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HPTLC fingerprinting and quantification of lignans as markers in sesame oil and its polyherbal formulations

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ABSTRACT

Sesamum (*Sesamum indicum*) seed and its oil have been in use in Indian traditional medicine, 'Ayurveda' since antiquity. However, there has been no attempt to standardize the polyherbal formulations containing sesamum oil as the main ingredient in terms of its active principle or marker compound. Biologically active lignans in sesamum oil are identified as the marker compound for the oil and its formulations. In this report, a simple, rapid and sensitive HPTLC method is described for the first time to identify and quantify sesamin and sesamolin, the major lignans of the sesamum oil and the method was applied to polyherbal formulations containing the oil for their quantitative estimation. The method was validated in terms of its calibration curve, limits of detection and quantification, precision, accuracy and robustness following standard protocols. The method thus developed was applied to sesamum oil and its commercial herbal formulations to quantify sesamin and sesamolin. The method for fingerprints of the formulations in the form of densitogram following charring of the chromatographic plate was also developed that could be useful for marker-based quality assurance of the polyherbal products containing sesamum oil.

1. Introduction

Sesame seed (*Sesamum indicum* Linn.), the oldest oil seed crop known to man is considered as the 'queen of oil seed crops' due to its high nutritional and therapeutic values and is widely naturalized in tropical and subtropical regions. India and China are the major producers of sesame, contributing about 70% of the total world production. Sesame seed yields highly stable oil with a distinct flavor for domestic use and protein rich meal for confectionaries. The medicinal applications of sesame seed and oil are referred in the traditional medical texts of India and China. In Indian traditional medicine, 'Ayurveda', a large number of health rejuvenating formulations like 'Rasayana' and 'Chyavanaprasam' and massage oils under the class 'Thailam' contain sesame oil as major ingredient and are being used in large volume.

Recent studies using modern methods have revealed potential health benefits of sesame such as antioxidative [1], antihypertensive [2], hypocholesteremic [3], anticancer [4], immunoregulation [5], etc. The beneficial health effects of sesame are primarily attributed to lignans and their glycosides. Lignans, a class of secondary plant metabolites produced by oxidative dimerization of two phenyl propanoid units are major components of unsaponifiable matter in sesame oil. Sesamin and sesamolin are the major components of lignans in sesame oil (1–2%) (Fig. 1) [6–8]. These compounds possess strong antioxidant activity [1] and are also reported to regulate lipid metabolism that includes lowering of serum lipids and LDL cholesterol [3], modulation of immunity [5] and lowering of blood pressure [9].

Standardization of herbal formulations in terms of quality of raw materials, manufacturing practices, and composition is important to ensure quality and optimum levels of active principles for their bio-potency. Recently, the concept of marker-based standardization of herbal drugs is gaining momentum. Identification of major and unique compounds in herbs as markers and development of analytical methodologies for monitoring them are the key steps involved in marker-based standardization. Being the major active principles largely responsible for the bio-potency of sesame oil, lignans are recognized as the marker compounds. There are some reports on the application of TLC [10–15], GC [10,12] and HPLC [10,12–16] methods for the analysis of lignans, but attempts to apply these techniques for the profiling of lignans in herbal formulations are not available. Kamal-Eldin et al. reported the application of one-dimensional and two-dimensional TLC for the qualitative analysis and isolation of lignans from unsaponifiable matter of sesame oil [11,12]. Separation of sesamin and sesamolin as major antioxidants in sesame oil unsaponifiables using one-dimensional TLC is demonstrated by Shahidi et al. [14]. HPTLC has recently emerged as a preferred analytical tool for fingerprints and quantification of marker compounds in herbal drugs because of its simplicity, sensitivity, accuracy, suitability for high throughput screening, etc.

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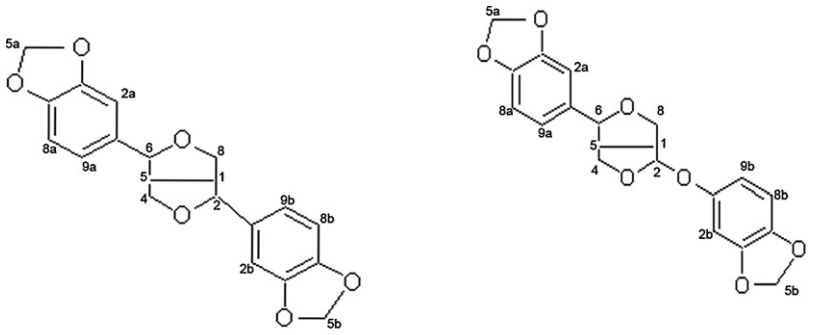

Fig. 1. Structure of seasmin and sesamolin.

HPTLC methods, hitherto, has not been reported for estimation of lignans either in sesame oil or its herbal formulations. In this paper development and validation of a HPTLC method for the quantitative analysis of sesamin and sesamolin is reported. The method was applied for the fingerprinting and quantification of lignans as markers of selected polyherbal formulations containing sesame oil.

2. Materials and methods

2.1. Reference compounds and reagents

Reference sesamin, monolinolein, dilinolein, triolein, γ-tocopherol, oleic acid and linoleic acid were purchased from Sigma–Aldrich (Germany). Sesamolin was isolated from unsaponifiable matter of sesame oil [14] and its purity was confirmed to 90% by HPLC. HPLC and analytical grade solvents were obtained from Merck (Mumbai, India).

2.2. Sesame oil and ayurvedic formulations

The sesame oil was gifted by Arjuna Aromatics (Cochin, India). Three major classes of commercial polyherbal formulations containing sesame oil manufactured by following the procedure laid down by the Indian traditional medicine (Ayurveda) were selected for the study. Samples of the same formulation in triplicate, manufactured by three different reputed ayurvedic drug manufacturers were collected from their authorized retail shops in Trivandrum, India.

2.3. Preparation of standard solutions

Standard stock solutions (1 mg/ml) of sesamin and sesamolin were prepared in methanol. Working solutions (0.04, 0.07, 0.1, 0.20, 0.40, 0.70, 1.00 and 2.00 μg/μl) of analytes were prepared by appropriate dilutions of the stock solutions with methanol.

2.4. Chromatographic conditions

CAMAG HPTLC system (Switzerland) with a Linomat 5 sample applicator was used for the analysis. The analysis was performed in air-conditioned room maintained at 22 °C and 55% humidity. TLC was performed on precoated silica gel HPTLC aluminum plates $60F_{254}$ (20 cm × 20 cm, 0.2 mm thickness, 5–6 μm particle size, E-Merck, Germany). Five microliters of the standard solutions were spotted as bands of 6 mm width by using the auto sampler fitted with a 100 μl Hamilton syringe. The plates were developed using benzene–chloroform (50:1, v/v) in a CAMAG twin—trough plate development chamber which was lined with filter paper and presaturated with 30 ml mobile phase. The developed plates were air dried and scanned. A spectrodensitometer (Scanner 3, CAMAG) equipped with 'win CATS' planar chromatography manager (version 1.3.0) software was used for the densitometry measurements, spectra recording and data processing. Absorption/remission were the measurement mode at a scan speed of 20 mm/s. Spots of sesamin and sesamolin were scanned from 200 to 600 nm so as to record their UV–vis spectrum and to obtain their wavelengths of maximum absorption. Densitograms were recorded at the wavelength of maximum absorption (290 nm) of sesamin and sesamolin. Each concentration of the reference compounds was spotted two times on the plates and analyzed. The concentrations of reference compounds were plotted against peak area to obtain calibration curves.

2.5. HPTLC analysis of sesame oil and ayurvedic polyherbal formulations

Sesame oil (10 g) and formulations (10–100 g) were homogenized at 200 rpm with methanol (1:5, w/v) at 50 °C for 20 min. Then the mixture was centrifuged at 2000 × *g* for 20 min at 4 °C and the supernatants were collected. The residues were resuspended in methanol and the extraction was repeated five more times under the same conditions. The supernatants were pooled and concentrated under vacuum at ≤45 °C and made up to a known volume with methanol. The extracts were filtered through 0.45 μm filter and HPTLC was performed under the conditions optimized for the reference compounds. The plates were scanned at 290 nm and the UV–vis spectra of the bands corresponding to sesamin and sesamolin were also recorded. For the confirmation of identity and purity of bands, the bands corresponding to sesamin and sesamolin were scrapped and eluted with chloroform, and ^{1}H NMR (Avance DPX 300, Bruker, UK) and MS (MS Route JMS 600H, JEOL, Japan) spectra were acquired. The amounts of sesamin and sesamolin were quantified using calibration curves plotted with the reference compounds.

2.6. HPTLC fingerprinting of sesame oil and formulations by charring method

After scanning at 290 nm, the plates were dipped in 5% methanolic H_2SO_4 solution for 5 s and then kept at 100 °C for 20 min for charring. After cooling, the plates were scanned at 450 nm to record their fingerprints.

2.7. HPLC analysis of sesamin and sesamolin

HPLC analysis [14] was performed in a Shimadzu HPLC system (Shimadzu, Japan) with a LC-10AD model pump, a Rheodyne injector fitted with 20 μl sample loop and a SPD-10A UV–vis detector. A reverse phase column (Phenomenex, C-18, ODS-2, 5 μm,

250 × 4–6 mm) with an extended guard column was used as stationary phase and an isocratic elution of methanol–water (70:30) at 1 ml/min as mobile phase. Chromatograms were recorded at 290 nm. Methanol extracts of sesame oil were appropriately diluted with mobile phase, filtered through 0.45 μm PTFE microfilters and injected in to HPLC. The amounts of sesamin and sesamolin were calculated by external standard calibration method. Calibration curves were plotted with reference sesamin (0.01–1.0 μg/μl) and sesamolin (0.01–0.8 μg/μl). The amounts of lignans were determined by triplicate analysis and mean ± S.D. was calculated.

2.8. Statistical analysis

The statistical analysis was performed using Microsoft Excel 2003 and Microcal Origin 6.0.

3. Results and discussion

3.1. Validation parameters

3.1.1. Optimization of chromatographic conditions

Different proportions of benzene and chloroform were tried as mobile phase for the resolution of sesamin and sesamolin on silica gel HPTLC plates. Benzene and chloroform in a ratio of 50:1 (v/v) was found to give good resolution between sesamin and sesamolin and their separation from other lipids (Fig. 3). In a previous report, Kamal-Eldin et al. demonstrated the application of one-dimensional and two-dimensional TLC for the profiling of lignans and other sesame unsaponifiables [11,12]. In their report, in one-dimensional TLC sesamin is well resolved from other unsaponifiables, but sesamolin band is merged with tocopherol band. Two-dimensional TLC is effective in resolving both sesamin and sesamolin, but is not recommended for quantitative analysis [12]. Shahidi et al. reported a modified TLC method for the separation of unsaponifiables, in which sesamin and sesamolin are well resolved from other components [14]. In the present study, the separation of sesamin and sesamolin from other lipid components was verified by profiling reference samples of monolinolein (monoglyceride), dilinolein (diglyceride), triolein (triglyceride), γ-tocopherol and mixture of oleic acid and linoleic acid (free fatty acids) under the proposed HPTLC conditions. HPTLC profile of methanol extract of sesame oil spiked with these reference compounds is given in Fig. 3. In the earlier reports unsaponifiable matter of sesame oil was used for the TLC profiling of lignans. The method using HPTLC developed here does not require saponification step and the oil extract can directly be used for analysis. Avoidance of long and tedious saponification step therefore make this method more amenable to the high throughput screening of sesame oil and its formulations. Under the optimized conditions using precoated HPTLC plates with 0.2 mm thickness and 5–6 μm particle size, with modified solvent system, well resolved and symmetric bands were obtained for sesamin and sesamolin as demonstrated for methanol extracts and reference compounds (Figs. 3 and 4). In order to further establish the selectivity of the method the bands corresponding to sesamin and sesamolin were separated and subjected to NMR and MS spectral analysis. NMR and MS (FAB) spectra obtained are presented below.

Sesamin: ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 3.05 (2H, m), 4.75 (2H, d), 3.84 (2H, dd), 4.16 (2H, dd), 5.94 (4H, s), 6.85 (6H, m).

MS (FAB): 354 (M^+), 338, 234, 203, 161, 149, 135 (base peak), 77.

Sesamolin: ^{1}H NMR (300 MHz,$CDCl_3$) δ (ppm): 2.93 (1H, m), 3.31 (1H, m), 3.6 (1H, d,), 3.94 (2H, dd), 4.23 (1H, d), 4.45 (2H,d), 5.92 (2H, s), 5.96 (2H, s), 6.49 (2H, d), 6.52 (1H, s), 6.62 (1H, d), 6.72 (1H, d), 6.80 (1H, d), 6.88 (1H, s).

MS (FAB): 370(M^+), 234, 203, 149, 135 (base peak), 91.

^{1}H NMR and MS (FAB) signals obtained for the bands of sesamin and sesamolin were compared with those of reference compounds

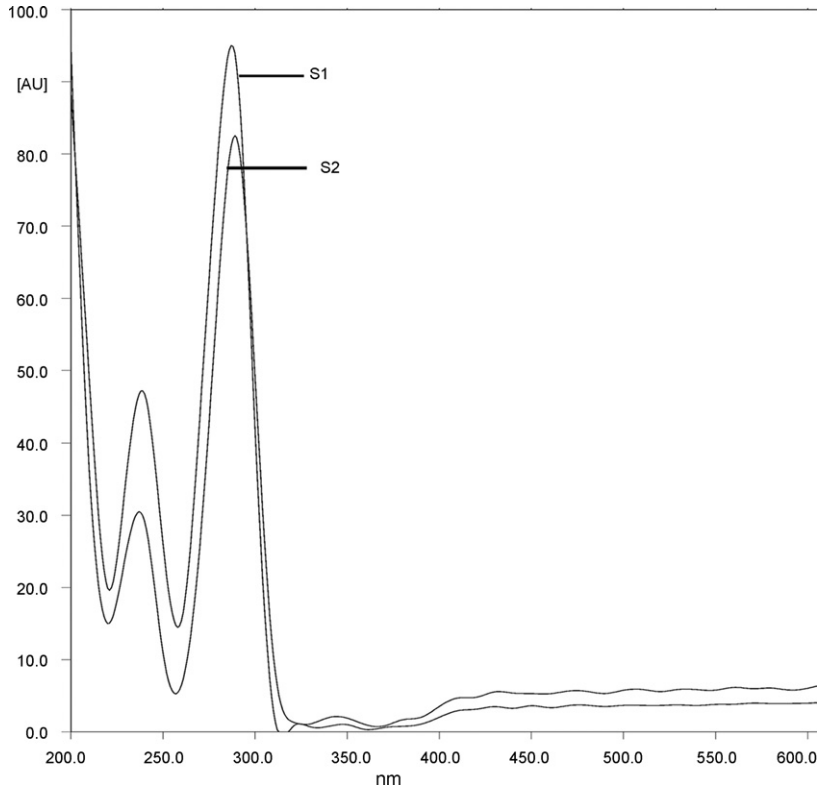

Fig. 2. UV–vis spectra of seasmin (S1) and sesamolin (S2).

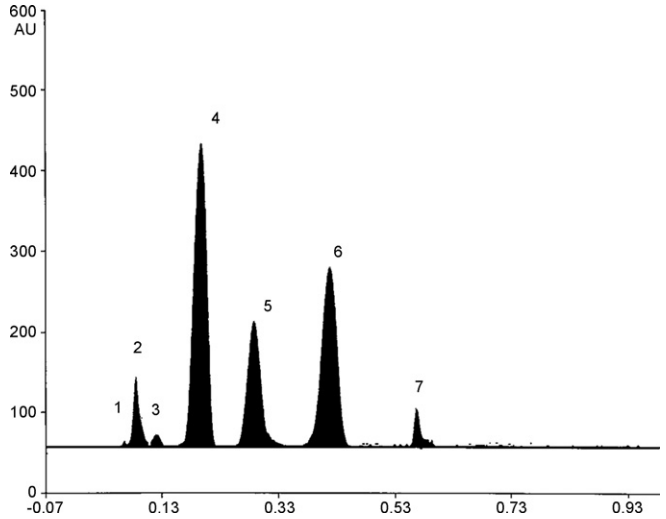

Fig. 3. HPTLC densitogram (at 290 nm) of sesame oil methanol extract spiked with reference compounds: (1) monolinolein (monoglyceride); (2) mixture of oleic acid and linoleic acid (free fatty acid); (3) dilinolein (diglyceride); (4) sesamin; (5) sesamolin; (6) γ-tocopherol; (7) triolein (triglyceride).

and previous reports [1,12,17,18]. All the signals obtained in the NMR spectra were found to belong to the respective molecules which indicated the identity and purity of bands. Molecular ion peaks, base peaks and fragments obtained from MS(FAB) spectra of these bands further confirmed the purity and identity of the bands. Comparison of the NMR and MS spectral data of the sesamin and sesamolin bands from the HPTLC bands with those of their reference compounds thus could establish the identity and purity of the lignan bands.

The spectrodensitometer of the HPTLC system enables monitoring densitograms at different wavelengths and can be used to record the UV–vis spectra of the analytes directly from the plates. The spectra, thus recorded in conjunction with R_f can be used to identify the analytes. The bands of the sesamin and sesamolin in HPTLC plates were scanned from 200 to 600 nm with the auto scanner (spectrodensitometer) so as to record the UV–vis spectra (Fig. 2). The wavelengths of maximum absorption were obtained from the spectrum and this wavelength (290 nm for both sesamin and sesamolin) was selected as the scanning wavelength for the subsequent experiments. The R_f values obtained for sesamin and sesamolin were 0.19 and 0.29, respectively (Fig. 3).

3.1.2. Calibration parameters

Different concentrations of the reference compounds (sesamin and sesamolin) were spotted on HPTLC plates and developed under the optimized conditions. The analysis was performed in triplicates and mean peak area responses to the concentrations were recorded at 290 nm to establish their correlation. The linearity between the peak area and concentration of sesamin and sesamolin in this experimental range was not satisfactory ($r < 0.98$). Therefore, according to the ICH guidelines [19], the analytical responses of these compounds were represented as second order polynomial equation as $y = a + b_1x + b_2x^2$ where y = peak area, x = concentration of sesamin or sesamolin, $a = y$ intercept, b_1 and b_2 were constants. The regression equation for the sesamin was $y = 558 + 4522x - 254x^2$ when $|x|$ is between 0.04 and 2.0 μg/μl and that for sesamolin, it was $y = 717 + 3751x - 278x^2$ when $|x|$ is between 0.04 and 0.4 μg/μl. For the given range of concentrations of sesamin and sesamolin, their correlation coefficients were 0.990 and 0.998, respectively showing good correlation with calibration equations.

Table 1
Precision of the method

	Concentration (μg/μl)	Sesamin (CV%)	Sesamolin (CV%)
Instrumental precision	0.05[a]	1.62	1.40
	0.10[a]	0.91	0.79
	0.40[a]	0.85	0.98
Intra-assay precision	0.70[b]	1.98	1.59
	1.40[b]	1.35	1.41
	5.60[b]	1.01	1.21
Inter-assay precision	0.70[b]	2.71	2.49
	1.70[b]	1.91	1.90
	5.60[b]	2.31	2.12

[a] Concentration of reference compounds applied.
[b] Concentration of methanol extract of sesame oil applied.

3.1.3. LOD and LOQ

The limits of detection and quantification were determined as signal to noise ratio using the equations LOD = $3.3\sigma/S$ and LOQ = $10\sigma/S$ where, σ is standard deviation of response and S is the slope of calibration curve. The LOD and LOQ were respectively 0.08 and 0.24 μg for sesamin and 0.02 and 0.06 μg for sesamolin.

3.1.4. Precision

Repeatability of the method was validated as instrumental, intra-assay and inter-assay precisions. Instrumental precision was measured for 10 spots for each of the reference compounds at three different concentrations (0.05, 0.1 and 0.4 μg/μl). Intra-assay precision was studied by analyzing repeatedly in the same lab on the same day, five solutions of three concentrations (0.7, 1.4 and 5.6 μg/μl) of sesame oil methanol extract, each of them independently prepared and each of them applied three times. Inter-assay precision included analysis of the same three concentrations of each solution analyzed three times on the same day for three different days. The concentrations of the methanol extract were selected such a way that their lignan contents were in experimental range.

The results of precision analysis are summarized in Table 1. Instrumental precision was found to be ≤1.62% (CV) and intra-assay and inter-assay precisions were ≤1.98 and 2.71% (CV), respectively. An instrumental precision of ≤1% (CV) and intra-assay precision of ≤2% are recommended as precision criteria for a method [20]. Among the instrumental precision values, one value for each compound was higher than 1% (CV). Intra-assay precision values were in acceptable range.

3.1.5. Accuracy and recovery

The accuracy was determined by the standard addition technique. Known amounts of the reference compound in a range of low, medium and high were added to the sample (methanol extract of oil) and conducted chromatography under the optimized conditions. The accuracy was then calculated from the test results as the percentage of analyte recovered by the assay (Table 2). The results indicated the accuracy of the method was very good as supported by the recovery of 95.7–102.0% on the concentrations used here for sesamin and sesamolin.

3.1.6. Robustness

The robustness of the method was determined by introducing small changes in certain chromatographic parameters. The amount of mobile phase, composition of mobile phase, temperature and

Table 2
Results of recovery studies

Amount added (μg/μl)	Recovery ± S.D. (%)	
	Sesamin	Sesamolin
0.02	97.8 ± 1.1	102 ± 0.4
0.08	100.1 ± 0.9	98.9 ± 2.1
0.30	95.7 ± 1.3	97.1 ± 1.8

Table 3
Results from robustness studies

Parameters	R.S.D. % in peak area	
	Sesamin	Sesamolin
Mobile phase composition	1.23	0.76
Amount of mobile phase	0.97	0.89
Temperature	0.87	1.14
Chamber saturation time	0.65	0.71
Chamber dimensions	0.81	1.06

the chamber saturation time were varied in the range ±5% and the effects on the analytical values were studied. The effect of variation in chamber dimension (20 × 20 and 10 × 10) was also studied by varying one parameter at a time. The S.D. of peak areas was calculated for each parameter and the %R.S.D. was found to be less than 2% in each case. The low values of %R.S.D. show the robustness of the method (Table 3).

3.1.7. HPTLC versus HPLC

In order to verify the reliability of the HPTLC densitometry analysis, five independently prepared methanol extracts of sesame oil was analyzed simultaneously by HPTLC and HPLC methods. Each sample was analyzed in triplicate and mean values were compared by matched pair students *T* test. The data were treated as paired data. The observed T (T_{obs}) was calculated using the equation

$$T_{(obs)} = \frac{|\bar{d}|}{\sqrt{\left(\sum d_i^2 - (1/n)\left(\sum d_i\right)^2\right)/n(n-1)}}$$

where d_i is the difference between two pairs of measurements for the same observation. For five pairs of analysis the T_{obs} values for sesamin and sesamolin were 2.27 and 2.21, respectively. Student's distribution table gives $T(95, 5, 4) = 2.78$ for a risk factor of 5%. Since the T_{obs} values for both sesamin and sesamolin were lower than the critical T value, the means of differences between HPTLC and HPLC were not significant. Therefore, the methods give identical results with a risk factor of 5%. This established the reliability of HPTLC densitometry method and could be used for routine analysis of sesame lignans.

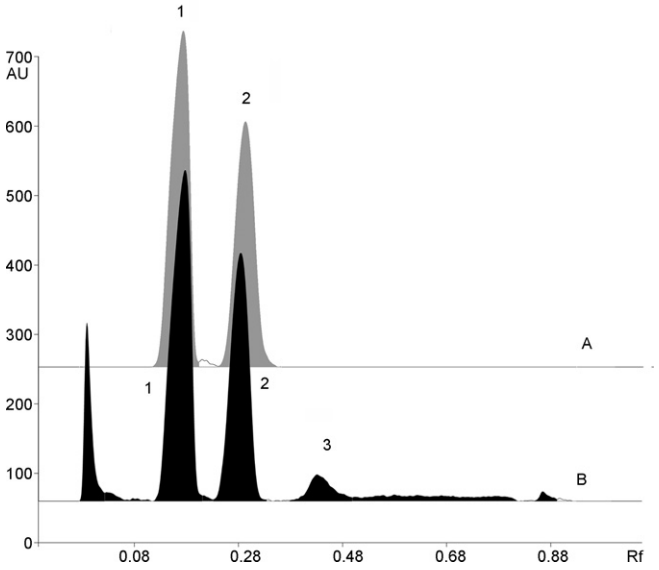

Fig. 4. HPTLC densitograms at 290 nm: (A) reference compounds (1: sesamin, 2: sesamolin) and (B) methanol extract of sesame oil (1: sesamin; 2: sesamolin; 3: γ-tocopherol).

3.2. Estimation of sesamin and sesamolin in sesame oil

The HPTLC method was used for the quantification of sesamin and sesamolin in methanol extract of sesame oil. HPTLC profile of the methanol extract of oil at 290 nm is given in Fig. 4. The peaks corresponding to sesamin and sesamolin in the densitogram were identified by comparing their R_f and UV spectra with those of reference compounds and also by spiking experiments. The amounts of sesamin and sesamolin in oil were determined from the calibration graphs and the results are given in Table 4. The contents of sesamin (0.72%) and sesamolin (0.40%) in sesame oil obtained here by HPTLC method are in accordance with the previous reports. Hemalatha et al. reported 0.73 and 0.47% of sesamin and sesamolin, respectively by HPLC method in sesame oil of Indian origin [8]. 0.23–0.72% of sesamin and 0.39–0.66% of sesamolin were reported for sesame oil collected from different locations of Sudan [13]. Sesame oils prepared from black, brown and white varieties of sesame collected from Japan contained 0.45–0.66% and 0.42–0.54% of sesamin and sesamolin, respectively [15].

Table 4
Determination of sesamin and sesamolin in sesame oil and ayurvedic formulations using the proposed HPTLC method

Sesame oil/formulation	Brand	Content of sesame oil (%)[a]	Sesamin[b] (mg/100 g)	Sesamolin[b] (mg/100 g)
Sesame oil			720 ± 10.9	400 ± 11.1
Agasthyarasayanam	1	4.8	28 ± 3.1	11 ± 3.2
	2	4.0	15 ± 4.2	4 ± 0.3
	3	3.3	13 ± 1.1	6 ± 0.9
Chyavanaprasam	1	7.6	46 ± 2.3	22 ± 6.2
	2	6.0	32 ± 1.7	20 ± 7.1
	3	4.4	26 ± 5.4	11 ± 2.0
Dhanwantharam Thailam	1	100[c]	427 ± 20.1	314 ± 22.8
	2	100[c]	359 ± 12.4	242 ± 14.0
	3	100[c]	312 ± 16.5	276 ± 13.4

[a] According to the ingredients list provided with the formulations.
[b] Mean ± S.D. ($n = 3$).
[c] Prepared by boiling herbs with oil.

3.3. HPTLC fingerprinting of polyherbal ayurvedic formulations and estimation of sesamin and sesamolin

A large number of ayurvedic formulations containing sesame oil are described in ayurvedic texts and many of them are commercially produced and marketed in large volumes. For the present study, three different classes of the formulations, namely 'Chyavanaprasam', 'Agasthyarasayanam' and 'Dhanwantharam Thailam' were selected and analyzed for their lignan contents. The peaks corresponding to sesamin and sesamolin in HPTLC plates were identified by comparing their R_f and UV spectra with those of reference compounds. The bands were separated and mass spectra were recorded and the purity was confirmed.

'Agasthyarasayanam' is recommended for the treatment of respiratory problems. The labeling indicated that, 'Agasthyarasayanam', samples analyzed here contained 30 herbs and 3.3–4.8% of sesame oil. The three major brands analyzed in HPTLC were found to contain 13–28 mg of sesamin and 6–11 mg of sesamolin in 100 g of the formulations (Table 4). 'Chyavanaprasam', a health rejuvenating formulation used in this study contained 50 herbs and 4.4–7.6% sesame oil as indicated in the label. The amounts of lignans in 'Chyavanaprasam' collected from three manufacturers varied from 26 to 46 mg for sesamin and 11–22 mg for sesamolin per 100 g. 'Dhanwantharam Thailam' is medicated oil prepared by boiling sesame oil with 60 herbs. It is common massage oil recommended for neurological and rheumatic problems, chronic arthritis and for pre- and post-natal care. HPTLC analysis showed that sesamin content in its three brands ranged from 312 to 427 mg and sesamolin from 242 to 314 mg in 100 g of formulation (Table 4).

The HPTLC fingerprints of the methanol extracts of oil and formulations were obtained by charring the developed plate with 5% H_2SO_4 in methanol since many of the bands were not UV active and hence had lower absorption maxima values. Partial carbonization resulted in visible bands of all organic compounds and the densitogram could be recorded by absorption at visible region. HPTLC profiles of the formulations at 290 nm, and at 450 nm after charring are given in Fig. 5. From the figure it is clear that many bands which are not detectable at 290 nm are visible at 450 nm. However, peaks of lignans were well resolved and distinct from the

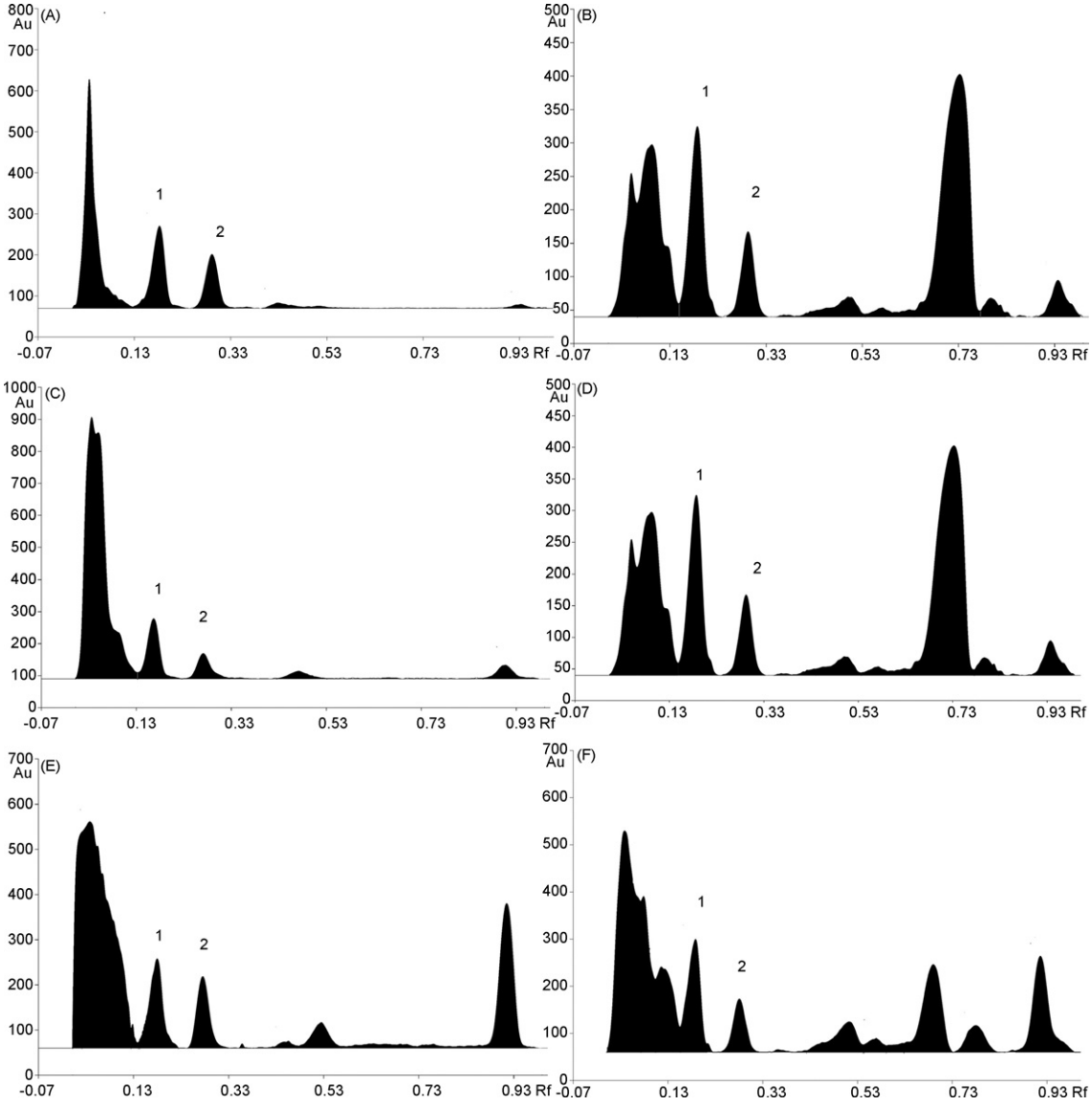

Fig. 5. HPTLC densitograms (finger prints) of polyherbal formulations: (A, C and E) Thailam; Chyavanaprasam and Agasthyarasayanam respectively at 290 nm; (B, D and F) Thailam; Chyavanaprasam and Agasthyarasayanam respectively at 450 nm after post-chromatographic charring with 5% sulphuric acid in methanol (1: sesamin; 2: sesamolin).

background at their absorption maxima 290 nm, than that in at 450 nm obtained after charring. Therefore, scanning at 290 nm is recommended for the quantitative analysis of lignans and charring method for fingerprints. In this report partial carbonization was attempted for the qualitative profiling of formulations containing sesame oil. Considerable variations were observed between the fingerprints of different formulations and within samples of different brands. These variations could be attributed to the quality of raw materials, processing and storage.

Separation and quantification of lignans in sesame oil were reported earlier [10,12–14]. Sesame oil was saponified and the unsaponifiable matter was used by these authors for separation and quantification of lignans. The present method was developed for the direct estimation of lignans in the sesame oil and their formulations without saponification and therefore is simple, accurate and suitable for routine analysis for quality assurance. Moreover precoated HPTLC plates with uniform particles of lower size (5–6 μm) afforded excellent resolution and reproducibility.

4. Conclusions

The application of a simple, rapid and accurate HPTLC method for the quantification of sesamin and sesamolin in sesame oil and its polyherbal formulations without saponification was demonstrated. This method may be recommended for quality assurance and fingerprinting to establish the authenticity of sesame oil and its formulations using sesame lignans as markers. The post-chromatographic charring was also performed so as to obtain a complete HPTLC fingerprint of various components present in sesame oil and its formulations. The method was validated to track the active principles (sesamin and sesamolin) in complex mixture of herbal ingredients used in traditional medicines like 'Ayurveda'. The method could be extended for the marker-based standardization of other herbal products containing sesame oil. Unlike the earlier TLC methods, the present method does not require saponification step; and therefore highly suitable for high throughput analysis.

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