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An Assessment of *in vitro* Antioxidant Potential of *Camelina sativa L.* Seed Oil and Estimation of Tocopherol Content using HPTLC Method

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Abstract

The present study was aimed to analyze the physicochemical properties of *Camelina sativa L*. seed oil in order to identify its utilization as a primary feedstock for biofuel production. Efforts were also made to identify and quantify the amount of α- tocopherols in Camelina seed oil and evaluated *in vitro* antioxidant potential of *Camelina sativa* seed oil and were compared with *α*–tocopherol standard. Physicochemical properties such as oil yield content (36.66 %), less acid value (5.39 mg KOH/g) make it a prominent feedstock for biodiesel production. Saponification value (182.66 mg KOH/g) also makes this oil useful in soap and cosmetic industries. To check *in vitro* antioxidant potential of Camelina seed oil H_2O_2, DPPH and ABTS were used as free radical inducers. Oil showed remarkable inhibition potential of trapping these free radicals. Tocopherol content was analyzed through HPTLC. *Camelina sativa* seed oil was found to contain 59.34 mg/100 g of tocopherol content. It is evident from this study that Camelina oil has high antioxidant potential and there is no need to add other antioxidant substances in the products formed by using *Camelina sativa* seed oil.

Keywords: *Camelina sativa*; Biodiesel; Acid Value; HPTLC; Free radicals.

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1. Introduction

Brassicaceae family is one of the most important families in the entire plant kingdom in terms of its diverse nature and agro-economic values. This angiosperm family has vast distribution across the globe with 338 genera and nearly 3700 species [1]. Many of these species have been consumed by people as vegetables and oils from the ancient time. It includes significant seed oil species, for example, canola, mustard, Crambe and vegetables

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like cauliflower, cabbage, radish, turnip and broccoli and so forth [2]. There is one more ancient oil seeded species belongs to this family i.e. *Camelina sativa*, (L.) Crantz is a short seasoned annual crop, which can thrive in any environment even under very harsh conditions with the low water consumption. This plant does not require any nutrient from outside and can be intercropped between the plants as well [3]. Addition to this, oil yield quantity of Camelina seeds is much higher almost 32-38 % which shows its efficacy as an oilseed crop [4]. Considering all these characteristics, Camelina crop can be utilized as a prominent source of biofuel which reduces the agro-economic inputs as well as offers some extraordinary environment benefits.

Camelina seed oil (CSO) contains high unsaturation in its moiety due to which it may be utilized as a biodiesel feedstock, because higher unsaturation prompts to the lower viscosity. However, higher unsaturation index makes oils more susceptible to oxidative degradation or rancidity [5]. Rancidity is a chemical reaction caused due to the aerial oxidation of unsaturated fats which inversely affects the quality of fats and oils [6]. Rancidity effect is analyzed in terms of peroxide value (titrimetric analysis) and as well as using rancimat test which measures the oxidation stability of oil. Camelina oil contains higher unsaturation, so it is expected that it has low oxidation stability yet despite of that CSO showed high oxidation stability and peroxide value [7].

The stability of CSO against the oxidation reactions is mainly due to presence of various antioxidants such as sinapic acids, quercetin, glucosinolate etc. [8, 9, 10]. In our previous studies, it has been also observed that Camelina seeds contains various secondary metabolites such as phenolics, flavonoids, terpenes, tannins etc. [4,11]. These components trap the free radicals and end the chain reactions by the elimination of the free radical intermediates. Kumar *et al*. studied the antioxidant potential of Camelina seed polar solvent extracts and observed that extracts were showed remarkable antioxidant activity in terms of APX and GPX assay [12]. Camelina seed and meal extracts was also analyzed by Mierina *et al.,* in which peroxide value was used for the antioxidant determination [13]. In the present investigation, antioxidant activity of Soxhlet extracted CSO was studied in terms of H_2O_2, DPPH (2,2-Diphenyl-1-picrylhydrazyl) and ABTS (2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) assay.

CSO rich in tocopherols, which acts as an important antioxidant for reducing oxidation reactions. Tocopherols are the fat-soluble components and are also very essential for the seed storage and the germination as well [14]. Ratusz *et al.,* quantified the amount of tocopherol in CSO using high performance liquid chromatography (HPLC) techniques and concluded that it contains 55-76 mg/100 g of tocopherol [15]. Belayneh *et al*. estimated in their study that Camelina contains 760 mg/kg of tocopherol in the oil [16]. Present study was also aimed to analyze and quantify the tocopherol content present in CSO grown in Indian climatic conditions using a novel HPTLC technique. The comparison between the *in vitro* antioxidant potential of *Camelina sativa* seed oil and *α*-tocopherol standard was also studied.

2. Materials and Methods

2.1. *Materials*

Camelina seeds (Fig. 1) were collected from the DIBER field station Haldwani - Uttarakhand, India, where the plants were grown under natural environment. Collected seeds were washed and dried for 2 days in hot air oven (35 °C) followed by grinding in a mixture grinder and sealed in a closed glass chamber for further processing. Hexane, methanol and chloroform (SRL, India), H_2O_2, DPPH, ABTS and α- tocopherol standard (Merck, Germany) were used without further purification.

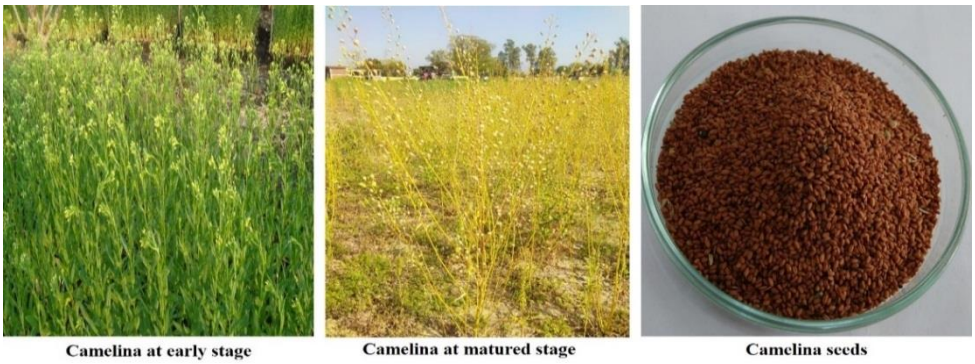

Fig. 1. Digital images of *Camelina sativa* plant grown at DIBER Field Station, Haldwani.

2.2. *Extraction of oil*

50 g of the seed powder was accurately weighed and filled inside a manually prepared thimble (Whatman paper 00) and extraction was done via Soxhlet apparatus using hexane solvent, after completion of three cycles oil was collected in a round bottom flask and excess of solvent was recovered using Rota evaporator (IKA RV10, Germany) and stored at room temperature for further analyses.

2.3. *Physicochemical Properties of the oil*

A fresh sample of Camelina oil was analyzed for the different physicochemical properties such as moisture content, saponification value, acid value, iodine value etc. Moisture content was analyzed using Halogen Moisture Analyzer (Mettler TOLEDO HE 53, United States), density was measured using Density/Specific Gravity Meter (DA- 130N, Kyoto electronics, Japan) and the Refractive Index (RI) was calculated using Refractometer (RA – 600, Kyoto electronics, Japan). All other properties were analyzed using the method described by IS: 548 (Part 1) [17].

2.4. *Antioxidant assay*

2.4.1. *H_2O_2 radical scavenging activity*

Camelina oil sample was analyzed using the hydrogen peroxide as a free radical generator, 1.0 mg of oil sample and reference (α- tocopherol) was dissolved in the methanol to prepare 1.0 mg/mL of stock solution, after that samples and reference (0 to 50 µL) were added to 2.0 mL of H_2O_2 (43 mM of 30 % H_2O_2) mixture was vortexed well and then 2.4 mL of 0.1 M phosphate buffer (pH 7.4) was added to it. This mixture was incubated in a closed chamber at 28 °C for 10 min and then absorbance (abs) was recorded at 230 nm.

2.4.2. *DPPH radical scavenging activity*

In this method, 2 mL of DPPH solution was added in sample and reference (0 to 50 µL) and volume was adjusted to 1.0 mL by adding the respective solvent (i.e. methanol in our study), mixture was incubated in a closed chamber for 40 min and absorbance was taken at 517 nm.

2.4.3. *ABTS + radical cation scavenging activity*

ABTS (7 mM) was dissolved with $K_2S_2O_8$ (2.45 mM) in equal proportions. This mixture was kept in dark for overnight. The mixture was further vortexed and diluted with methanol until the absorbance of 0.700 (±0.002) was achieved at 734 nm. After achieving the desired absorbance, 1.0 mL of the solution was added to the sample and reference (0 to 50 µL) and kept the mixture in dark for 10 min, finally, absorbance was taken at 734 nm.
FRSA for all three methods were calculated using the formula : -

$$FRSA\ (\%) = [(A_0 - A_1) / A_0] \times 100$$

2.5. *HPTLC instrumentation and procedure*

For quantifying the tocopherol content, HPTLC (CAMAG, Switzerland) instrument was used at DIBER laboratory Haldwani, India. 10 mg of Camelina oil dissolved in 10 mL of methanol to form 1mg/mL of the solution, shaked well and centrifuged (Eppendorf 5330 R, Germany) at 3000 rpm (25 °C) for 5 min. 1.0 mg of α-tocopherol was dissolved in 1.0 mL of methanol and was used as a reference.

A pre coated silica gel aluminum plate (60F- 254, Make: Merck, Germany) was used as a stationary phase. Before, spotting the samples, plate was washed with methanol in TLC Chamber (TTC 20X10) and further activated at 60 °C for 10 min. Samples were loaded in the form of bands (width 8 mm) with the help of Camag µL syringe injector

using LINOMAT V at the speed of 0.15 µL/sec with the help of nitrogen. First track position was set at 15 mm and band gap among the spots were 18.4 mm. TLC chromatogram was developed in ascending order in twin through glass chamber of 20 × 10 mm using the mobile solvent chloroform. The solvent was run up to 90 mm to the plate. Developed Chromatographic plate was dried in TLC spray Cabinet. After complete drying of the plate, densitometric analysis (TLC Scanner) and photo documentation (TLC visualizer) were done at the wavelength of 254 - 336 nm (U-V range) using Deuterium and Tungsten lamp. Programming and documentation of HPTLC was operated on Vison CATS 2.5 software. Whole HPTLC procedure was operated under standard laboratory conditions {(25 ± 2 ºC), RH 50 ± 5 % (MEXTECH TM-2)}.

2.6. *Calibration curve of α-tocopherol*

1 mg/mL solution of tocopherol was prepared in methanol as a standard. 1 to 6 µL of this solution was spotted on to the plate and the obtained calibration curve was validated by the coefficient of variation (CV %) and correlation coefficient (R %) values.

2.7. LOD and LOQ determination

To validate the performed experiment, LOD (Limit of Detection) and LOQ (Limit of Quantification) were also measured, these are the performance parameter that are related to the ability of the method to detect, identify and quantify low analyte levels in sample. LOD is the lowest concentration of the analyte that can be distinguished from zero. LOQ is the lowest concentration at which analyte can be quantified with an acceptable repeatability. Both values were calculated by the following formula:

$$LOD = \frac{3.3 \times \sigma}{S}\ ;\ LOQ = \frac{10 \times \sigma}{S}$$

Where, σ is the standard deviation and S is the slope of the related calibration curve.

2.8. *Statistical analysis*

All the treatments were carried out in triplicates and entered in MS excel 2016 and data were exported to SPSS 16.0 software for statistical analysis, mean values and standard deviation. One-way ANOVA was applied among the different means and was compared by the least significant difference (LSD) test at a significance level of $p \leq 0.05$.

3. Results and Discussion

3.1. *Physicochemical properties of oil*

All the analyzed physicochemical properties of CSO are given in Table 1, observed oil percentage of Camelina oil was 36.67 % (± 0.252) which exhibits that Camelina seeds have high oil yield content than many other oilseed crops. Moisture content is the primary test for processing the food or oil. Observed mean moisture percentage was 0.0616 (± 0.003), high moisture content indicated about the sensitivity of the oil towards the oxidative degradation. In the present study, efforts were done to keep the moisture percentage as low as was possible for the preservation of the oil.

Acid value of the CSO was 5.396 (± 0.012), this value indicates about the fatty acid formed due the decomposition of triglyceride molecules. Observed saponification value of the CSO was 182.66 (± 0.472), SV indicates about the number of saponifiable components present in oil, higher the SV, more soap is expected from the particular oil. It provides a basic idea about the applicability of the oil for the formation of soaps and cosmetic products. The iodine value of the CSO was 153.300 (± 0.458), this value is the measure of the unsaturation present in the oil or fat. Analyzed value showed the presence of higher unsaturation in Camelina oil, due to which the oil is light and of lower viscosity. But, higher unsaturation also prompts to the free radical reactions which adversely affects the quality of oil. However, some antioxidants such as tocopherols lowers these free radical reactions and makes oil more stable.

Table 1. Physicochemical properties of *Camelina sativa* seed oil.

Sr No.	Properties	Unit	Observed Values
1.	Color	-	Mustard yellow
2.	Odor	-	Pungent
3.	Oil Yield	%	36.667 (± 0.252)
4.	Moisture	%	0.0616 (± 0.003)
5.	Density	g/cm^3	0.926 (± 0.028)
6.	Refractive Index	-	1.424 (± 0.0036)
7.	Acid Value	mg KOH/g	5.396 (± 0.012)
8.	FFA (In terms of oleic acid)	%	2.698 (± 0.0104)
9.	Saponification Value	mg KOH/g	182.660 (± 0.472)
10.	Iodine Value	gI_2 / 100g	153.300 (± 0.458)

*Each value is expressed as mean ± standard deviation (n=3)

3.2. *Antioxidant potential of Camelina seed oil*

Oxidation of the lipids is the main cause for the oil deterioration. Oxidation leads to the free radicals due to which oil becomes rancid and unsuitable for the long term uses. Natural antioxidants play crucial role to prevent these undesirable chemical reactions and stop the excess formation of free fatty acids in the oil. In the present investigation, the antioxidant potential was evaluated using three different methods i.e. H_2O_2, DPPH and ABTS methods.

H_2O_2, DPPH and ABTS assays are the most extensively utilized methods for observing the antioxidant capacity of any substance, biomolecule or plant extract. All these three

works as free radical generators as shown in their graph and their color shows a characteristic peak at 234, 517 and 734 nm, respectively.

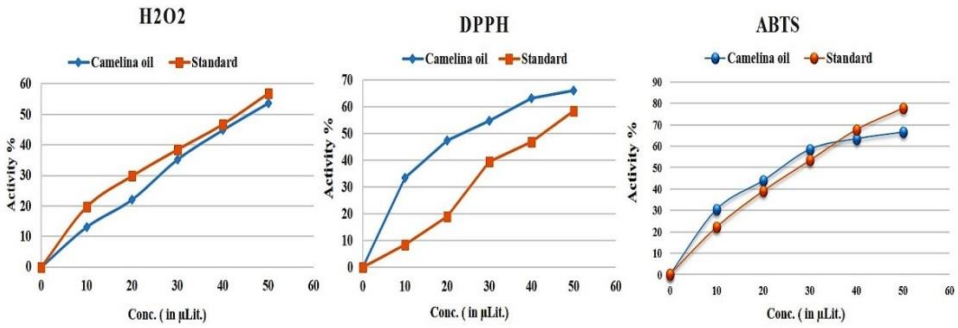

Fig. 2. Inhibition percentage of Camelina oil and α-tocopherol at different concentrations.

Table 2. FRSA Activity (H_2O_2 method).

Sr No	Conc. (µL)	Scavenging Activity % (Oil)	Scavenging Activity % (Reference)
1	10	13.100 ± 0.218	19.723 ± 0.126
2	20	22.125 ± 0.455	29.840 ± 0.630
3	30	35.226 ± 0.334	38.501 ± 0.252
4	40	44.905 ± 0.827	46.798 ± 0.126
5	50	53.712 ± 0.378	56.914 ± 0.455
	MSE	0.398	0.305

*Each value expressed as mean ± standard deviation (n=3); MSE: Mean Standard Error
*The mean difference is significant at the 0.05 level

Table 3. FRSA Activity (DPPH method).

Sr No	Conc. (µL)	Scavenging activity % (Oil)	Scavenging activity % (Reference)
1	10	33.426 ± 0.283	8.437 ± 0.240
2	20	47.487 ± 0.160	18.903 ± 0.319
3	30	54.726 ± 0.080	39.604 ± 0.319
4	40	63.117 ± 0.080	46.934 ± 0.211
5	50	66.252 ± 0.138	58.552 ± 0.160
	MSE	0.184	0.210

*Each value expressed as mean ± standard deviation (n=3); MSE: Mean Standard Error
*The mean difference is significant at the 0.05 level

Table 4. FRSA Activity (ABTS method).

Sr No	Conc. (µL)	Scavenging activity % (Oil)	Scavenging activity % (Reference)
1	10	30.440 ± 0.225	22.135± 0.172
2	20	43.931 ± 0.130	39.046± 0.427
3	30	58.549 ± 0.172	53.363± 0.235
4	40	63.472 ± 0.195	67.757± 0.298
5	50	66.554 ± 0.260	77.790± 0.113
	MSE	0.165	0.222

*Each value expressed as mean ± standard deviation (n=3); MSE: Mean Standard Error
*The mean difference is significant at the 0.05 level

When the biomolecule or compound bound these free radicals, the color of these assays turn out to be lighter. This color change is considered as a positive result and indicates that substance or biomolecules have antioxidant capabilities. In all three FRSA, Camelina oil showed remarkable percentage inhibition (Fig. 3). In case of H_2O_2 at lower concentration (10 µL), *α*-tocopherol showed high efficacy than the Camelina oil, but as the concentration increased oil showed almost equal activity to trap the free radicals (Table 2). When DPPH was used as a stable free radical, Camelina oil showed remarkable percentage inhibition than the *α*-tocopherol at lower as well as at higher concentration (Table 3). Free radicals when generated by ABTS method, *α*-tocopherol worked more effectively and showed higher percentage of inhibition (Table 4) than the Camelina oil, however, Camelina oil also showed a significant inhibition percentage against the ABTS scavenging activity. Antioxidant activity of Camelina seed extract was also studied by Rahman *et al*. In this study, they also observed an extraoridanry antioxidant capabilities of Camelina seed extracts in terms of trolox equivalent antioxidant capacity (TEAC) 14.11 µmole TE/g and metal ion chelation activity 37.5 µmole EDTA Eq/g [18]. These values strongly support that Camelina has a remarkable antioxidant potential. The obtained FRSA activities also suggest that CSO contains many effective antioxidants for trapping these free radicals generated from H_2O_2, DPPH and ABTS assay.

3.2. *Quantification of tocopherol by HPTLC method*

3.2.1. *Extracting solvent selection*

For quantification and isolation of tocopherols, hexane was used as an extracting solvent because tocopherols are the fat-soluble compounds and may be present in the lipid content of the Camelina seeds. This extract contains many fat-soluble vitamins and components including *α*-tocopherol.

3.2.2. *Chromatogram development*

For mobile phase, different solvents were studied as previously reported in the literature, however, appropriate chromatogram was obtained only when chloroform was used as a single mobile solvent. After saturating the chamber for 20 min, dried spotted plate was placed into the chamber and run the chromatogram till the solvent reached the 90 mm position followed by drying for the imaging purpose. There were no spots seen in white light, but at U-V light clear spots were visible for the standard and the oil. At the R_f value of 0.61 there was clear band visible in the Camelina oil at the same R_f value of *α*-tocopherol, that confirmed the presence of tocopherol in the Camelina oil (Fig. 3).

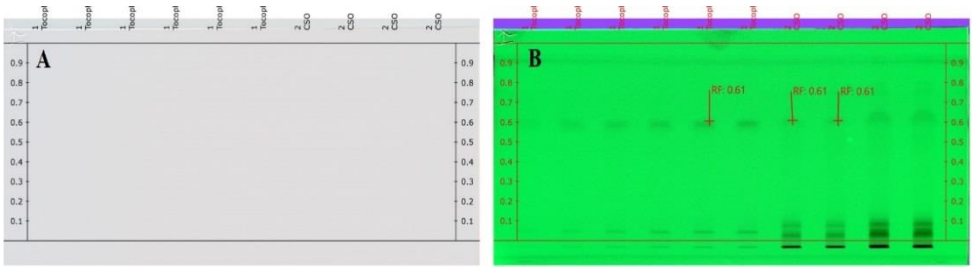

Fig. 3. Chromatographic plate at white light (A); at UV light (B).

3.2.3. *Scanning of the plate*

Scanned images of standard and oil extract showed a characteristic band at the R_f value of 0.59 to 0.61 (Table 5). Spectrum scanning of the plate further confirm the strong absorbance of the *α*-tocopherol at the wavelength of 290 nm (Fig. 4). R_f values of the oil confirm the presence of tocopherol in the oil (Fig. 5). After the confirmation, the quantity was determined by using the standard curve.

Table 5. Table showing R_f peak of reference and Camelina oil for tocopherol.

Sr. No.	Track	Sample	R_f Value			
			Starts at	Peak Point	Max	Height (AU)
1	1	*α*-Tocopherol (Reference)	0.551	0.600	0.632	0.1244
2	3	*α*-Tocopherol (Reference)	0.551	0.598	0.639	0.3036
3	5	*α*-Tocopherol (Reference)	0.551	0.601	0.645	0.4084
4	7	*α*-Tocopherol (Sample)	0.569	0.615	0.651	0.2220
5	9	*α*-Tocopherol (Sample)	0.575	0.635	0.675	0.2495

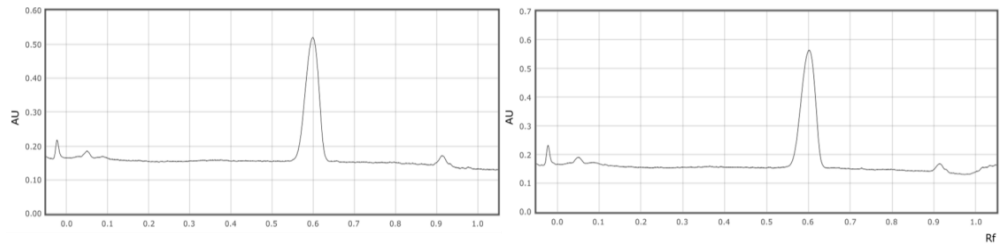

Fig. 4. Chromatogram showing two different peaks of α tocopherol standard at different tracks with different concentrations.

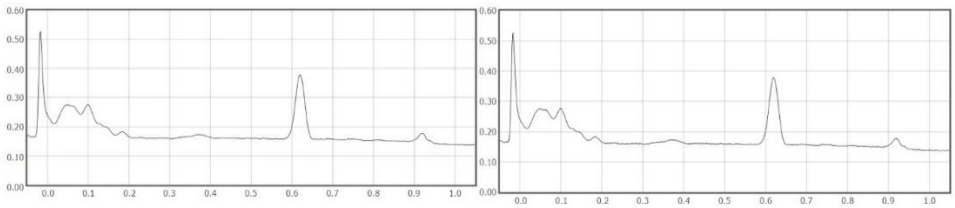

Fig 5: Chromatogram showing two different peaks of extract at different tracks with different concentrations.

3.2.4. *Quantification and validation of α*-tocopherol

The quantity of *α*-tocopherol was determined through statistical analyses. Calibration curve (Fig. 6) was plotted over a concentration range of 100-600 ng/spot. Calibration curve was developed by plotting peak area versus concentration (n = 6) with the help of vison cat software.

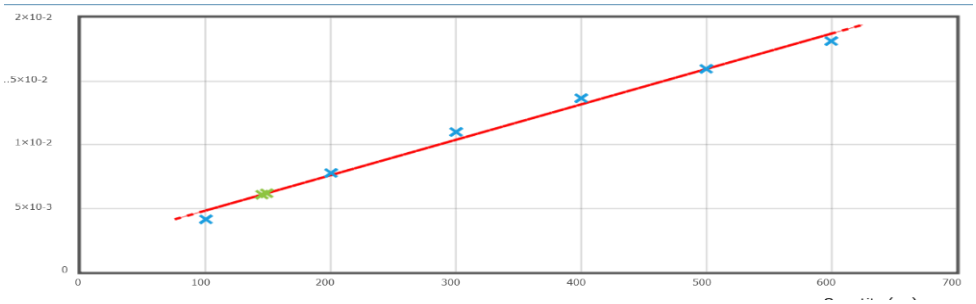

Fig. 6. Calibration curve of tocopherol.

Table 6. Validation parameters for alpha tocopherol estimation.

Parameters	Results
Correlation Coefficient	0.994
Linearity Range (ng)	100-600
Range deviation	5%
LOD (ng/Spot)	6.44
LOQ (ng/spot)	19.5

Summary of all validated parameters are listed in Table 6. Correlation coefficient value was 0.994 which validates and confirms that method is reproducible. *Camelina sativa* seed oil was found to contain 59.34 mg/100 g tocopherol content. LOD and LOQ were determined to be 6.44 and 19.5 ng/spot which indicate that the developed chromatographic method is accurate and satisfactory.

4. Conclusion

Physicochemical properties of *Camelina sativa* seed oil makes it a prominent feedstock for biodiesel production. The presence of the high amount of antioxidants also exhibits its high antioxidant potential which was confirmed in the present investigation via different radical scavenging methods. α-Tocopherol is generally used for increasing the oxidation stability of vegetable oil. Camelina oil itself has a high amount of α-tocopherol which is responsible for its high efficacy against the free radical inducers. So, it can be concluded from the study that Camelina oil can be used for the formulation of biodiesel as well as for other industrial applications. This study also gives a very rapid and easy method for identifying the tocopherol amount in vegetable oils using HPTLC technique.

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References

1. N. Anjum, S. S. Gill, I. Ahmad, M. Pacheco, A. C. Duarte, S. Umar, N. A. Khan, and M. E. Pereira, Environ. Pollut. 1–33. (2012).
2. M. Berti, R. Gesch, C. Eynck, J. Anderson, and S. Cermak, Ind. Crops Prod. **94**, 690 (2016).
3. D. H. Putnam, J. T. Budin, L. A. Field, and W. M. Breene, Camelina: A Promising Low-Input Oilseed, in New Crops, ed. J. Janick et al. (Wiley, New York, 1993) pp. 314-322. https://hort.purdue.edu/newcrop/proceedings1993/V2-314.htmL
4. K. Kumar and R. Pathak, Ind. J. Plant Physiol. **21**, 255 (2016). https://doi.org/10.1007/s40502-016-0223-6
5. P. Patil, V. Gude, and S. Deng. Ind. Eng. Chem. Res. **48**, 10850 (2009). https://doi.org/10.1021/ie901146c
6. L. Botella, F. Bimbela, L. Martan, J. Arauzo, and J. Sajnchez, Front. Chem. **2**, (2014). https://doi.org/10.3389/fchem.2014.00043
7. K. Ratusz, E. Popis, H. Ciemniewska-Zytkiewicz, and M. Wroniak, J. Therm. Anal. Calorim. **126**, 343 (2016). https://doi.org/10.1007/s10973-016-5642-0
8. P. Terpinc, T. Polak, D. Makuc, P. N. Ulrih, and H. Abramovic, Food Chem. **131**, 580 (2012). https://doi.org/10.1016/j.foodchem.2011.09.033
9. M. A Berhow, S. F. Vaughn, B. R Moser, D. Belenli, and U. Polat, Recent Adv. Phytochem. **44**, 129 (2014). https://doi.org/10.1007/978-3-319-04045-5_7
10. D. Kurasiak-Popowska and K. Stuper-Szablewska, Acta Agric. Scand. B Soil Plant Sci. **70**, 39 (2020). https://doi.org/10.1080/09064710.2019.1665706
11. R. Pathak, A. Kumari, M. Mohsin, G. Bisht, and M. Bala, AJRC **13**, 38 (2020). https://doi.org/10.5958/0974-4150.2020.00009.7
12. K. Kumar, S. M. Gupta, M. C. Arya, and M. Nasim, Proc. Natl. Acad. Sci. India Sect. B Biol. Sci. **87**, 521 (2017). https://doi.org/10.1007/s40011-015-0631-9
13. I. Mierina, L. Adere, K. Krasauska, E. Zoltnere, D. Z. Skrastina, and M. Jure, Proc. Latv. Acad. Sci. Sect. B Natl. Exact Appl. Sci. **71**, 515 (2017). https://doi.org/10.1515/prolas-2017-0089

14. S. Duncan and H. Chang, Adv. Food Nutr. Res. **67**, 25 (2012). https://doi.org/10.1016/B978-0-12-394598-3.00002-2
15. K. Ratusz, E. Symoniuk, M. Wroniak, and M. Rudzinska, Appl. Sci. **8**, 2606 (2018). https://doi.org/10.3390/app8122606
16. H. D. Belayneh, R. L. Wehling, Y. Zhang, and O. N. Ciftci, Food Sci. Nutr. **6**, 440 (2018). https://doi.org/10.1002/fsn3.572
17. Indian Standard Methods of Sampling and Test for Oils and Fats, IS: 548 (Part 1) 1964 Reaffirmed (2010).
18. M. J. Rahman, A. C. de Camargo, and F. Shahidi, Food Chem, **240**, 917 (2018). https://doi.org/10.1016/j.foodchem.2017.07.098