oil across the genotypes noticeably varied with promising amino and fatty acid profiles. Further, the genotype CG17 obtained greater quantities of β-carotenoids, tocopherols and vitamin values compared to the other genotypes.

Key words: plant oil seeds, oil production, lipid peroxidation, oil quality, product development

1 Introduction

Garden cress (*Lepidium sativum* L.) seed, of Brassicaceae family (Cruciferae), besides commonly cultivated in India, North America, western Asia and some parts of Europe, is well established crop in some parts of Africa. Specifically considered an annual, erect herb farmed across different regions/soil types across regions of Ethiopia, the garden cress is considered a culinary vegetable edible for its seeds[1], which are physically characterised by small size, oval shaped, smooth textured and brownish-red colour[2]. Garden cress seeds comprise important/medicinally enriched essential oils[3], with antimicrobial and antifungal efficacy[1, 4, 5]. Due to the high nutritional and functional content, garden cress seeds help to fortify foods[2, 6]. Moreover, oil crops remain the second most important source of edible calories after cereals[7], producing millions of metric tonnes annually and occupying among global agricultural commodities[8]. Generally, oilseed crops possess unique fatty acids profiles[9], with majority having roughly 20-45% (w/w) oil[7]. Additionally, the dominant fatty acids in seed oils largely found in seed kernel include linoleic, linolenic, oleic, palmitic, and stearic acids[8]. Upregulating the carbon flux towards the oil by increased activities of one or more key rate-limiting enzymes can help accumulate more seed oil, reasonably[7].

Largely, the extraction of fat and oils from plant seed matrices has been based primarily on factors/techniques like choice/selection/type of solvent (polarity), extraction time, heat/temperature, and use of heat and agitation[10–12], which serve as prerequisites in achieving reasonable oil yield. When (these above-named factors) not properly controlled, good quality and yield of the oil would most likely

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be compromised[12]. In particular, the Soxhlet extraction, among the oldest most referenced technique of promising performance still in use today, is able to separate compounds of interest from insoluble high-molecular-weight fractions. To implement the Soxhlet process, the correct choice of solvents, extensive use of maceration as well as extraction protocols have to be followed. Additionally, Soxhlet extraction methods include the automated, conventional, high-pressure, and ultrasound-assisted types[11], and the type to use largely depends on affordability, and availability. Nonetheless, there are innumerable oilseeds that have been extracted by the Soxhlet method, and with continued success. Examples of recent Soxhlet method extraction studies involving oilseeds include the likes of garden cress (*Lepidium sativum* L.)[1], *Sapindus mukorossi* seed oil[13], sweet passion fruit (*Passiflora alata* Curtis) seed oil[14], Krabok (*Irvingia malayana*) seed oil[15], caper seed oil[16], Neem seed oil[17], grape seed oils[18] as well as corn germ oil[19]. Notably, the objectives of the above-mentioned studies clearly differ despite the various oilseeds being subject to the Soxhlet method.

Generally, plant oils are considered more oxidized/unsaturated with a weak spot of its central β-CH group situated in the oil molecule (triacylglycerol), providing them limited hydrolytic, oxidation, and thermal stability[8]. Therefore, to take advantage of existing plant species as domesticated oil crops would provide a major opportunity to produce technical oil qualities and great diverse fatty acids[8]. However, to obtain a reasonable yield of crop oil, an extraction method that is of high performance is required. Plausibly, the characteristic attributes of plant seed oil might differ by location, crop varieties, topographical altitude of cultivated field, application/non-application of fertilizer, and type, among others. Until now, there is paucity of relevant information about the quality attributes of extracted essential oil obtained from the different varieties of garden cress seeds specifically cultivated in Ethiopia. Supplementing the existing information, therefore, this current work investigated the physiochemical properties, lipid breakdown, β-carotenoids, tocopherols, and vitamins (A and C) as well as amino and fatty acid profiles of Soxhlet extracted oil obtained from five different garden cress seed (*Lepidium sativum*) genotypes in Ethiopia.

2 Materials and Methods

Figure 1 shows a schematic overview of the experimen-

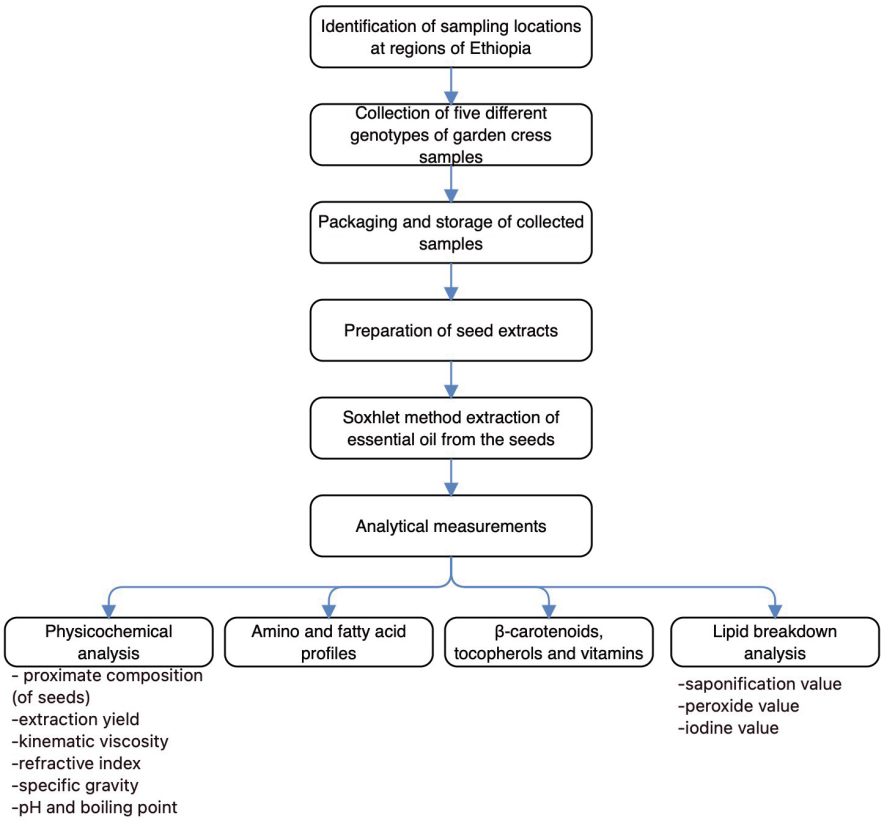

Fig. 1 A schematic overview of the experimental program, depicting the major stages of this current work, from the identification of sample location across the regions of Ethiopia, collection of five different genotypes of garden cress, packaging and storage, to the analytical methods.

tal program depicting the major stages, from the identification of sample location across the regions of Ethiopia, collection of five different genotypes of garden cress, packaging and storage, to the analytical methods. All chemicals and reagents used were of analytical grade standard. Specifically, the five different genotypes of garden cress (*L. sativum* L.), namely CG8, CG7, CG17, CG4, and 207910, given their availability (during the 2020 harvest season), were collected across the Oromia, Amhara, Tigray, as well as Southern Nations, Nationalities, and Peoples' (SNNP) regions of Ethiopia. Table 1 describes the garden cress seed genotypes. After harvest, the seeds packed into plastic net bag/lot of approximately 2 kg weight per genotype were transported to Wollo University (Ethiopia), where there were kept in cold room (4-8℃) until required.

Extract preparation required about 100 g of garden cress (*L. sativum* L.) seed sample, which were cleaned with distilled water, air dried, ground by mortar and pestle, and subsequently sieved into particle size (1.8 mm), prior to storage in plastic containers. Next, Soxhlet extraction of the oil followed steps described by Schuten *et al.*[20] with slight modifications. Prepared sample (50 g) of ground garden cress seed were submitted to extraction chamber, which used hexane (200 mL) as a solvent. A rotary evaporator under vacuum at 65℃ was employed to separate the solvent and oil mixture, after which the obtained oil was stored at −20℃ until required. Analytical measurements involved the determinations of physiochemical (proximate composition, extraction yield, kinematic viscosity, refractive index, specific gravity, pH value and boiling point) properties, lipid breakdown (iodine, peroxide and saponification values), β-carotenoids, tocopherols, and vitamins (A and C), as well as amino and fatty acid profiles.

Proximate determinations of ash, carbohydrate, crude protein, crude fat, crude fibre, and moisture contents of garden cress seed followed the AOAC method[21] with slight modifications where deemed appropriate. Ash determination utilised the muffle furnace at 600℃ for 3 h, whereas crude protein determination employed the Kjeldahl digestion with the protein content calculated by correction factor (% N×6.25). Crude fat determination utilised the Soxhlet apparatus, which required about 16 h extraction time after which evaporation at 70℃ helped to remove the remaining solvent. Moisture determination was performed by oven at 110℃ to a constant weight. Total carbohydrate (COH) was calculated by difference method. To establish the crude fibre, fresh oil sample (2 g) had to be subjected to diluted H_2SO_4, boiled (30 min), filtered, pre-heated with 150 mL KOH, drops of octanol added, then boiling for 30 min, followed by filtering. Using cold extraction unit, acetone helped to wash the sample, then drying (130℃ for 1 h), followed by ashing using furnace at 500℃. With the resultant ash weighed, the percentage crude fibre was determined.

After Soxhlet extraction had been performed, the extraction yield of oil from garden cress seed was achieved, and expressed as a percentage. Kinematic viscosity required 35 mL sample of oil inserted into the viscometer's sample holder, and its sensor immersed therein, after which at a temperature of 20℃, the dynamic viscosity displayed on the screen[22]. The kinematic viscosity was calculated using the equation 1 below:

$$\text{Kinematic viscosity} = \frac{\textit{dynamic viscosity of the sample}}{\textit{Density of the sample}} \quad \text{(Eqn. 1)}$$

Refractive index of garden cress seed oil was determined using a refractometer at 25℃. After, the specific gravity was determined via the density bottle method[22] with slight modifications. A washed, dried and weighed density bottle was filled with the oil sample and placed in a water bath for a temperature of 25℃. The weight of the oil was recorded after which the bottle was emptied, washed and dried again before refilling with water maintained at a temperature of 25℃, the weight of water recorded, and specific gravity calculated.

Boiling point and pH of garden cress seed oil were determined by AOAC method[21]. Whereas the pH electrode was standardised with a buffer, the boiling point of garden cress seed oil (25 mL) was measured by inserting the thermometer, and data read out as boiling started.

Saponification value of garden seed oil was determined by method of Ogungbenle and Afolayan[23] with slight modifications. About 2 g of oil were mixed with 25 mL of ethanoic potassium hydroxide (0.1 M), allowed to boil for about 1 h with gentle/regular swirling at 5 min intervals. A drop of phenolphthalein indicator was added, followed by titra-

Table 1 Description of garden cress seed genotypes used for the study.

No	Genotypes	Regional State	Zone	District/Location
1	CG8	Oromia	Jimma	Limu Seka
2	CG7	Amhara	South Wollo	Debresina
3	CG17	SNNP	North Omo	Basketo
4	CG4	Oromia	Bale	Agarfa
5	207910	Tigray	Central Tigray	Adwa

tion with 0.5 M HCl, to achieve endpoint. The same procedure was followed for the blank (that is, without the oil). The saponification value (SV) is calculated using the equation 2 below:

$$\text{Saponification value (SV)} = 56.1 \times \frac{N(V0 - V1)}{m} \quad \text{(Eqn. 2)}$$

where: V0 = volume of HCl solution used for the blank test, V1 = volume of HCl solution for the determination, N = actual molarity of HCl used, using 56.11 value as the molecular weight of KOH, and m = mass of a sample.

Peroxide value of garden seed oil followed the method of Ogungbenle and Afolayan[23] with slight modifications. Approx. 1 g of oil and 30 mL of chloroform were mixed, and saturated potassium iodide solution (20 mL) added for titration, and 0.5 mL starch indicator solution. Titration with 0.02 M sodium thiosulphate solution followed until the dark blue color disappeared. Blank test was conducted in parallel under identical conditions. Peroxide value expressed in millimoles of active oxygen per kilogram (mEq. O_2/kg) was calculated using the equation 3 below:

$$\text{Peroxide value} = \frac{(TB - TS) \times N}{\textit{Weigh to fsample oil}} \times 100 \quad \text{(Eqn. 3)}$$

where: N = molarity of thiosulphate, TS = volume of thiosulphate used in the sample test, and TB = volume of thiosulphate used in the blank.

Iodine value of garden cress seed oil followed the method of Boukhatem *et al.*[24] with slight modifications. Approx. 0.1 g garden cress seed oil was dissolved in 10 mL of chloroform. Then 25 mL of iodine bromide solution was added and allowed to stand for 30 min in dark. After that, 30 mL of 1 N potassium iodide and 100 mL of distilled water were added, and titrated with 0.1 N sodium thiosulphate with constant swirling. When the iodine color became quite pale, 1 mL of 1% starch solution was added and the titration was continued until the blue color was discharged. A blank test was also carried out parallel under identical conditions. The iodine value was calculated using the equation 4 below:

$$\text{Iodine value} = 12.69 \times \frac{V1 - V2}{w} \quad \text{(Eqn. 4)}$$

where: W is the weight of the sample, V1 is the number of mL of thiosulphate consumed by the blank; and V2 is the number of mL of thiosulphate consumed by the test sample.

The AOAC method[21] with slight modifications was used to determine the β-carotenoids, tocopherols, and vitamins of garden cress seed oil. For the β-carotene, a mix with aqueous solution (containing β-carotene) were submitted to UV-VIS Spectrophotometer Model 6305 (Bibby Scientific Ltd, Stone, UK), and the absorbance read at a wavelength of 450 nm. For the tocopherols, vitamins A and C, the HPLC (L-6200 intelligent pump, AS-2000A autosampler, and L-5025 column thermostat) system equipped with an UV/VIS and fluorescence detector.

Consistent with previously published methods[25-27] with some modifications was used to determine the amino acid profile of garden cress seed oil. The Hewlett Packard AminoQuant II system was used, equipped with temperature controlled compartment, variable volume injector, and an autosampler. The AminoQuant that analysed the peptides and proteins operated by pre-column derivatization of hydrolyzed samples with o-phthalaldehyde (OPA), as well as 9-fluoromethyl-chloroformate (FMOC). A known amount of essential oil was mixed with internal standards (norvaline and sarcosine) in a vacuum concentrator and subjected to vapor phase hydrolysis by 6 N HCl at 110℃ for 24 h under argon atmosphere in phenol. This was reconstituted subsequently in borate buffer, and thereafter, transferred to the AminoQuant auto-sampler for automated derivatization/loading. A total of 17 naturally occurring amino acids were identified. The derivatised amino acids has been eluted from a reversed-phase column (20 cm × 2.1 mm) (Hewlett-Packard Amino Acid Analysis column) by simple linear gradient. In addition, the analytical column has been guarded with a 2 cm × 2.1 mm (guard) column. A UV-vis diode-array detector helped to detect the primary amino acids at a wavelength of 338 nm (reference at 390 nm), and the secondary amino acids at a wavelength of 262 nm (reference at 324 nm).

The method described by Ogungbenle and Afolayan[23] with modification was used to determine the fatty acid profile of garden cress seed oil, conducted in the context of fatty acid methyl esters (FAMEs) via gas chromatograph (GC). The FAMEs have been prepared by the methylation/saponification with sodium methylate, consistent with the European regulations (EEC 2568/91). The chromatographic analysis employed Perkin-Elmer Clarus 500 gas chromatograph, equipped with 30 m × 0.25 mm × 0.25 μm film thickness HP INNOWax, coupled with a flame ionization detector (FID) and column temperature of 210℃. Both the injector as well as detector were eventually maintained at temperatures between 230 and 250℃, respectively. The carrier gas was nitrogen, and volume injection was 1 μL with a split ratio of 100:1. The first ramping was at 12℃/min, which lasted for 15 min, and maintained for 2 min. The second ramping was at 15℃/min, which lasted for 5 min, and maintained for 8 min. The retention times have been verified against standard compounds.

Minitab software package (version 18) (Minitab Ltd., Coventry CV3 2TE, United Kingdom) was used to run the data analysis. The resultant data, from triplicate determinations given to analysis of variance (ANOVA), were expressed as the mean ± standard deviation. Probability level of statistical significance was set at $p < 0.05$ (95% confidence).

3 Results and Discussion

Table 2 shows the proximate composition of five genotypes of garden cress seed (CG8, CG7, CG17, CG4, and 207910). The moisture contents ranged from 3.1 ± 0.4% (genotype CG7) to 4.2 ± 0.3% (genotype 207910) with no statistical difference ($p > 0.05$). More so, seeds with a low moisture content would provide improved stability and shelf life[28]. The amount of oil extracted from the garden cress ranged from 19.8 ± 0.4% (genotype 207910) to 27.2 ± 0.3% (genotype CG17), with significant differences ($p < 0.05$), which somewhat agree with data of Diwakar *et al.*[1]. Moreover, the crude protein ranged from 22.1 ± 0.8% (genotype 207910) to 28.4 ± 0.7% (genotype CG8), with genotypes CG7, CG17, CG4 and 207910 resembling one another ($p > 0.05$), except CG8 being significantly higher ($p < 0.05$). The presence of protein content depicts amino acid as major abundant compounds[29]. The crude fiber obtained from five genotypes of garden cress seed ranged from 8.1 ± 0.5% (genotype CG17) to 12.5 ± 0.8% (genotype CG4), with those of CG4, CG7 and CG8 resembling ($p > 0.05$), and the others significantly lower ($p < 0.05$). With respect to ash contents, genotypes CG7 and 207910 obtained significantly higher ($p < 0.05$) values compared to the others (CG8, CG17 and CG4) resembled ($p > 0.05$) one another. The total carbohydrate ranged from 28.6 ± 1.4% (genotype CG8) to 37.3 ± 0.5% (genotype 207910) with significant ($p < 0.05$) differences between them. The crude fiber, ash and total carbohydrate values of garden cress seed herein seem to compete well with data of Al-Jasass *et al.*[30].

Table 3 shows the extraction yield, kinematic viscosity, specific gravity, refractive index, lipid breakdown, and boiling point of the extracted oil from five garden cress seed genotypes. Extraction yield ranged from 18.5 ± 1.1% (genotype 207910 = least extraction yield) to 23.4 ± 0.8% (genotype CG17 = peak extraction yield) with significant ($p < 0.05$) differences among them. Kinematic viscosity obtained at constant extraction temperature ranged from 52.10 ± 0.26 m^2/s (genotype 207910 least viscosity) to 58.8 ± 0.6 m^2/s (genotype CG17 = peak viscosity), with the genotype CG17 significantly higher ($p < 0.05$) than others (genotypes CG7, CG8, CG4, and 207910). Both refractive index and specific gravity seemed not to differ considerably ($p < 0.05$), with refractive index ranging from 1.2 ± 0.0 (genotype 207910 = least refractive index) to 1.5 ± 0.0 (genotype CG17 = peak refractive index), and specific gravity ranging from 0.8 ± 0.0 g/mL (genotype 207910 = least specific

Table 2 Proximate composition of five genotypes of garden cress seed (CG8, CG7, CG17, CG4, and 207910).

Genotypes	Moisture (%)	Oil (%)	Protein (%)	Crude fiber (%)	Ash (%)	COH (%)
CG8	3.5 ± 0.7^{a}	22.9 ± 1.7^{b}	28.5 ± 0.7^{a}	11.6 ± 1.9^{a}	4.9 ± 0.4^{b}	28.6 ± 1.4^{c}
CG7	3.1 ± 0.4^{a}	20.8 ± 1.1^{bc}	23.8 ± 0.9^{b}	11.6 ± 1.4^{a}	7.1 ± 0.8^{a}	33.6 ± 0.3^{b}
CG17	4.0 ± 0.4^{a}	27.2 ± 0.3^{a}	22.5 ± 0.8^{b}	8.1 ± 0.5^{b}	4.8 ± 0.5^{b}	33.5 ± 0.8^{b}
CG4	3.1 ± 0.3^{a}	26.9 ± 0.7^{a}	23.7 ± 0.4^{b}	12.5 ± 0.8^{a}	4.2 ± 0.5^{b}	29.5 ± 0.8^{c}
207910	4.2 ± 0.3^{a}	19.8 ± 0.4^{c}	22.1 ± 0.8^{b}	9.4 ± 0.4^{ab}	7.1 ± 0.3^{a}	37.3 ± 0.5^{a}

COH= Total carbohydrate; Data are expressed as the mean ± standard deviation;
Values in the same column having different letters differ significantly ($p < 0.05$).

Table 3 Extraction yield, kinematic viscosity, specific gravity, refractive index, lipid breakdown, and boiling point of extracted oil from five garden cress seed genotypes.

Attributes	Garden cress seed genotypes				
	CG8	CG7	CG17	CG4	207910
Extraction yield (%)	20.5 ± 0.8^{bc}	19.3 ± 1.1^{bc}	23.4 ± 0.8^{a}	21.5 ± 0.7^{ab}	18.5 ± 1.1^{c}
Kinematic viscosity (m^2/s)	53.7 ± 0.6^{c}	53.5 ± 0.6^{c}	58.8 ± 0.6^{a}	55.4 ± 0.5^{b}	52.1 ± 0.3^{d}
Specific gravity (g/mL)	0.9 ± 0.1^{abc}	0.8 ± 0.0^{bc}	1.0 ± 0.1^{a}	0.9 ± 0.0^{ab}	0.8 ± 0.0^{c}
Refractive index at 25℃	1.4 ± 0.0^{b}	1.3 ± 0.0^{bc}	1.5 ± 0.0^{a}	1.4 ± 0.0^{b}	1.2 ± 0.0^{c}
Saponification value (mg KOH/g)	174.3 ± 2.1^{bc}	173.1 ± 1.2^{c}	182.3 ± 2.2^{a}	178.6 ± 2.0^{ab}	171.2 ± 1.9^{c}
Iodine value (mg iodine/100 g)	129.3 ± 3.8^{a}	120.7 ± 2.5^{b}	136.0 ± 3.0^{a}	132.7 ± 2.5^{a}	111.7 ± 3.1^{c}
pH value	6.5 ± 0.2^{ab}	6.7 ± 0.3^{ab}	7.0 ± 0.2^{a}	6.4 ± 0.3^{ab}	6.1 ± 0.3^{b}
Peroxide value (mEq. O_2/kg)	3.5 ± 0.2^{cd}	3.8 ± 0.1^{bc}	4.9 ± 0.3^{a}	4.2 ± 0.2^{b}	3.2 ± 0.1^{d}
Boiling point (℃)	173.1 ± 1.0^{a}	169.2 ± 0.8^{b}	175.9 ± 0.4^{a}	173.7 ± 0.8^{a}	167.2 ± 2.3^{b}

Data are expressed as the mean ± standard deviation; values in the same row having different letters differ significantly ($p < 0.05$).

gravity) to 1.0 ± 0.1 g/mL (genotype CG17 = peak specific gravity). In addition to depicting the degree of saturation and presence of unusual hydroxyl components/groups, the refractive index provides useful information about the oil purity, where the peak value would signal spoilage due to oxidation[1]. The pH value ranged from 6.1 ± 0.3 (genotype 207910 = least pH) to 7.0 ± 0.2 (genotype CG17 = peak pH), which is acidic. Oil with a pH value below seven is considered as preferred for healthy hair/skin[31, 32].

The iodine values ranged from 111.7 ± 3.1 mg iodine/100 g (genotype 207910 = least iodine value) to 136.0 ± 3.0 mg iodine/100 g (genotype CG17 = peak iodine value). When the iodine value is high, there is increased unsaturation, which would help to lengthen the shelf life[25–27], which connotes positives for the CG17 genotype. Moreover, the peroxide values at CG8, CG7, and 207910 respectively appear low, with 3.5 ± 0.2, 3.8 ± 0.1, and 3.2 ± 0.1 mEq. O_2/kg, which might suggest increased stability to oxidative degradation[33], compared to higher peroxide values at genotypes CG4 (4.2 ± 0.2 mEq. O_2/kg) and CG17 (4.9 ± 0.3 mEq. O_2/kg). Saponification values ranged from 171.2 ± 1.9 mg KOH/g (genotype 207910 = least saponification value) to 182.3 ± 2.2 mg KOH/g (genotype CG17 = peak saponification value) with significant differences ($p < 0.05$) among them. Elsewhere, varied saponification values in garden cress (*L. sativum* L.) and *Lannea kerstingii* seed oil respectively from 174.0 to 182.2 mg KOH/g, as well as 189.7 mg KOH/g, have been reported[1, 34]. High saponification value signals an oil with a greater proportion of low molecular weight fatty acids[1, 35].

Table 4 shows the β-carotenoids, tocopherols, and vitamins A and C values of the extracted oil from five garden cress genotypes. Besides β-carotene content ranging from 3.2 ± 0.0 mg/100 g (genotype 207910 = least β-carotene content) and 4.6 ± 0.0 mg/100 g (genotype CG17 = peak β-carotene content), the resembling ($p > 0.05$) three CG8, CG7, and CG4 genotypes appeared significantly higher ($p < 0.05$) than CG17 and 207910 genotypes. The tocopherols varied from 122.6 ± 0.0 mg/100 g (genotype CG7 = least tocopherol content) to 133.7 ± 0.0 mg/100 g (genotype CG17 = peak tocopherol content), with significant differences ($p < 0.05$) among them. Besides tocopherol content of garden cress seed oil herein agreeing with data reported by Singh and Paswan[2], a much higher tocopherol content (about 578.6 mg/kg) had been reported elsewhere[34]. Whereas vitamin A ranged from 0.2 ± 0.0 mg/100 g (genotype 207910 = least vitamin A content) to 0.4 ± 0.0 mg/100 g (genotype CG17 = peak vitamin A content), the vitamin C ranged from 50.2 ± 0.0 mg/100 g (genotype 207910 = least vitamin C content) to 73.1 ± 1.7 mg/100 g (genotype CG7 = peak vitamin C content). The vitamin C contents of garden cress oil from Dadas (54 mg/100 g) and Izmir species (74 mg/100 g)[36] appear to concur with those of the current study. The presence of β-carotenoids, tocopherols, and vitamins A and C in the garden cress seed oil would make it useful for healthy and smooth skin[37–39], given its capacity to improve (skin) water retention[40]. Additionally, vitamin C can help alleviate hair loss associated with iron deficiency[41].

Table 5 shows the amino acid profile of the extracted oil from garden cress seed of different genotypes. Specifically, there were nine essential amino acids (namely: histidine, arginine, methionine, phenyl alanine, valine, isoleucine, lysine, threonine and leucine), and seven non-essential amino acids (namely: tyrosine, alanine, serine, glutamine, asparagin, proline and glycine). Among the non-essential amino acids, both glutamine and asparagine appeared as the major abundant compounds, whereas among the essential amino acids, the leucine, valine, and lysine appeared the major abundant compounds[2, 27, 29]. The considerable presence of amino acids in garden cress seed oils makes it promising for cosmetic formulations, hair conditioning/skin agents, etc[25]. Table 6 shows the fatty acid profile of extracted oil from garden cress seed of different genotypes. On the one hand, both myristic and arachidonic acid appeared as the least abundant of saturated and unsaturated fatty acids, respectively. On the other hand, both linoleic and oleic appeared as the most abundant of unsaturated fatty acids, whereas both palmitic and stearic were the most abundant of saturated fatty acids, in agreement with data reported by Moser *et al.*[42]. Elsewhere, linoleic acid has been considered as a predominant fatty acid in black cumin, fenugreek, black pepper, and clove seed oils[43–45].

Table 4 β-carotenoids, tocopherols, and vitamins (A and C) (reported in mg/100 g) of oil extracted from five garden cress seed genotypes.

Parameters (mg/100 g)	Garden cress seed genotypes				
	CG8	CG7	CG17	CG4	207910
β-Carotenoids	4.2 ± 0.0^{b}	4.2 ± 0.0^{b}	4.6 ± 0.0^{a}	4.0 ± 0.0^{b}	3.2 ± 0.0^{c}
Tocopherols	126.7 ± 0.0^{c}	122.6 ± 0.0^{d}	133.7 ± 0.0^{a}	127.4 ± 0.0^{c}	130.4 ± 0.0^{b}
Vitamin A	0.4 ± 0.0^{a}	0.3 ± 0.0^{b}	0.4 ± 0.0^{a}	0.3 ± 0.0^{b}	0.2 ± 0.0^{c}
Vitamin C	67.3 ± 1.8^{b}	73.1 ± 1.7^{a}	54.6 ± 0.9^{d}	62.0 ± 0.8^{c}	50.2 ± 0.0^{e}

Data are expressed as the mean ± standard deviation; values in the same row having different letters differ significantly ($p < 0.05$).

Table 5 Amino acid profile (reported in g/100 g) of oil extracted from five garden cress seed different genotypes.

Amino acid	Garden cress seed genotypes				
	CG8	CG7	CG17	CG4	207910
Essential amino acid					
Histidine	3.5 ± 0.3^{a}	3.9 ± 0.1^{a}	2.7 ± 0.2^{b}	4.1 ± 0.1^{a}	3.6 ± 0.5^{a}
Arginine	4.5 ± 0.1^{c}	3.6 ± 0.3^{d}	6.9 ± 0.1^{a}	5.5 ± 0.2^{b}	4.5 ± 0.3^{c}
Methionine	0.9 ± 0.0^{a}	0.5 ± 0.1^{bc}	1.0 ± 0.1^{a}	0.8 ± 0.3^{ab}	0.3 ± 0.1^{c}
Phenylalanine	5.4 ± 0.1^{b}	5.5 ± 0.2^{ab}	5.8 ± 0.2^{a}	4.4 ± 0.2^{c}	5.2 ± 0.1^{b}
Valine	8.3 ± 0.2^{a}	6.6 ± 0.2^{b}	5.8 ± 0.3^{c}	6.8 ± 0.3^{b}	5.8 ± 0.1^{c}
Isoleucine	5.0 ± 0.1^{a}	4.3 ± 0.2^{b}	5.1 ± 0.1^{a}	3.8 ± 0.2^{c}	4.5 ± 0.2^{b}
Lysine	6.6 ± 0.1^{a}	5.9 ± 0.1^{b}	6.4 ± 0.3^{a}	6.4 ± 0.1^{a}	5.9 ± 0.1^{b}
Threonine	2.3 ± 0.1^{d}	3.9 ± 0.1^{b}	4.4 ± 0.1^{a}	3.3 ± 0.1^{c}	3.7 ± 0.1^{b}
Leucine	8.2 ± 0.2^{b}	7.2 ± 0.2^{c}	8.4 ± 0.3^{b}	9.1 ± 0.1^{a}	7.9 ± 0.1^{b}
Non-essential amino acid					
Tyrosine	2.8 ± 0.1^{bc}	2.7 ± 0.1^{c}	3.0 ± 0.1^{b}	3.0 ± 0.1^{b}	3.4 ± 0.0^{a}
Alanine	4.6 ± 0.1^{b}	4.5 ± 0.2^{b}	5.1 ± 0.1^{a}	5.2 ± 0.1^{a}	4.7 ± 0.1^{b}
Serine	5.1 ± 0.2^{ab}	4.4 ± 0.2^{d}	4.8 ± 0.1^{bc}	5.3 ± 0.1^{a}	4.4 ± 0.1^{cd}
Glutamine	21.3 ± 1.3^{c}	24.7 ± 0.3^{a}	19.2 ± 0.3^{d}	23.3 ± 0.4^{ab}	22.8 ± 0.8^{bc}
Asparagin	10.4 ± 0.4^{b}	12.1 ± 0.2^{a}	9.8 ± 0.3^{bc}	9.9 ± 0.3^{bc}	9.5 ± 0.1^{c}
Proline	4.5 ± 0.2^{c}	6.3 ± 0.1^{a}	5.3 ± 0.7^{bc}	3.0 ± 0.1^{d}	6.2 ± 0.2^{ab}
Glycine	5.5 ± 0.1^{ab}	5.2 ± 0.2^{b}	4.5 ± 0.2^{c}	6.0 ± 0.3^{a}	5.7 ± 0.1^{a}

Data are expressed as the mean ± standard deviation; values in the same row having different letters differ significantly ($p < 0.05$);

Table 6 Fatty acid profile (reported in g/100 g) of oil extracted from five garden cress seed genotypes.

Fatty acid attributes (g/100 g)	Garden cress seed genotypes				
	CG8	CG7	CG17	CG4	207910
Linolenic acid ($C_{18}H_{30}O_2$)	32.4 ± 0.3^{c}	32.7 ± 0.1^{c}	33.9 ± 0.1^{a}	33.3 ± 0.3^{b}	32.2 ± 0.1^{c}
Oleic acid ($C_{17}H_{33}COOH$)	28.9 ± 0.1^{c}	29.9 ± 0.1^{a}	30.1 ± 0.1^{a}	29.3 ± 0.1^{b}	29.3 ± 0.1^{b}
Eicosanoic acid ($C_{20}H_{38}O_2$)	12.8 ± 0.4^{a}	11.3 ± 0.1^{c}	9.2 ± 0.1^{d}	13.0 ± 0.1^{a}	12.2 ± 0.1^{b}
Linoleic acid ($C_{17}H_{31}COOH$)	8.6 ± 0.5^{c}	10.1 ± 0.1^{a}	10.4 ± 0.1^{a}	9.4 ± 0.1^{b}	9.3 ± 0.1^{b}
Palmitic acid ($C_{15}H_{31}COOH$)	9.7 ± 0.4^{a}	9.9 ± 0.1^{a}	8.7 ± 0.1^{c}	8.4 ± 0.1^{c}	9.2 ± 0.1^{b}
Arachidonic acid ($C_{19}H_{31}COOH$)	3.3 ± 0.3^{b}	3.3 ± 0.1^{b}	4.0 ± 0.2^{a}	2.5 ± 0.1^{c}	3.2 ± 0.1^{b}
Stearic acid ($C_{17}H_{35}COOH$)	3.1 ± 0.1^{a}	2.0 ± 0.0^{b}	2.1 ± 0.1^{b}	3.2 ± 0.1^{a}	3.0 ± 0.0^{a}
Palmitoleic acid ($C_{15}H_{29}COOH$)	0.9 ± 0.1^{a}	0.4 ± 0.0^{b}	1.0 ± 0.1^{b}	0.4 ± 0.1^{a}	0.5 ± 0.0^{b}
Myristic acid ($C_{14}H_{28}O_2$)	0.2 ± 0.0^{b}	0.4 ± 0.1^{a}	0.4 ± 0.4^{a}	0.2 ± 0.0^{b}	0.2 ± 0.1^{b}

Data are expressed as the mean ± standard deviation; values in the same row having different letters differ significantly ($p < 0.05$).

In fact, the presence of arachidonic acid ($C_{19}H_{31}COOH$), stearic acid ($C_{17}H_{35}COOH$), palmitoleic acid ($C_{15}H_{29}COOH$), palmitic acid ($C_{15}H_{31}COOH$), linoleic ($C_{17}H_{31}COOH$) and oleic acid ($C_{17}H_{33}COOH$) acids in the garden cress seed oil herein makes this product very promising for the cosmetic industry[46, 47]. Extracted oils with high concentration of oleic and linoleic acids would help in keeping the skin calm, nourished and moisturized[46]. Additionally, the abundance of such fatty acids helps to stimulate hair growth, maintain healthy scalp conditions, and control water loss from hair to keep it soft and pliable[48].

In conclusion, the garden cress seeds would be of benefi-

cial health resource given its demonstrated considerable nutrients. Moreover, despite the obtained differing peak and least proximate aspects of seeds, the (extracted) garden cress seed oil produced promising extraction yield, kinematic viscosity, refractive index, specific gravity, pH, and boiling point, lipid breakdown, β-carotenoids, tocopherols, and vitamins, as well as amino/fatty acid profiles. Differences in various tested parameters that occurred across garden cress seed genotypes could be due to such factors like cultivation methods, soil types, environmental conditions, and genotypic aspects. Additionally, the presence of arachidonic, stearic, palmitoleic, palmitic, linolenic and oleic acids in the garden cress seed oil of this study suggests it with high promise for the cosmetic industry.

Author Contributions

The authors' contributions are as follows: A.T.: sample preparation and performing analyses, contribution to the design of the work, preparation of the manuscript draft, interpretation of data, formatting of the tables; B.D.: contribution to the design of the work, preparation of the manuscript draft, interpretation of data, formatting of the tables, revising of manuscript; C.S.N.: preparation of the manuscript draft, interpretation of data, revising of manuscript; Author C.O.R.O: preparation of the figure, manuscript formatting, revising and final manuscript check, manuscript submission, final manuscript check, contribution to the design of the work, improving text and scientific quality. All authors read and approved the final manuscript.

Conflict of Interest

The authors declare there are no conflicts of interest.

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References

1) Diwakar, B.T.; Dutta, P.K.; Lokesh, B.R.; Naidu, K.A. Physicochemical properties of garden cress (*Lepidium sativum* L.) seed oil. *J. Am. Oil Chem. Soc.* **87**, 539-548 (2010).
2) Singh, C.S.; Paswan, V.K. The potential of garden cress (*Lepidium sativum* L.) seeds for development of functional foods. *Adv. Seed Biol.* (2017). doi:10.5772/intechopen.70355
3) Jabeen, A.; Rani, S.; Ibrahim, M.; Mohammad, A.S. A review on *Lepidium sativum*. *Indo Am. J. Pharm. Sci.* **4**, 2223-2227 (2017).
4) El-Haggar, M.; El-Hosseiny, L.; Ghazy, N.M.; El-Fiky, F.K.; El-Hawiet, A. Phytochemical investigation, antimicrobial and cytotoxic activities of suspension cultures of *Lepidium sativum* L. *South African J. Bot.* **138**, 500-505 (2021).
5) Gawlik-Dziki, U. Changes in the antioxidant activities of vegetables as a consequence of interactions between active compounds. *J. Funct. Foods* **4**, 872-882 (2012).
6) Bhuiyan, M.; Shams-Ud-Din, M.; Islam, M. Development of functional beverage based on taste preference. *J. Environ. Sci. Nat. Resour.* **5**, 83-87 (2012).
7) Murphy, D. Engineering oil production in rapeseed and other oil crops. *TIBTECH* **14**, 206-213 (1996).
8) Carlsson, A. Plant oils as feedstock alternatives to petroleum – A short survey of potential oil crop platforms. *Biochimie* **91**, 665-670 (2009).
9) Attia, Z.; Pogoda, C.S.; Reinert, S.; Kane, N.C. Breeding for sustainable oilseed crop yield and quality in a changing climate. *Theor. Appl. Genet.* **134**, 1817-1827 (2021).
10) Danlami, J.M.; Arsad, A.; Zaini, M.A.A.; Sulaiman, H. A comparative study of various oil extraction techniques from plants. *Rev. Chem. Eng.* **30**, 605-626 (2014).
11) Luque de Castro, M.D.L.; Priego-Capote, F. Soxhlet extraction: Past and present panacea. *J. Chromatogr. A* **1217**, 2383-2389 (2010).
12) Suwari; Kotta, H.Z.; Buang, Y. Optimization of soxhlet extraction and physicochemical analysis of crop oil from seed kernel of Feun Kase (*Thevetia peruviana*). *AiP Conf. Proceed* **1911**, 020005 (2017).
13) Hu, B.; Xi, X.; Li, H.; Qin, Y.; Li, C. *et al.* A comparison of extraction yield, quality and thermal properties from *Sapindus mukorossi* seed oil between microwave assisted extraction and Soxhlet extraction. *Ind. Crops Prod.* **161**, 113185 (2021).
14) Pereira, M.G.; Hamerski, F.; Andrade, E.F.; Scheer, A. de P.; Corazza, M. Assessment of subcritical propane,

ultrasound-assisted and Soxhlet extraction of oil from sweet passion fruit (*Passiflora alata* Curtis) seeds. *J. Supercrit. Fluids* **128**, 338-348 (2017).
15) Roschat, W.; Phewphong, S.; Pholsupho, P.; Namwongsa, K.; Wongkaa, P.; Moonsin, P.; Yoosuk, B.; Promarak, V. The synthesis of a high-quality biodiesel product derived from Krabok (*Irvingia malayana*) seed oil as a new raw material of Thailand. *Fuel* **308**, 122009 (2022).
16) Mohamed Ahmed, I.A.; Özcan, M.M.; AlJuhaimi, F.; Ghafoor, K.; Babiker, E.E. *et al.* Quality characteristics of caper seed oils the impact of extraction: Soxhlet versus cold pressing. *J. Food Process. Preserv.* **45**, e15266 (2021).
17) Tesfaye, B.; Tefera, T. Extraction of essential oil from neem seed by using Soxhlet extraction methods. *Int. J. Adv. Eng. Manag. Sci.* **3**, 646-650 (2017).
18) Al Juhaimi, F.; Özcan, M.M. Effect of cold press and soxhlet extraction systems on fatty acid, tocopherol contents, and phenolic compounds of various grape seed oils. *J. Food Process. Preserv.* **42**, e13417 (2018).
19) Azevedo, A.Q.P.L.; Juchen, P.T.; Hamerski, F.; Ramos, L.P.; Santos, A.F.; Corazza, M. Corn germ oil extraction with compressed propane compared with Soxhlet extraction. *Brazilian J. Chem. Eng.* (2021). doi:10.1007/s43153-021-00184-5
20) Schuten, H.; van Gijssel, J.; Slotboom, E. Effect of frying conditions on the fat content of French fries. Ref. Nr. OPD 03/336 (2004).
21) AOAC *Official Methods of Analysis*. 17th ed. The Association of Official Analytical Chemists, Gaithersburg, MD, USA (2000).
22) Amin, M.Z.; Islam, T.; Mostofa, F.; Uddin, M.J.; Rahman, M.; Satter, M.A. Comparative assessment of the physicochemical and biochemical properties of native and hybrid varieties of pumpkin seed and seed oil (*Cucurbita maxima* Linn.). *Heliyon* **5**, e02994 (2019).
23) Ogungbenle, H.N.; Afolayan, M.F. Physical and chemical characterization of roasted cashew nut (*Anacardium occidentale*) flour and oil. *Int. J. Food Sci. Nutr. Eng.* **5** (1), 1-7 (2015).
24) Boukhatem, M.N.; Amine, F.M.; Kameli, A.; Saidi, F.; Walid, K.; Mohamed, S.B. Quality assessment of the essential oil from *Eucalyptus globulus* Labill of Blida (Algeria) origin. *Int. Lett. Chem. Phys. Astron.* **17**, 303-315 (2014).
25) Gokavi, S.S.; Malleshi, N.G.; Guo, M. Chemical composition of garden cress (Lepidium sativum) seeds and its fractions and use of bran as a functional ingredient. *Plant Foods Hum. Nutr.* **59** (3), 105-111 (2004).
26) Kamel, B.S.; Kakuda, Y. Characterization of the seed oil and meal from apricot, cherry, nectarine, peach and plum. *J. Am. Oil Chem. Soc.* **69**, 492-494 (1992).
27) Ingale, S.; Shrivastava, S.K. Amino acid profile of some new varieties of oil seeds. *Adv. J. Food Sci. Technol.* **3**, 111-115 (2011).
28) Deaker, R.; Hartley, E.; Gemell, G. Conditions affecting shelf-life of inoculated legume seed. *Agriculture* **2**, 38-51 (2012).
29) Zia-Ul-Haq, M.; Ahmad, S.; Calani, L.; Mazzeo, T.; Del Rio, D. *et al.* Compositional study and antioxidant potential of *Ipomoea hederacea* Jacq. and *Lepidium sativum* L. seeds. *Molecules* **12**, 10306-10321 (2012).
30) Al-Jasass, F.M.; Al-Jasser, M.S. Chemical composition and fatty acid content of some spices and herbs under Saudi Arabia conditions. *Sci. World J.* 859892 (2012).
31) Tessema, S.S. Physicochemical characterization and evaluation of castor oil (*R. communis*) for hair biocosmetics. *Am. J. Appl. Chem.* **7** (4), 110-115 (2019).
32) Kolhe, S.; Indore, P.; Shewale, S.; Pol, A.; Hanmante, L. Formulation and evaluation of polyherbal hair oil for alopecia. *J. Drug Deliv. Ther.* **9**, 387-390 (2019).
33) Saeed, M.; Shola, E. Extraction and physicochemical properties of some edible seed oils sampled in Kano metropolis, Kano state. *Bayero J. Pure Appl. Sci.* **8**, 239-244 (2015).
34) Ouilly, J.T.; Bazongo, P.; Bougma, A.; Kaboré, N.; Lykke, A.M. *et al.* Chemical composition, physicochemical characteristics, and nutritional value of *Lannea kerstingii* seeds and seed oil. *J. Anal. Methods Chem.* **2017**, 2840718 (2017).
35) Odoom, W.; Edusei, V.O. Evaluation of saponification value, iodine value and insoluble impurities in coconut oils from Jomoro District of the Western Region of Ghana. *Asian J. Agric. Food Sci.* **3**, 494-499 (2015).
36) Sat, I.G.; Yildirim, E.; Turan, M.; Demirbas, M. Antioxidant and nutritional characteristics of garden cress (*Lepidium sativum*). *Acta Sci. Pol. Cultus.* **12**, 173-179 (2013).
37) Al-Niaimi, F.; Chiang, N.Y.Z. Topical vitamin C and the skin: Mechanisms of action and clinical applications. *J. Clin. Aesthet. Dermatol.* **10** (7), 14-17 (2017).
38) Dattola, A.; Silvestri, M.; Bennardo, L.; Passante, M.; Scali, E. *et al.* Role of vitamins in skin health: A systematic review. *Curr. Nutr. Rep.* **9**, 226-235 (2020).
39) Pullar, J.M.; Carr, A.C.; Vissers, M. The roles of vitamin C in skin health. *Nutrients* **9**, 866 (2017).
40) Stuetz, W.; Schlörmann, W.; Glei, M. B-vitamins, carotenoids and α-/γ-tocopherol in raw and roasted nuts. *Food Chem.* **221**, 222-227 (2017).
41) Almohanna, H.M.; Ahmed, A.A.; Tsatalis, J.P.; Tosti, A. The role of vitamins and minerals in hair loss: A review. *Dermatol. Ther.* (*Heidelb*), **9**, 51-70 (2019).
42) Moser, B.R.; Shah, S.N.; Winkler-Moser, J.K.; Vaughn, S.F.; Evangelista, R.L. Composition and physical properties of cress (*Lepidium sativum* L.) and field pennycress (*Thlaspi arvense* L.) oils. *Ind. Crops Prod.*

30, 199-205(2009).
43) Nergiz, C.; Ötleş, S. Chemical composition of *Nigella sativa* L. seeds. *Food Chem.* **48**, 259-261(1993).
44) Sultan, M.T.; Butt, M.S.; Anjum, F.M.; Jamil, A.; Akhtar, S.; Nasir, M. Nutritional profile of indigenous cultivar of black cumin seeds and antioxidant potential of its fixed and essential oil. *Pak. J. Bot.* **41**, 1321-1330 (2009).
45) Tulukcu, E. A comparative study on fatty acid composition of black cumin obtained from different regions of Turkey, Iran and Syria. *African J. Agric. Res.* **6**, 892-895(2011).
46) Amjad Khan, W.; Chun-Mei, H.; Khan, N.; Iqbal, A.; Lyu, S.-W.; Shah, F. Bioengineered plants can be a useful source of omega-3 fatty acids. *Biomed Res. Int.* **2017**, 7348919(2017). doi:10.1155/2017/7348919
47) Yang, M.; Zhou, M.; Song, L. A review of fatty acids influencing skin condition. *J. Cosmet. Dermatol.* **19**, 3199-3204(2020).
48) Choi, J.-S.; Jeon, M.-H.; Moon, W.-S.; Moon, J.-N.; Cheon, E.J. *et al. In vivo* hair growth-promoting effect of rice bran extract prepared by supercritical carbon dioxide fluid. *Biol. Pharm. Bull.* **37**, 44-53(2014).