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Research Article

Rapid Quantitative Determination of Sinapic Acid in *Camelina sativa* L. Seed Cake using High Performance Thin Layer Chromatography

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ABSTRACT

A rapid and very accurate method of High performance thin layer chromatography was developed for the validation and quantification of Sinapic acid in methanolic extract of Camelina defatted seed cake. There are various HPLC methods have been reported previously for the identification and quantification of Sinapic acid in different extracts, but HPTLC provides an ideal confirmatory method which is cost effective and environmentally friendly as compared to HPLC. Methanolic extract of defatted cake was prepared using Soxhlet method. Analyses and quantification of sinapic acid was performed in pre coated aluminium sheet TLC plate (Merck, Silica gel F254) as stationary phase. Samples were sprayed onto TLC plate in the form of bands with Nitrogen (Linomat 5) at the speed of 150 nl/sec. Development of Chromatogram was carried out in twin through glass chamber saturated with the developing mobile solvent i.e. Ethyl acetate: Ethyl methyl ketone: Formic acid: water at room temperature (25˚C ± 2). Densitometric evaluation (TLC Scanner 4) of developed chromatogram was done at 254 -336 nm (U-V range) using Deuterium and Tungsten lamp. System identified a clear spot of Sinapic acid at the R_f value of 0.91 (± 0.02). Linear graph shows the correlation coefficient (R) was 99.9952 %. The result observed clearly showed the intense band of sinapic acid with the value of 250.4 µg/mg. Statistical analysis of the data showed that the method is reproducible and selective for estimation of sinapic acid and its derivatives.

Keywords: Camelina defatted seed, HPTLC, Sinapic acid, Chromatogram, Mobile solvents.

INTRODUCTION

Camelina sativa L. is very well-known plant from the family of Brassicaceae and best known for excellent oil content and capability of growing at any climatic condition[1]. Unsaturated fatty acid of the oil makes it a prominent source of biofuel. Owing to this, it is under the study of biofuel R&D; key advantage of this plant is that it is short seasoned crop with minimum requirement of nutrition and pesticides[2]. However, Camelina is not still well explored and some of its parts are not studied yet. It has various neutraceutical components such as Phytoalexins, sinapine, phenolic components, terpenes etc. Camelina seeds are highly rich with the phenolic compounds, these phenols are almost remains in the defatted part of the seed after oil extraction. In the present investigation, efforts are made to identify and quantify the amount of sinapic acid present in Camelina deoiled seed cake.

Sinapic acid is main polar phenolic component of mustard family and responsible for the high antioxidant potential of these oils[3]. It is the derivative of Cinammic acid generally found in ester form. It has many bioactive, antimicrobial[4], anticancer[5], anti-anxiety[6] and anti-inflammatory[7] activities. Decarboxy product of Sinapic acid i.e. vinyl syringol has very high antioxidant property by which it easily traps the free radicals which can cause harm to the cells of the organism also it acts as antimutagenic agent which down regulates oncogenesis as well as suppresses the induction of inflammatory cytokines[8]. Sinapine also act as an acetylcholinesterase inhibitor which shows high therapeutic values in alzheimer disease, ataxia and parkinson's disease treatment[9]. Sinapic acid and its derivatives can also be used in cosmetics, food preservations and in pharmaceutical applications[10]. There are several methods are reported for the identification and quantification of sinapic acid using HPLC methods and other techniques[11-12]. But in the best of the knowledge, there was no literature found about the method of HPTLC for quantification and identification of Sinapic acid.

High Performance Thin Layer Chromatography (HPTLC) is a unique technique which is based on the principle of adsorption chromatography. Mobile phase solvents are used on the basis of like dissolves like concept; also a particular standard method is prepared on the basis of some previously reported scientific literature[13]. This technique is the more extended form of thin layer chromatography so the selection of mobile solvents for validation of a particular compound becomes easy. After some trials of the solvents in different ratio, optimize condition obtained. Stationary phase is the plate of silica gel which gives higher resolution and easily quantifies using densitometer and standard curve. In this article, HPTLC method is validated for the quantitative determination of Sinapic acid in Camelina de fatted cake.

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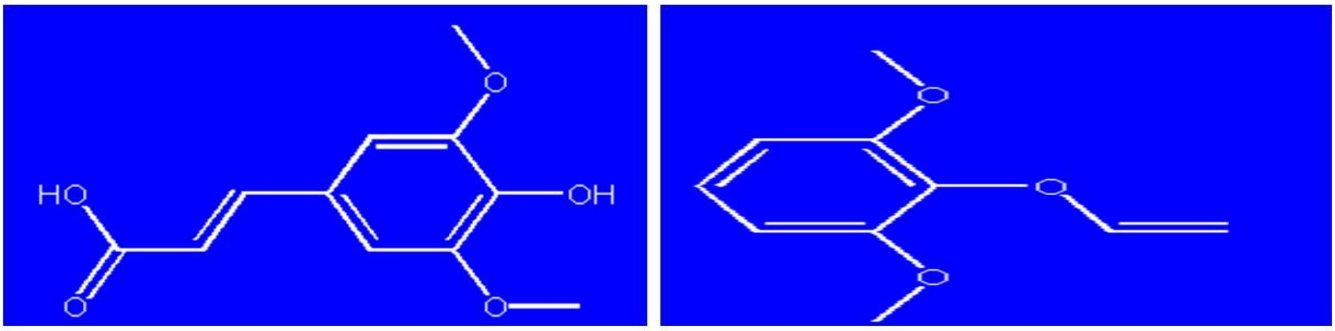
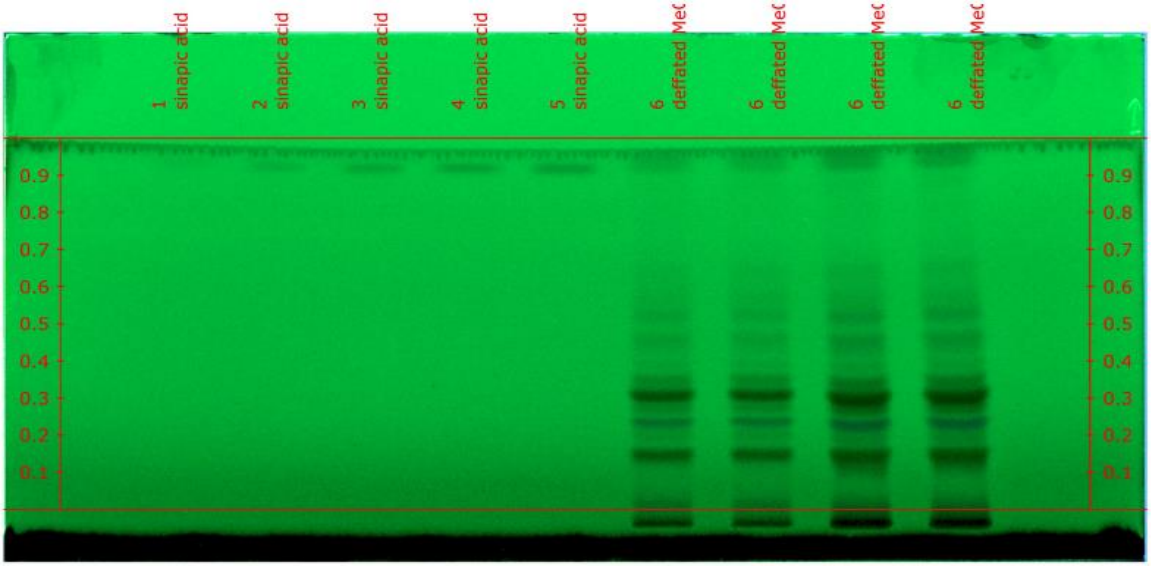


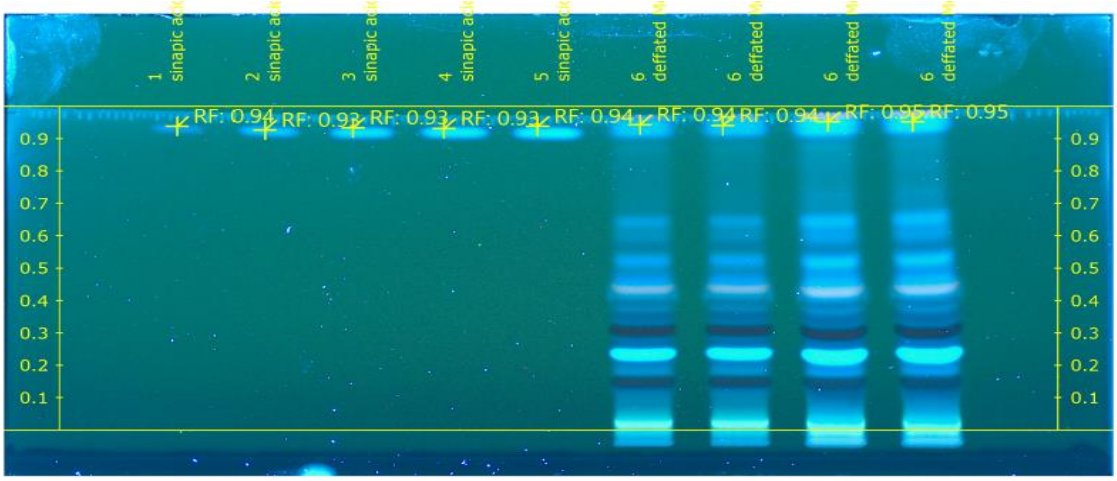
Sinapic acid **Vinyl Syringol**

Figure 1: Structures of Sinapic acid and Vinyl syringol (Chem draw ultra 7.2 (Software)).



Exposure	0.304 s
Contrast	1
Normalized exposure	Disabled
Clarify	Disabled
White balance	1.00, 1.00, 1.00

Figure 2: TLC Chromatogram at 254 nm.



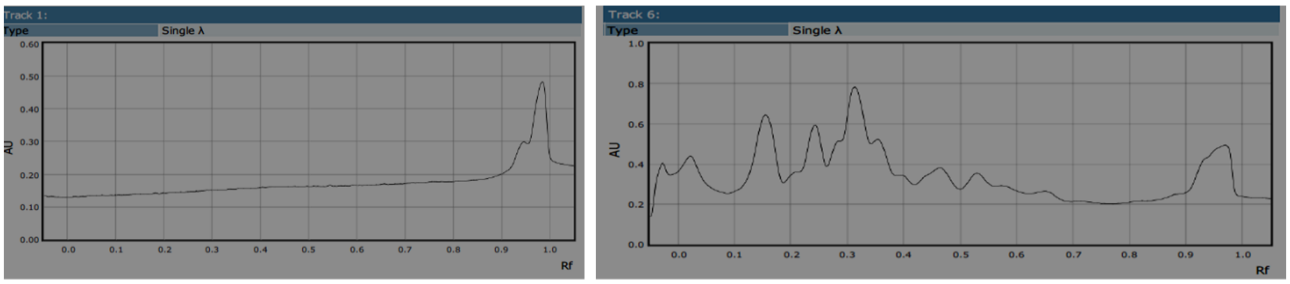
Exposure	2.922 s
Contrast	1
Normalized exposure	Enabled
Clarify	Disabled
White balance	1.00, 1.00, 1.00

Figure 3: TLC Chromatogram at 366 nm.

EXPERIMENTAL

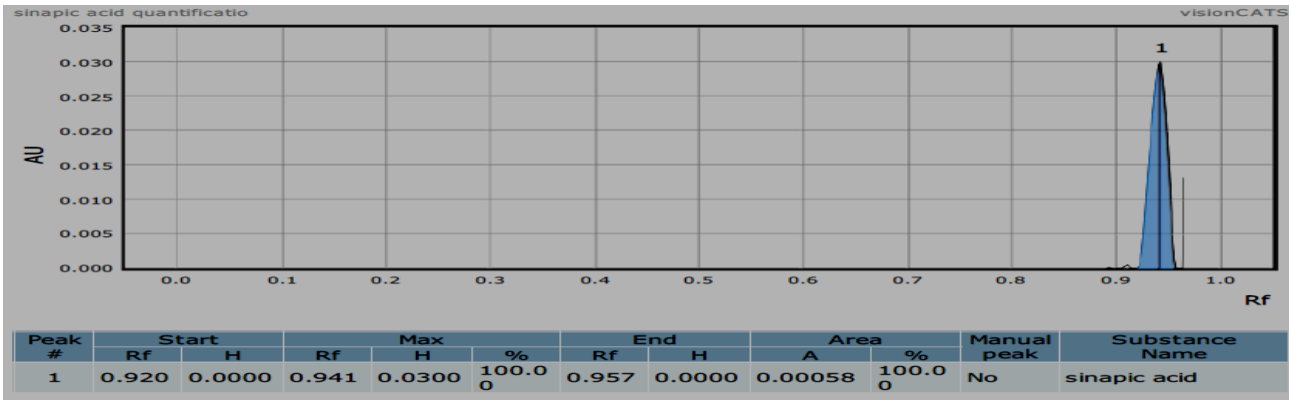
Plant material

Camelina seeds were collected from the field station of DIBER hq. Haldwani (29.22˚N, 79.52 ˚E, 424 m asl) where the plants were grown under natural environment.



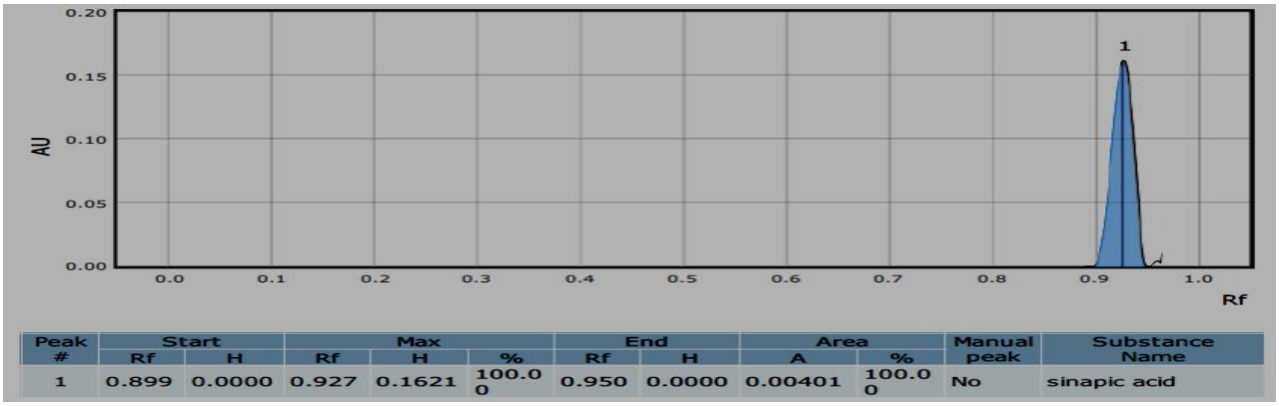
A **B**

Figure 4: Image showing the band scanning of Reference (A) and Extract (B).



Peak #	Start		Max			End		Area		Manual peak	Substance Name
	Rf	H	Rf	H	%	Rf	H	A	%		
1	0.920	0.0000	0.941	0.0300	100.00	0.957	0.0000	0.00058	100.00	No	sinapic acid

Figure 5: Sinapic acid Standard.



Peak #	Start		Max			End		Area		Manual peak	Substance Name
	Rf	H	Rf	H	%	Rf	H	A	%		
1	0.899	0.0000	0.927	0.1621	100.00	0.950	0.0000	0.00401	100.00	No	sinapic acid

Figure 6: Sinapic acid in Defatted Camelina seed methanolic extract.

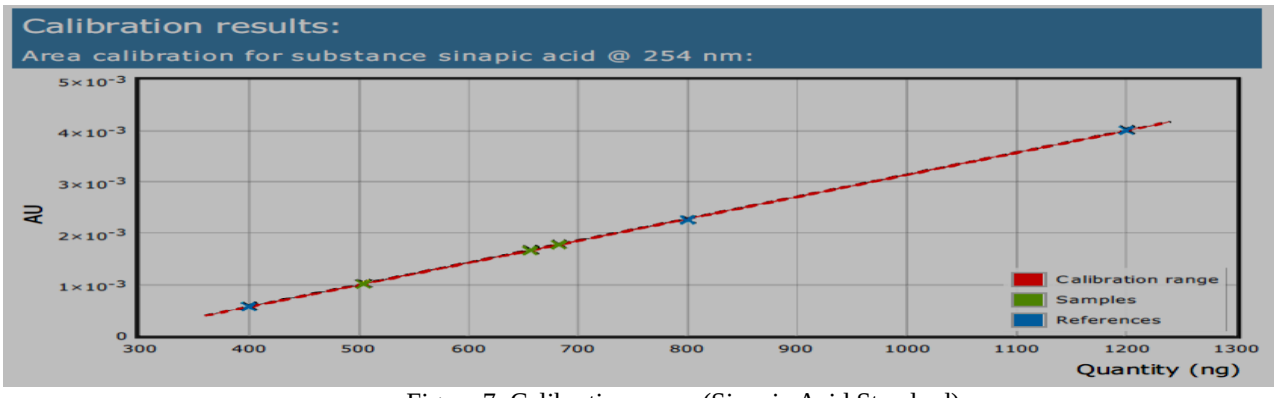


Figure 7: Calibration curve (Sinapic Acid Standard).

sinapic acid quantificatio visionCATS

sinapic acid	(3 sample assignments) @ 254 nm		
Sample '6'	250.4 µg/ml	CV=31.497 %	(3 applications)
Volume: 2.0 µl	290.3 µg/ml	CV=18.526 %	(2 replicas)
Track 6	328.3 µg/ml	656.7 ng	
Track 7	252.3 µg/ml	504.6 ng	
Volume: 4.0 µl	170.6 µg/ml	(CV unavailable)	(1 replicas)
Track 8	170.6 µg/ml	682.5 ng	

Figure 8: Table showing the final results of HPTLC with the obtained value.

Table 1: R_f value and Height of the peaks of reference and extract.

Sr. No.	Track	Sample	R_f Value		
			Starts at	Max	Height (AU)
1.	1	Sinapic acid (Reference)	0.92	0.941	0.03
2.	2	Sinapic acid (Reference)	0.906	0.933	0.099
3.	3	Sinapic acid (Reference)	0.899	0.927	0.1621
4.	6	Sinapic acid (Sample)	0.909	0.93	0.0559
5.	7	Sinapic acid (Sample)	0.911	0.933	0.0433

Table 2: Showing Calibration curve data.

Sr. No.	Calibration curve data	Obtained values
1.	Regression Mode	Linear -2
2.	Range Deviation	5.00%
3.	Related substances	default
4.	No. of References	3
5.	Calibration Function	$Y = 4.299 * 10^{-9} X – 1.154 * 10^{-3}$
6.	Coefficient of variation	CV 0.6018
7.	Correlation coefficient	R = 99.9952 %

Seed were washed thoroughly with the sterile distilled water to remove undesired material and dried under hot air oven at 35 ˚C. All the chemicals (AR grade) procured from Sigma Aldrich, USA and used without further purification.

Extraction procedure

Seeds were grounded into fine power using mixture grinder (Remi), about 50 g of powder was taken into the manually prepared thimble (Whatmann filter paper 00) and the oil extraction was done in Soxhlet apparatus using Hexane as a non polar solvent. After oil extraction, defatted seed powder was collected from the thimble dried well in hot air oven for two hours. Again, about 30 g of defatted sample filled for the extraction using methanol as a polar solvent and after 24 hours cycle extract was collected, solvent evaporation done by rota vapor (IKA RV 10) and then sample is lyophilized (LABCONCO) for the further studies.

HPTLC instrumentation and working conditions

For the present investigation, CAMAG HPTLC (Switzerland) model was used. Initially, the 10 mg of lyophilized sample was dissolved in 10 ml of methanol (1 mg/ml), shake well and centrifuged (Eppendorf 5330 R) at 3000 rpm for 10 minutes (Maintained temp. 20 º C). A precoated silica gel aluminum sheet 60 F 254 (Merck) with the dimension of (200 *100 mm) & 0.2 mm thickness was used as a stationary phase. The plate was washed with methanol under the chamber (TTC 20*10) and activated at 60 º C for 10 minutes before injecting the samples. Samples were loaded in the form of bands (width 8 mm) with the help of Camag µlit syringe injector using LINOMAT V at the speed of 150 nl/sec with the help of nitrogen. first track position was set at 29 mm and the band gap between the spots were 17.4 mm. TLC Chromatogram was developed in ascending order in twin through glass chamber of 20*10 mm, Solvent takes almost 20 minutes to flow at the top of the chromatogram (80 mm), the developed chromatogram was dried in TLC spray cabinet II, Densitometric analysis (TLC Scanner IV) and photo documentation (TLC visualize II) of developed chromatogram was done at 254 - 336 nm (U-V range) using Deuterium and Tungsten lamp after spraying the developed plate with the Alcoholic Ferric chloride. Programming and documentation of HPTLC operated on Vison CATS 2.5 software. Whole HPTLC procedure was operated under standard laboratory conditions {(Temp. 25 ± 2 º C), RH 50 ± 5% (MEXTECH TM-2)}.

Calibration curve of sinapic acid

For the quantification of sinapic acid in camelina defatted seed a reference is also required to formulate the calibration curve. For this purpose, 1mg/ml stock solution of sinapic acid prepared in methanol, from this stock solution 200, 400, 600, 800, and 1000 µg/ml was spotted on TLC plate to obtain concentration in nanogram range. Accuracy and precision of the calibrated curve was validated in terms of Coefficient of variation (CV %) and Correlation coefficient (R %).

RESULTS AND DISCUSSION

Selection of extracting solvent

Camelina sativa L. seeds are highly rich in the oil content, mainly seeds are considered as the potential source of biodiesel production and DIBER is also working on biofuel production with different sources, main motive of the Organization is to achieve potential sources for the biofuel formulation. In this practice, Camelina is also a part of these studies. But Camelina have not only the biofuel potential but its seeds are also contains various bioactive substances. HPTLC provide a better route for quantification of sinapic acid. Firstly, seed oil (non polar behavior) was obtained using hexane on the basis of like dissolves like. After oil removal, Defatted powder was dried in oven; moisture percentage (3.24 %) was calculated

Table 3: Values of sinapic acid at different tracks.

Sr No.	Linearity range (300 - 1300 ng)	Sample (ng/spot) Sample 6 (250.6 µg/ml Calculated value)	
1.		Track 6	656.7 ng
2.		Track 7	504.6 ng
3.		Track 8	682.5 ng

using Halogen moisture analyzer (Mettler TOLEDO HE 53). Phenolic compounds remained in the defatted part. To extract these polar compounds methanol used as a polar solvent.

Selection for the mobile phase (Developing solvents)

Initially, the different solvent system was analyzed for the optimization and better resolution of the phenolic compounds obtained from methanolic extract. Desired chromatogram was developed by using filtered solvent (Whatmann filter no. 00) of Ethyl acetate: Ethyl Methyl Ketone: Formic acid: water (5:3:3:1 v/v/v/v), this solvent system was mixed well and 20 ml of it poured in twin through glass chamber for 20 minutes. It shows a clear TLC images at 254 and 366 nm.

Chromatogram development and imaging

The developed TLC Chromatogram was analysed in TLC visualizer II, A band of standard was clearly found which was clearly visible, these standard spots (first six) becoming darker as the quantity of standard was increased. Developed chromatogram, was not visible in white light, but at 254 nm clear spots are confirming the presence of sinapic acid in the defatted camelina seed.

Plate was also documented at 366 nm which shows clear intense band of sinapic acid in the defatted seed, with the R_f value of 0.93 ± 0.02.

Scanning of the spots (Standard & extract)

After the chromatogram development, Scanning of the each spot was done using Scanner (TLC Scanner 4 (S/N: 241075), the band of sinapic acid standard showed clear peaks at the value of R_f 0.899 to 0.92 range, the defatted extract showed number of compounds in the Chromatogram and an intense band found at R_f 0.3. However, a band which matches the standard in the chromatogram falls under the range of R_f 0.909 to 0.911 range. This shows the presence of sinapic acid in the defatted seed, the height (AU) of the peak tells about the quantity of the sinapic acid which further quantify by calibration curve.

Calibration curve

Area calibration curve of sinapic acid was linear in the concentration range of 300- 1300 ng range. All the values of the curve showed in the table below:

Statistical analyses of the chromatogram suggest that sinapic acid present in the methanolic extract of defatted camelina seeds and the total amount was 250.4 µg/g.

CONCLUSION

Sinapic acid and their derivatives are main phenolic components found in almost every species of Brassicaceae family. After the extraction of oil from Camelina seeds, around 60 percent of de oiled cake obtained, which contains protein, fiber content and many phenolic acids. The conclusion of the present investigation suggests that Camelina de oiled cake could be a potential source of natural antioxidant and can be used in nutraceutical activities; however it needs some more study to develop it as a source of various drugs. Study also suggests that the HPTLC method for quantification of Sinapic acid is more efficient and results are reproducible.

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