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Research Article

DETERMINATION OF LINOLEIC ACID IN PITAYA EXTRACTS VIA HIGH-PERFORMANCE THIN-LAYER CHROMATOGRAPHY: A STANDARDIZATION APPROACH

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ABSTRACT

Objectives: To develop and validate a robust high-performance thin-layer chromatography (HPTLC) method for the determination and standardization of linoleic acid in *Hylocereus polyrhizus* (dragon fruit [DF]) extracts. For quality control and standardization, linoleic acid—a polyunsaturated omega-6 fatty acid vital for human health-was selected as a marker compound in herbal formulations.

Methods: Chromatographic separation was carried out on silica gel 60 F_{254} HPTLC plates. The optimized mobile phase used was *n*-hexane: ethyl acetate: benzene:methanol in the ratio of 4:3:2.5:0.5 (v/v/v/v). Detection was performed at 230 nm. Method validation parameters included linearity, precision, accuracy, robustness, limit of detection (LOD), and limit of quantification (LOQ).

Results: The method demonstrated excellent linearity in the range of 2000–14000 ng/band with a correlation coefficient (R^2) of 0.9954. Precision showed a % relative standard deviation of <1.2%. Accuracy ranged between 99.03% and 99.64%. LOD and LOQ were found to be 210.83 ng/band and 638.88 ng/band, respectively. Quantitative analysis of two commercial DF extract samples revealed linoleic acid concentrations of 30.28 µg/mg and 25.14 µg/mg.

Conclusion: The developed and validated HPTLC method is reliable, precise, and accurate for the quantification of linoleic acid in *H. polyrhizus* extracts. This method can be effectively applied for the standardization and quality control of herbal formulations containing DF extracts.

Keywords: Linoleic acid, Dragon fruit extracts, Finger print, High-performance thin-layer chromatography.

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INTRODUCTION

Hylocereus polyrhizus, which is a species of dragon fruit (DF) plant known for producing fruit with deep red or magenta-colored flesh and bright pink to red skin. The DF plant is a member of the Cactaceae family, also known as the cactus family, and is classified under the genus *Hylocereus*. The colorful DF, pitaya or pitahaya, is produced by this tropical climbing cactus. It does best in warm temperatures with adequate drainage. It is known as the "moonflower" or "queen of the night" because of its huge, fragrant, bat- or moth-pollinated night-blooming blossoms [1-4].

Human health depends on linoleic acid, a polyunsaturated omega-6 fatty acid that must be obtained through diet because our systems are unable to produce it. Its chemical formula is $C_{18}H_{32}O_2$, and it plays a key role in maintaining healthy skin, supporting cell membrane structure, and regulating inflammation. Linoleic acid is commonly found in plant oils such as sunflower, safflower, soybean, and grapeseed oils, as well as in seeds and nuts. In skincare, it is known for its ability to strengthen the skin barrier, reduce acne, and improve moisture retention without clogging pores [5-11].

High-performance thin-layer chromatography (HPTLC) standardization and biological activity assessment are essential for ensuring the efficacy and quality control of herbal products and natural extracts. HPTLC provides a fast, accurate, and cost-effective method to identify, quantify, and fingerprint bioactive compounds within a complex plant matrix, ensuring batch-to-batch consistency. Generating characteristic chromatographic profiles helps detect adulteration or variations in raw material quality. However, chemical standardization alone is not sufficient – linking these profiles to biological activity assays (e.g., antioxidant, antimicrobial, and anti-inflammatory tests) allows researchers to correlate specific compounds or profiles with therapeutic effects. This dual approach enhances the scientific validity, safety, and regulatory acceptance of herbal medicines and extracts, bridging the gap between traditional knowledge and modern pharmacological standards [12,13].

METHODS

Instrumentation and reagents

S. No.	List of instruments	Model	Company name
1	Analytical Balance	AP-BP	SHIMADZU Corporation
2	pH Meter	EQ-614A Deluxe	Equiptronics, India
3	FTIR	Platinum-ATR	Bruker ALPHA II
4	Hot Air Oven	SSI-50	Balaji Scientific Instruments
5	HPTLC Instrument	Linomat 5, TLC Scanner 4, Visualizer 2	CAMAG, Switzerland
6	UV-Visible Spectrophotometer	UV-1900i	SHIMADZU Corporation

Chromatographic condition

Pre-treatment of high-performance thin-layer chromatography plates

Methanol was used as the mobile phase, and HPTLC plates were stored in a glass twin-trough chamber. The rising approach was used to allow

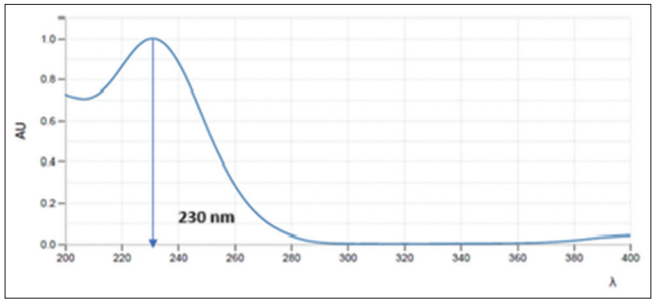

Fig. 1: Ultraviolet spectrum of linoleic acid (2000 ng/spot) showing a maximum absorbance (λmax) at 230 nm

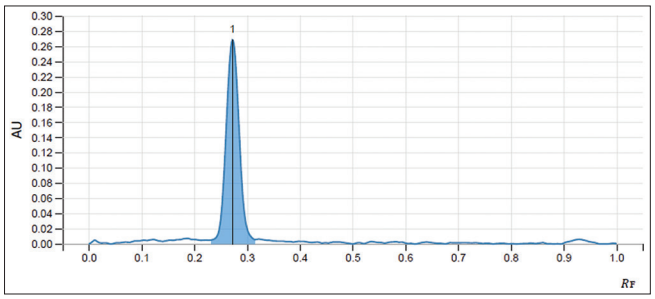

Fig. 2: Densitogram of standard linoleic acid (2000 ng/spot) recorded at 230 nm using high-performance thin-layer chromatography

Fig. 3: High-performance thin-layer chromatography plate of linoleic acid under 254 nm ultraviolet light

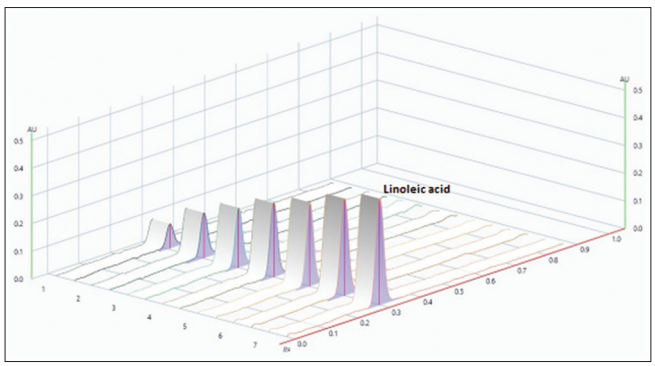

Fig. 4: Three-dimensional densitogram of linoleic acid (2000 ng/spot to 14,000 ng/spot) via high-performance thin-layer chromatography

Table 1: Optimized HPTLC conditions for estimation of linoleic acid

Parameters	Conditions
Stationary phase	Merck, HPTLC silica gel 60 F254
Mobile Phase composition	n-hexane: ethyl acetate: benzene: methanol (4:3:2.5:0.5 mL) v/v/v
Saturation Time	20 min
Rf values	Linoleic acid: 0.300
Band width	6 mm
Micro-Syringe	Hamilton syringe (100 µL)
Silica gel thickness	100 µm
Development	Linear Ascending
Slit size	6×0.45 mm
Chamber	Twin trough glass chamber
Migration distance	8 cm
Temperature	Room temperature
Band length	8 mm
Scanning speed	100 mm/s
Scanning Wavelength	230 nm

HPTLC: High-performance thin-layer chromatography

Table 2: Mobile phase trials for optimization of linoleic acid detection via HPTLC

Trial no.	Mobile phase composition	Result
1.	n-Hexane: ethylacetate: benzene: methanol (5:2:2.5:0.5 mL, v/v/v)	Linoleic acid: Detected, but tailing is there
2.	Benzene: methanol: n-hexane: ethylacetate (2.5:0.5:4:3 mL, v/v/v)	Linoleic acid: Properly detected without tailing at Rf value 0.300

HPTLC: High-performance thin-layer chromatography

Table 3: Calibration data for linoleic acid standard

Track no.	Volume (µL)	Concentration (ng/spot)
1	2	2000
2	4	4000
3	6	6000
4	8	8000
5	10	10,000
6	12	12,000
7	14	14,000

Table 4: Linearity of linoleic acid standard in the HPTLC method

S. No.	Concentration of linoleic acid (ng/band)	Mean Area	SD	% RSD
1	2000 ng	0.00335	4.98E-05	1.48
2	4000 ng	0.00513	5.08E-05	0.98
3	6000 ng	0.00649	8.17E-05	1.27
4	8000 ng	0.00782	3.71E-05	0.48
5	10000 ng	0.00909	3.29E-05	0.36
6	12000 ng	0.01084	1.52E-05	0.14
7	14000 ng	0.01168	3.49E-05	0.30

Regression equation-y=7E-06x+0.0022

R^2=0.9954

HPTLC: High-performance thin-layer chromatography

the methanol to reach the plate's upper edge. The plates were taken out and dried in the oven for 5 min at 110°C before being used right away for the experiment.

Table 5: Precision studies of linoleic acid (intra-day and inter-day)

S. No.	Precision (Intra-day)					Precision (Inter-day)			
	Conc.(ng/band)	Time interval	Peak area	SD	% RSD	Time interval	Peak area	SD	% RSD
1	4000	Morning	0.0051	2.52E-05	0.5	1 day	0.00303	3.61E-05	1.2
2	4000	Afternoon	0.00512			2 day	0.0031		
3	4000	Evening	0.00515			3 day	0.00305		
		Average	0.005123			Average	0.00306		
1	8000	Morning	0.00781	4.16E-05	0.5	1 day	0.00779	5.03E-05	0.6
2	8000	Afternoon	0.00779			2 day	0.00783		
3	8000	Evening	0.00787			3 day	0.00789		
		Average	0.007823			Average	0.007837		
1	12000	Morning	0.01084	2.65E-05	0.2	1 day	0.01084	5.51E-05	0.5
2	12000	Afternoon	0.0108			2 day	0.01079		
3	12000	Evening	0.01079			3 day	0.0109		
		Average	0.01081			Average	0.010843		

Table 6: Recovery study of linoleic acid (Standard addition method)

Level	Amount of sample taken (ng/spot)	Amount spiked (ng/spot)	Drug recovered (ng/spot)	% Recovered
Linoleic acid				
80%	2000	1600	3585.71	99.60
100%	2000	2000	3985.71	99.64
120%	2000	2400	4357.14	99.03

Table 7: Repeatability study of linoleic acid (n=5)

Phytomarkers	Concentration	Area	SD	%RSD
Linoleic acid	4000 ng/spot	0.00505	4.95E-05	1.0
		0.0051		
		0.00515		
		0.00518		
		0.00512		
	Average	0.00512		

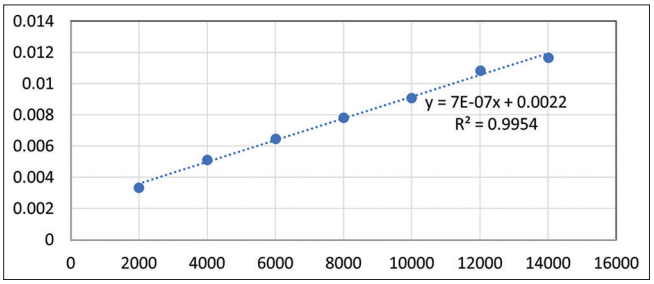

Fig. 5: Calibration curve of linoleic acid (n=5)

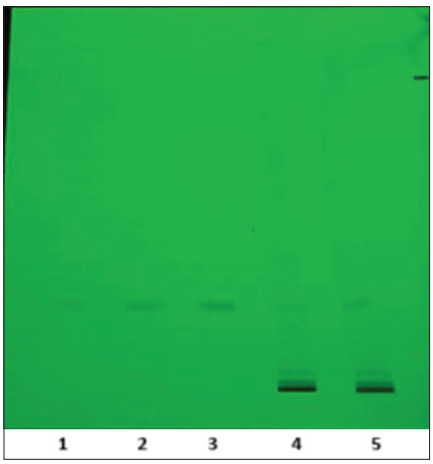

Fig. 6: High-performance thin-layer chromatography chromatogram of standard linoleic acid and *Hylocereus polyrhizus* (dragon fruit) extract samples, visualized under ultraviolet light at 254 nm. Track 1: Standard linoleic acid (4 µL), Track 2: Standard linoleic acid (8 µL), Track 3: Standard linoleic acid (12 µL), Track 4: Dragon fruit extract sample 1, Track 5: Dragon fruit extract sample 2

Methods

Solution preparation

Preparation of the mobile phase

Mobile phases with different ratios were mixed to prepare a mobile phase for different trials. Before usage, the mobile phase was kept in a twin-trough glass chamber with a lid for 20 min to soak.

Preparation of the mobile phase (for optimized conditions)

4 mL n-hexane, 3 mL ethyl acetate with 2.5 mL benzene, and 0.2 mL of ammonia were mixed to prepare the mobile phase. The mobile phase was saturated for 20 min before use by keeping it in a twin-trough glass chamber covered with a lid.

Preparation of standard solution

To prepare a stock solution of approximately 1000 µg/mL, 10 mg of standard linoleic acid was properly weighed and diluted in 10 mL of methanol.

Preparation of working standard solution for method development

Standard solutions of linoleic acid (2 µL corresponding to 2000 ng/band) were applied to the plate for method development by changing the mobile phase ratio.

High-performance thin-layer chromatography fingerprinting (quantification of linoleic acid in *H. polyrhizus* [dragon fruit] extracts)

H. polyrhizus (DF) extract sample 1(purchased from vital herbs, Delhi): An accurately weighed 100 mg of extract was dissolved in 10 mL of methanol in a 10 mL volumetric flask. *H. polyrhizus* (DF) extract sample 2(purchased from yucca enterprises, Mumbai): An accurately weighed 100 mg of extract was dissolved in 10 mL of methanol in a 10 mL volumetric flask.

- From standard solutions of linoleic acid 14 µL volume and above, extract solutions 10 µL volume were applied on the TLC plate
- The plates were outlined with filter paper and immersed in mobile phase vapor for 20 min at ambient temperature (25°C ± 2°C) before being developed in a 20 cm × 10 cm Camag twin-trough chamber. For plates, the development distance was maintained at 8 cm. Hot air was used to dry the plates that had been taken out of the chamber.

Table 8: Robustness study of linoleic acid at 4000 ng/spot

Chromatographic parameters		Linoleic acid (4000 ng/spot)			
Chamber saturation time	**Variation**	**Area (n=3)**	**Mean**	**SD**	**% RSD**
18 min	−2	0.005193	0.00521	2.33E-05	0.4
20 min	0	0.0052			
22 min	+2	0.005237			
Change in wavelength					
228 nm	−2	0.00522	0.005177	2.52E-05	0.5
230 nm	0	0.005173			
232 nm	+2	0.00521			
Change in mobile phase					
Benzene: methanol: n-hexane: ethyl acetate (2.45:0.45: 3.95:2.95) mL	−0.05	0.00517	0.005143	2.3E-05	0.5
n-hexane: ethyl acetate: benzene: methanol (4:3:2.5:0.5) mL	0	0.00513			
n-hexane: ethyl acetate: benzene: methanol (4.05:3.05:2.55:0.55) mL	+0.05	0.005133			

Table 9: Summary of HPTLC method validation parameters for linoleic acid

Parameters	Linoleic acid
Linearity	2000–14,000 ng/band
Regression equation	−y=7E-06x+0.0022
R^2	0.9954
LOD (ng/band)	210.83
LOQ (ng/band)	638.88
Accuracy	99.03–99.64%
Solution Stability	Stable for (24 h)
Specificity	Specific
System Suitability	1.39
Repeatability	1.0
Intraday (n=3)	0.2–0.5
Interday (n=3)	0.5–1.2
Robustness (% RSD) Robust	
Change in chamber saturation time	0.4
Change in wavelength	0.5
Change in mobile phase	0.5

HPTLC: High-performance thin-layer chromatography, LOD: Limit of detection, LOQ: Limit of quantification

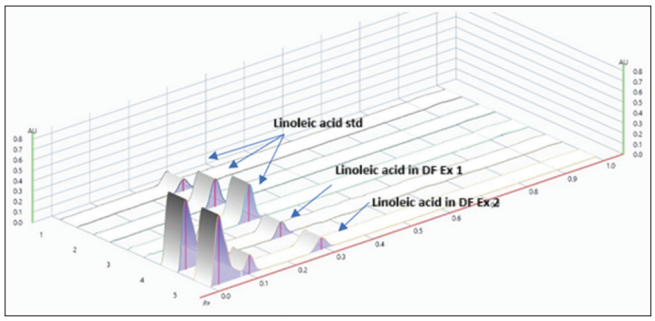

Fig. 7: Three-dimensional densitogram obtained by high-performance thin-layer chromatography analysis at 230 nm, showing the presence of linoleic acid in *Hylocereus polyrhizus* (dragon fruit) extracts

Using the TLC Scanner IV and the winCATS software, the produced plates were scanned at λ=230 nm.

RESULTS

Development and validation of analytical techniques for estimating linoleic acid

Optimization of mobile phase and detection wavelength

Spectra of the linoleic acid were recorded in the range of 200–800 nm using the CAMAG TLC scanner IV, where linoleic acid exhibited maximum absorbance at 230 nm. The detection wavelength for linoleic acid was selected as 230 nm.

The first HPTLC trial, which used a phase of mobile n-hexane, ethyl acetate, benzene, and methanol (5:2:2.5:0.5 mL, v/v/v), cannot produce the desired outcome. In addition, the composition of the mobile phase can be changed by altering the ratio of the mobile phase with an acceptable Rf value. N-hexane: benzene:methanol: ethyl acetate (4:3:2.5:0.5 mL, v/v/v) was selected as the optimized mobile phase as it provided a good resolution of the band at Rf of 0.300 as shown in Table 1.

Selection of the wavelength

For chromatographic estimation of linoleic acid, the optimized mobile phase consisting of benzene:methanol:n-hexane:ethyl acetate (2.5:0.5:4:3, v/v/v/v) was employed, and 230 nm was selected as the detection wavelength based on the maximum absorbance of linoleic acid as shown in Fig. 1.

Chromatographic trials

A 1000 µg/mL stock 10 mg of standard linoleic acid was dissolved in 10 mL to create the solution in volumetric flasks. These solutions were stored at 4°–6°. Using 2 µL stock solutions of these linoleic acids, HPTLC trials were performed as shown in Table 2.

Chromatographic estimation of linoleic acid

Chromatographic estimation of linoleic acid was carried out using HPTLC on silica gel 60 F254 plates with benzene:methanol:n-hexane:ethyl acetate (2.5:0.5:4:3, v/v/v/v) as the mobile phase, and detection at 230 nm as shown in Fig. 2.

Method validation

According to ICH Q2 (R1) guidelines. The calibration curve was plotted in the range of 2000–14,000 ng/band for linoleic acid. Detection limit for linoleic acid was found to be 0.01054 ng/spot, whereas the quantitation limits were 0.03194 ng/spot.

Linearity

Linearity for linoleic acid was observed in the concentration range of 2000–14000 ng/spot, as shown in Figs. 3-5 and Tables 3-4, the calibration curve demonstrated a good correlation between concentration and peak area with a regression coefficient (r^2) of 0.9954.

High-performance thin-layer chromatography fingerprinting (quantification of linoleic acid in dragon fruit extracts)

HPTLC fingerprint of DF extracts indicated an intense band at 230 nm for linoleic acid (Rf 0.272). A reference standard band was compared to the marker compounds identified in dragon fruit extracts using the HPTLC fingerprint, as shown in Figs. 6 and 7.

DF sample 1 or DF extract sample is DF extract purchased from Vital Herbs Delhi, and DF sample 2 or DF extract sample 2 is DF extract purchased from Yucca Enterprises.

Linoleic acid was quantified in DF extracts (DF sample 1 and DF sample 2) by comparing the band with bands of standard linoleic acid.

Table 10: Quantification of linoleic acid in dragon fruit extract samples

Track 3 (Rf value of standard linoleic acid)	Track 4 (Rf values found in dragon fruit extract sample 1)	Area	Concentration
0.274	0.269	0.00432	Linoleic acid (3028.57 ng)
Track 3 (Rf value of standard Linoleic acid)	**Track 5(Rf values found in dragon fruit extract sample 2)**	**Area**	**Concentration**
0.274	0.287	0.00396	Linoleic acid (2514.29 ng)

Table 11: Quantification of linoleic acid in *Hylocereus polyrhizus* (dragon fruit) extract samples

Extracts	Company (Vendor)	Marker	Area	Conc.(ng/spot) Found±RSD	Dried extract (µg/mg)±%RSD
Dragon fruit extract	Vital Herbs, Delhi	Linoleic acid	0.00432	3028.57	30.28
	Yucca Enterprises, Mumbai	Linoleic acid	0.00396	2514.29	25.14

The analysis was conducted to determine the presence and concentration of linoleic acid in DF extract samples. In Track 3, the standard linoleic acid exhibited an Rf value of 0.274.

For Sample 1 (Track 4), the detected Rf value was 0.269, which was closely aligned with the standard. The area under the peak was recorded as 0.00432, corresponding to a linoleic acid concentration of 3028.57 ng.

For Sample 2 (Track 5), the observed Rf value was 0.287, slightly deviating from the standard. The area under the peak was 0.00396, with a calculated linoleic acid concentration of 2514.29 ng.

These findings confirmed the presence of linoleic acid in both DF extract samples, with Sample 1 containing a higher concentration than Sample 2 as shown in Tables 10 and 11.

CONCLUSION

The HPTLC method was successfully developed and validated for the estimation of linoleic acid in *H. polyrhizus* (DF) extract and a polyherbal formulation. Chromatographic separation was achieved on silica gel 60 F254 plates using an optimized mobile phase of ethyl acetate:benzene:n-hexane:methanol (4:3:2.5:0.5, v/v/v/v),ml which resulted in a well-resolved band of linoleic acid at an Rf value of 0.272. The method was found to be linear in the range of 2000–14,000 ng/spot with a correlation coefficient (R^2 = 0.9954) (Tables 3 and 4; Figures 3-5). Validation studies performed as per ICH Q2(R2) guidelines confirmed high precision (%RSD <1.2%, Table 5), accuracy (99.03–99.64% recovery, Table 6), robustness (Table 7), and suitable sensitivity parameters (LOD and LOQ, Tables 8 and 9). Quantitative analysis revealed linoleic acid content of 3.0% and 2.5% in two DF extract samples. Thus, the developed method is novel, reliable, and suitable for the standardization and quality control of dragon fruit extracts and related polyherbal formulations.

AUTHOR'S CONTRIBUTION

The research work was done at the Centre for Research for Development (CR4D), Parul Institute of Medical Sciences and Research (PIMSR), Parul University, Waghodia, Vadodara. The manuscript editing and preparation were carried out by Sweta Patel, Falguni Tandel, and G.S. Chakraborthy.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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REFERENCES

1. Chowdhury MM, Sikder MI, Islam MR, Barua N, Yeasmin S, Eva TA, *et al*. A review of ethnomedicinal uses, phytochemistry, nutritional values, and pharmacological activities of *Hylocereus polyrhizus*. J HerbMed Pharmacol. 2024;13(3):353-65. doi: 10.34172/jhp.2024.49411
2. De Menezes Abreu G, Simões GD, Da Silva TG, Oliveira JT, Pieniz S. Antioxidant characterization of fruits of the family Cactaceae: *Hylocereus undatus*, *Selenicereusmegalanthus*, and *Hylocereus polyrhizus*. Food Sci Technol. 2024 Nov;44:360-8. doi: 10.5327/fst.00360%20
3. Agraval M, Nandini D, Sharma V, Chauhan NS. Herbal remedies for treatment of hypertension. Int J Pharm Sci Res. 2010;1(5):1-21. doi: 10.13040/IJPSR.0975-8232.1(5).1-2
4. Chopra RN, Nayar SL, Chopra IC. Glossary of Indian Medicinal Plants. New Delhi: Council of Scientific and Industrial Research; 1956.
5. Badawy S, Liu Y, Guo M, Liu Z, Xie C, Marawan MA, *et al*. Conjugated linoleic acid (CLA) as a functional food: Is it beneficial or not? Food Res Int. 2023;172:113158. doi: 10.1016/j.foodres.2023.113158, PMID 37689911
6. Jackson KH, Harris WS, Belury MA, Kris-Etherton PM, Calder PC. Beneficial effects of linoleic acid on cardiometabolic health: An update. Lipids Health Dis. 2024;23(1):296. doi: 10.1186/s12944-024-02246-2, PMID 39267068
7. Wang X, Jia Y, He H. The role of linoleic acid in skin and hair health: A review. Int J Mol Sci. 2024;26(1):246. doi: 10.3390/ijms26010246, PMID 39796110
8. Qin Y, Li K, Zhang Q, Liu J, Xie Y, Zhang T, *et al*. Linoleic acid inhibits lipopolysaccharide-induced inflammation by promoting TLR4 regulated autophagy in murine RAW264.7 macrophages. J Appl Biomed. 2024;22(4):185-96. doi: 10.32725/jab.2024.023, PMID 40033806
9. Mousavi M, Hojjatoleslamy M, Mousavi ZE, Kiani H, Jalali SM. Optimization of conjugated linoleic acid and eicosapentaenoic acid production from sesame waste by response surface methodology. J Food Process Preserv. 2024;2024(1):1-13. doi: 10.1155/2024/3344932
10. Nayyar D, Said JM, McCarthy H, Hryciw DH, O'Keefe L, McAinch AJ. Effect of a high linoleic acid diet on pregnant women and their offspring. Nutrients. 2024;16(17):3019. doi: 10.3390/nu16173019, PMID 39275331
11. Gebereyowhans S. Potential strategies to enhance conjugated linoleic acid content of milk and dairy products: A review. Heliyon. 2024 Oct 2;10(19):e38844. doi: 10.1016/j.heliyon.2024.e38844, PMID 39435105
12. Gope ER, Begum SM, Anisetti PP, Kasa GG, Eedarada VG, Nalli J, *et al*. A review of principles, applications, and recent developments in HPTLC and HPLC. J Pharm Insights Res. 2024 Dec 5;2(6):56-64. doi: 10.69613/315vge42
13. Sharma S, Modi K, Shah M. Development and validation of high-performance thin-layer chromatography (HPTLC) and high-performance liquid chromatography (HPLC) methods for the simultaneous determination of myricetin and quercetin in *Manilkara hexandra*. J Planar Chromatogr Mod TLC. 2024;37(6):511-9. doi: 10.1007/s00764-024-00268-w