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Original Article

DEVELOPMENT, VALIDATION OF HPTLC METHOD FOR SIMULTANEOUS QUANTITATION OF LUTEOLIN, APIGENIN FROM CARDIOSPERMUM *HALICACABUM LINN*. AND HYDNOCARPUS PENTANDRA (BUCH.-HAM.) OKEN

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ABSTRACT

Objectives: To develop and validate High Performance Thin Layer Chromatographic (HPTLC) method for simultaneous quantitation of luteolin and apigenin from dried leaf powder of *Cardiospermum halicacabum* Linn. and dried seed hull powder of *Hydnocarpus pentandra* (Buch.-Ham.) Oken.

Methods: Chromatography was performed using methanolic extracts of dried leaf powder of *Cardiospermum halicacabum* Linn. and dried seed hull powder of *Hydnocarpus pentandra* (Buch.-Ham.) Oken. Separation of luteolin and apigenin from methanolic extracts of both the plant materials was achieved on Silica gel $60F_{254}$ TLC plates using a suitable solvent system. Detection and quantitation of luetolin and apigenin was done by densitometric scanning at λ=349 nm. The developed HPTLC method has been validated using International Conference on Harmonization (ICH) guidelines.

Results: The validated HPTLC method was used for simultaneous quantitation of luteolin and apigenin from methanolic extracts of dried leaf powder of *Cardiospermum halicacabum* Linn. and dried seed hull powder of *Hydnocarpus pentandra* (Buch.-Ham.) Oken using their respective calibration curves. Amounts of luteolin and apigenin present in dried leaf powder of *Cardiospermum halicacabum* Linn. are 0.2119mg/g and 0.9089mg/g respectively. Amounts of luteolin and apigenin present in dried seed hull powder of *Hydnocarpus pentandra* (Buch.-Ham.) Oken are 0.0696mg/g and 0.0320mg/g.

Conclusion: The developed method is simple, precise and accurate and can also be used for routine quality control analysis and for the quantitation of luteolin and apigenin in herbal raw materials as well as in their formulations.

Keywords: Luteolin, apigenin, *Cardiospermum halicacabum* Linn., *Hydnocarpus pentandra* (Buch.-Ham.) Oken, High Performance Thin Layer Chromatography, Method validation.

INTRODUCTION

Cardiospermum halicacabum Linn. is a climber which belongs to the family Sapindaceae. This plant exhibits a wide range of biological and pharmacological properties. The active constituents contributing extracts and powders from the leaves, roots and seeds of this plant are used in the preparation of shrubs and infusions in traditional medicine against diabetes and arthritis [1]. Indian system of medicine recommends leaves for rheumatism, chronic bronchitis, stiffness of limbs and snake bite [2]. The plant has also been listed among those possessing anti-obesity activity [3].

Hydnocarpus pentandra (Buch.-Ham.) Oken, syn. *Hydnocarpus wightiana* Blume. is a dioecious evergreen tree endemic to Western Ghats, India and it belongs to family Flacourtiaceae. It is commonly known as Chaulmoogra. Its seeds yield chaulmoogra oil which has been proved to be greatly efficient in treatment of leprosy. Seeds are also reported to have other major therapeutic uses against worm infestation and obesity [4]. Acetone extract of seed hulls has been reported to possess strong free radicals (DPPH and ABTS) scavenging activity. It has also been reported to possess inhibitory activity against the enzymes like α-glucosidase and N-acetyl-β-D-glucosaminidase which may be responsible for the antidiabetic property as advocated in traditional medicine [5].

Both the plants possess high medicinal value because they are reported to contain array of therapeutically important classes of phytochemicals such as triterpenoids, flavones, aglycones, glycosides and a range of fatty acids and esters. It has been reported that ethanolic extract of leaves of *Cardiospermum halicacabum* Linn. possesses antioxidant and hypolipidemic effects in diabetic rats, which may be due to the presence of flavones aglycones, such as luteolin and apigenin in the extract [6]. Flavone aglycones, luteolin and apigenin have been reported to be present in considerable amounts in leaves of *Cardiospermum halicacabum* Linn.[7] and seed hulls of *Hydnocarpus pentandra* (Buch.-Ham.) Oken [8].

Therefore, a High Performance Thin Layer Chromatographic method has been developed for simultaneous quantitation of these two flavonoids from leaves of *Cardiospermum halicacabum* Linn. and seed hulls of *Hydnocarpus pentandra* (Buch.-Ham.) Oken which may be used as an important quality control method for standardization of these two plant materials.

Some of the HPTLC methods reported for the qualitative and quantitative analysis of apigenin and luteolin from other plant materials are discussed herewith. A normal phase HPTLC method has been used for simultaneous separation of flavonoids such as apigenin, luteolin, apigenin-7-O-glucoside and luteolin-7-O-glucoside from methanolic extract of aerial parts of *Platycodon grandiflorum* A. DC. Chromatographic separation of flavonoids was carried on Silica gel TLC plate with mobile phase comprising toluene: ethyl formate: formic acid (5.0: 4.0: 1.0, v/v/v). After development, the plate was analysed under UV (254 nm and 366 nm) light before and after spraying with 2% $AlCl_3$ [9]. Another normal phase HPTLC method has been reported for simultaneous quantitation of fourteen flavonoids including luteolin and apigenin from *Woodfordia fruticosa, Adhatoda vasica, Chenopodium ambrosoides, Viburnum cotinifolium, Euphorbia hirta, Vitex negundo, Peganum harmala, Broussonetia papyrifera, Taraxacum officinale, Urtica dioica, Verbascum thapsus, Caryopteris grata and Mimosa rubicaulis*. Chromatographic separation of flavonoids was carried on Silica gel TLC plate, with mobile phase comprising of water: acetic acid: butanol (5.0: 1.0: 4.0, v/v/v).

After development, the plate was sprayed with 1% ethanolic 2-aminoethyl diphenyl borinate solution followed by ethanolic solution of polyethylene glycol- 400 and visualized under UV (254 nm and 365 nm) light [10]. A reverse phase HPTLC method has also

been developed for simultaneous quantification of flavonoids apigenin, luteolin, quercetin and quercitrin from *Bauhinia variegata, Bacopa monnieri, Centella asiatica, Ginkgo biloba, Lonicera japonica, Rosa bourboniana, Rosa brunonii,* and *Rosa damascena*. The HPTLC of flavonoids was performed on RP-18 F_{254} TLC plates with dual run, water (5% formic acid)/methanol (70:30) and water (5% formic acid)/methanol (50:50) as mobile phases. Densitometric determination of flavonoids was performed at λ = 280 nm in reflectance/absorbance mode [11].

However, no HPTLC method has been reported for simultaneous quantitation of luteolin and apigenin from dried leaf powder of *Cardiospermum halicacabum* Linn. and dried seed hull powder of *Hydnocarpus pentandra* (Buch.-Ham.) Oken.

Therefore, in the present research work, a normal phase high performance thin layer chromatographic method was developed and validated using International Conference on Harmonization (ICH) guidelines for simultaneous quantitation of luteolin and apigenin from dried leaf powder of *Cardiospermum halicacabum* Linn. and dried seed hull powder of *Hydnocarpus pentandra* (Buch.-Ham.) Oken.

MATERIALS AND METHODS

Experimental reagents

Analytical grade methanol (99.9%), chloroform (99.4%) and formic acid (99.0%) were procured from Qualigens Fine Chemicals (Mumbai, India).

TLC plates, precoated with Silica gel 60F_{254}, with aluminium sheet support were obtained from E. Merck (India).

Reference standards

The reference standards, luteolin (purity ≥ 98%) and apigenin (purity ≥ 95%), were purchased from Sigma-Aldrich Chemie GmbH (Aldrich Division, Steinbeim, Germany).

Plant material

Leaves of *Cardiospermum halicacabum* Linn. were procured from Keshav Shrushti, Mumbai, India. Seed hulls were separated from fruits of *Hydnocarpus pentandra* (Buch.-Ham.) Oken which were procured from Castle Rock, Karnataka, India. Both plant materials were authenticated from Botanical Survey of India (BSI), Pune, India. The authenticated herbarium of each plant is preserved in duplicate, one by Botanical Survey of India and one at the place of research, i.e., Ramnarain Ruia College, Mumbai, India, for future reference.

The plant materials were washed with water and dried in a hot air oven at temperature less than 45°C ± 2°C [12]. The dried plant materials were then finely powdered and sieved through BSS mesh size 85 and stored in separate airtight containers at room temperature (25°C± 2°C).

Preparation of solutions

Preparation of stock solution and working solution of standard luteolin (1000 µg/mL)

Stock solution of 1000 µg/mL of luteolin was prepared by dissolving 10.0 mg of accurately weighed luteolin in 5.0 mL of methanol in a 10.0 mL standard volumetric flask. The standard volumetric flask was then sonicated in an ultrasonic bath (Model: TRANS-O-SONIC, Frequency: 50 Hz) for 5.0 minutes for complete dissolution of luteolin. The contents were then diluted up to the mark with methanol to obtain a solution of 1000.0 µg/mL of luteolin. 0.50 mL of the above stock solution of luteolin was then transferred to 50.0 mL volumetric flask and the contents were diluted to 50.0 mL using methanol to obtain working solution of standard luteolin with concentration 10.0 µg/mL.

Preparation of stock solution and working solution of standard apigenin (1000 µg/mL)

Stock solution of 1000 µg/mL of apigenin was prepared by dissolving 10.0 mg of accurately weighed apigenin in 5.0 mL of methanol in a 10.0 mL standard volumetric flask. The standard volumetric flask was then sonicated in an ultrasonic bath (Model: TRANS-O-SONIC, Frequency: 50 Hz) for 5.0 minutes for complete dissolution of luteolin. The contents were then diluted up to the mark with methanol to obtain a solution of 1000.0 µg/mL of apigenin. 0.25 mL of the above stock solution of apigenin was then transferred to 50.0 mL volumetric flask and the contents were diluted to 50.0 mL using methanol to obtain working solution of standard apigenin with concentration 5.0 µg/mL.

Preparation of sample solutions

Sample solution of dried leaf powder of *Cardiospermum halicacabm* Linn

About 1.000 g of dried leaf powder of *Cardiospermum halicacabm* Linn. was accurately weighed and transferred to a stoppered conical flask and 10.0 mL of methanol was then added to it. The flask was then shaken at 50 rpm, on a conical flask shaker overnight at room temperature (25°C± 2°C). The contents of the flask were filtered through Whatman No.41 filter paper (E. Merck, Mumbai, India). The stock sample solution was stored in a glass stoppered tube. 1.0 mL of the stock sample solution was then transferred to a 10.0 mL standard volumetric flask and the contents of the flask were diluted upto the mark using methanol. This solution was further used as the sample solution for the assay experiment. The sample solution was filtered through 0.45µm filter paper before analysis.

Sample solution of dried seed hull powder of *Hydnocarpus pentandra* (Buch.-Ham.) Oken

About 1.000 g of dried seed hull powder of *Hydnocarpus pentandra* (Buch.-Ham) Oken was accurately weighed and transferred to a stoppered conical flask and 10.0 mL of methanol was then added to it. The flask was then shaken at 50 rpm, on a conical flask shaker overnight at room temperature (25°C± 2°C). The contents of the flask were filtered through Whatman No.41 filter paper (E. Merck, Mumbai, India). The filtrate was further used as sample solution for the assay experiment. The sample solution was filtered through 0.45µm filter paper before analysis.

Preparation of mobile phase

The mobile phase used in the present research work was prepared by mixing chloroform, methanol and formic acid in volume ratio of 8.0: 2.0: 1.0. It was then sonicated for 5 minutes.

Chromatographic conditions

Chromatographic separation was carried out on aluminium plates (20cm x 20cm), precoated with Silica Gel 60 F_{254}, with thickness of 200 µm. Sample solution and standard solutions of different concentrations were applied to plates, as 6 or 8 mm bands at 10 mm from the bottom edge of the plate by means of CAMAG Linomat V Automatic sample applicator fitted with a 100µL syringe (Hamilton, Bonaduz, Switzerland).

The plates were developed in a CAMAG (Muttenz, Switzerland) glass twin-trough chamber saturated with mobile phase comprising of chloroform: methanol: formic acid in the volume ratio of 8.0: 2.0: 1.0. The optimized chamber saturation time for the mobile phase was 20 minutes at room temperature (25°C± 2°C).

The plates were developed to a distance of 90 mm from the bottom edge of the plate. The plates were air dried for 10 min after development and scanned at λ=349 nm in absorbance/reflectance mode, using a CAMAG TLC Scanner III and winCATS software version 1.4.2.

METHOD VALIDATION

Linearity

Linear working range for luteolin

The linear working range of luteolin was determined by applying 10.0µg/mL of standard solution of luteolin in the volume range of 2µL, 4µL, 6µL, 8µL, 10µL, 12µL, 14µL, 16µL, 18µL, 20µL and 22µL as bands on TLC plate to obtain concentrations in the range of 0.02µg/band to 0.22µg/band.

Linear working range for apigenin

The linear working range of apigenin was determined by applying 5.0µg/mL of standard solution of apigenin, in the volume range of 6µL, 8µL, 10µL, 12µL, 14µL, 16µL, 18µL, 20µL and 22µL as bands on TLC plate to obtain concentrations in the range of 0.03µg/band to 0.11µg/band. Calibration curves of luteolin and apigenin were obtained by plotting mean peak areas against corresponding concentrations. Good correlation was obtained between mean peak areas and corresponding concentrations for both the standards. The results are listed in Table 1.

Limit of Detection (LOD) and Limit of Quantitation (LOQ)

The limit of detection (LOD) and limit of quantitation (LOQ) were determined at signal to noise ratios of 3:1 and 10:1, respectively. The LOD and LOQ values obtained for both the components are listed in Table 1.

System suitability

The system suitability test was carried out to confirm that the system used for the analysis gives precise, accurate and reproducible results. System suitability test was carried out by applying standard solutions of luteolin with concentration of 0.06µg/band and apigenin with concentration of 0.07µg/band on same TLC plate in six replicates under specified chromatographic conditions. The chromatograms for both standards were recorded.

The values of percent relative standard deviation (%R.S.D) for peak area and retention factor (R_f) of luteolin and apigenin were found to be less than 2, indicating that the method was suitable for analysis.

Precision

The precision of the method was studied by determining repeatability and intermediate precision.

Repeatability was carried out by analysing three different sample solutions of each plant material on same day. Each sample solution was applied in triplicate on same TLC plate and analysed using optimised chromatographic conditions. The peak areas of luteolin and apigenin were recorded for each applied sample solution of *Cardiospermum halicacabum* Linn. and *Hydnocarpus pentandra* (Buch.-Ham.) Oken.

Intermediate precision was carried out on three successive days. Each sample solution was applied in triplicate on a TLC plate each day and analyzed using the optimized chromatographic conditions. The peak areas of luteolin and apigenin were recorded for each applied sample solution of *Cardiospermum halicacabum* Linn. and *Hydnocarpus pentandra* (Buch.-Ham.) Oken.

The precision results were expressed as percentage relative standard deviations of peak areas of luteolin and apigenin and are listed in Table 2. The results indicate that the proposed method is precise and reproducible.

Stability of the standard luteolin and apigenin solution

The stabilities of standard luteolin and apigenin solution were determined by comparing the peak areas of standard solution of luteolin and apigenin at different time intervals, for a period of minimum 48 hours, at room temperature. Standard solutions of luteolin with concentration of 0.06µg/band and apigenin with concentration of 0.07µg/band were applied as bands on same TLC plate at different time intervals and analyzed under the optimized chromatographic conditions. Low values of percent relative standard deviation (less than 2) for peak areas of luteolin and apigenin indicated that the peak area values remained unchanged over a period of 48 hours and no significant degradation was observed within the given period, indicating that standard solutions of luteolin and apigenin were stable for a period of minimum 48 hours.

Assay

The validated HPTLC method was used for simultaneous quantitation of luteolin and apigenin from the sample solutions of dried leaf powder of *Cardiospermum halicacabum* Linn. and dried seed hull powder of *Hydnocarpus pentandra* (Buch.-Ham.) Oken.

10µL of methanolic extracts of dried leaf powder of *Cardiospermum halicacabum* Linn. and dried seed hull powder of *Hydnocarpus pentandra* (Buch.-Ham.) Oken. were applied as bands on the TLC plate. The plate was developed using optimized chromatographic conditions. The chromatograms were obtained. To ascertain the repeatability of the method, the assay experiment was repeated seven times. The values of amounts of luteolin and apigenin present in leaf powder of *Cardiospermum halicacabum* Linn. and seed hull powder of *Hydnocarpus pentandra* (Buch.-Ham.) Oken, values of standard deviation (S.D.) and the percent relative standard deviation (%R.S.D.) were calculated. Amounts of luteolin and apigenin present in the sample solutions were determined from their respective calibration curves, by using the peak areas of luteolin and apigenin recorded for both the sample solutions. The results of assay experiment are listed in Table 3.

Accuracy

The accuracy of the method was established by performing recovery experiment by using standard addition method at three different levels.

To about 1.000g of dried leaf powder of *Cardiospermum halicacabum* Linn., known amounts of luteolin (0.25, 0.50 and 0.75 mg) and apigenin (0.025, 0.050 and 0.075 mg) were added and the sample solution was prepared in methanol as described earlier.

To about 1.000g of dried seed hull powder of *Hydnocarpus pentandra* (Buch.-Ham.) Oken, known amounts of luteolin (0.025, 0.050 and 0.075 mg) and apigenin (0.020, 0.040 and 0.060 mg) were added and the sample solution was prepared in methanol as described earlier.

Each of the three different levels containing sample solution and standard were applied in seven replicates on the same plate. The plate was then developed and scanned under the specified chromatographic conditions, as described earlier. The values of amounts of luteolin and apigenin found in each level were determined in a manner similar to that in the assay experiment. The percent recovery values of luteolin and apigenin from both the sample solution were then calculated. The results of accuracy are listed in Table 3.

RESULTS

Different mobile phases were tried in order to resolve flavones, luteolin and apigenin from methanolic extracts of dried leaf powder of *Cardiospermum halicacabum* Linn. and dried seed hull powder of *Hydnocarpus pentandra* (Buch.-Ham.) Oken. However, the mobile phase comprising of chloroform: methanol: formic acid, 8.0: 2.0: 1.0 (v/v/v), resulted in a good separation of luteolin (R_f = 0.37) and apigenin (R_f = 0.49) from one another and also from other phytochemicals present in the methanolic extracts of selected plant materials. Detection was carried out densitometrically at λ = 349 nm as both luteolin and apigenin showed maximum response at this wavelength. The identity of the bands of luteolin and apigenin in the sample solution was confirmed by comparing their R_f value in sample with that of reference standards. Figure 1 represents a typical TLC plate and Figure 2 represents an overlay HPTLC chromatogram showing separation of luteolin and apigenin from methanolic extracts of leaf powder of *Cardiospermum halicacabum* Linn. and seed hull powder of *Hydnocarpus pentandra* (Buch.-Ham.) Oken. The developed method was validated in terms of precision, repeatability and accuracy. The linearity of luteolin was found to be in the range 0.02µg/band to 0.22µg/band, with correlation coefficient as 0.999. The linearity of apigenin was found to be in the range 0.03µg/band to 0.11µg/band, with correlation coefficient as 0.999. The proposed HPTLC method was found to be precise with percent relative standard deviation values for repeatability and intermediate precision being less than 2 indicating that the proposed method is precise and reproducible. The mean amounts of luteolin and apigenin present in dried leaf powder of *Cardiospermum halicacabum* Linn. are 0.2119mg/g and 0.9089mg/g (Table 3). The mean amounts of luteolin and apigenin present in dried seed hull

powder of *Hydnocarpus pentandra* (Buch.-Ham.) Oken are 0.0696mg/g and 0.0320mg/g (Table 3). The mean percent recoveries, at three different levels of luteolin and apigenin, from dried leaf powder of *Cardiospermum halicacabum* Linn. were 98.94 and 98.40 respectively (Table 3). The mean percent recoveries, at three different levels of luteolin and apigenin, from dried seed hull powder of *Hydnocarpus pentandra* (Buch.-Ham.)Oken. were 98.35 and 99.16 respectively (Table 3).

Table 1: Results of Method validation

Parameters	Observations	
	Luteolin	**Apigenin**
Linear Dynamic Range (µg/band)	0.01 - 0.30	0.01 - 0.15
Linear Working Range (µg/band)	0.02 - 0.22	0.03 - 0.11
Correlation coefficient (r)	0.999	0.999
Limit of Detection (LOD) (µg/band)	0.01	0.01
Limit of Quantitation (LOQ) (µg/band)	0.02	0.03

Table 2: Results of Precision

Precision	Luteolin	Apigenin
Repeatability (% R.S.D.)		
Leaf powder of *Cardiospermum halicacabum* Linn. (n=3) (on the same day)	1.06	0.92
Seed hull powder of *Hydnocarpus pentandra* (Buch.-Ham.) Oken (n=3) (on the same day)	1.04	1.06
Intermediate Precision (% R.S.D.)		
Leaf powder of *Cardiospermum halicacabum* Linn. (n=9) (For three successive days)	1.02	0.92
Seed hull powder of *Hydnocarpus pentandra* (Buch.-Ham.) Oken (n=9) (For three successive days)	1.03	1.03

Table 3: Results of Assay and Accuracy

Parameters	Luteolin	Apigenin
Assay (mg/g) (n=7)*		
Leaf powder of *Cardiospermum halicacabum* Linn.	0.2119±0.0020	0.9089±0.0080
Seed hull powder of *Hydnocarpus pentandra* (Buch.-Ham.) Oken	0.0696±0.0010	0.0320±0.0003
Percent Recovery (n=7)		
Leaf powder of *Cardiospermum halicacabum* Linn.	98.94	98.40
Seed hull powder of *Hydnocarpus pentandra* (Buch.-Ham.) Oken	98.35	99.16

*Mean±S.D. (n=7)

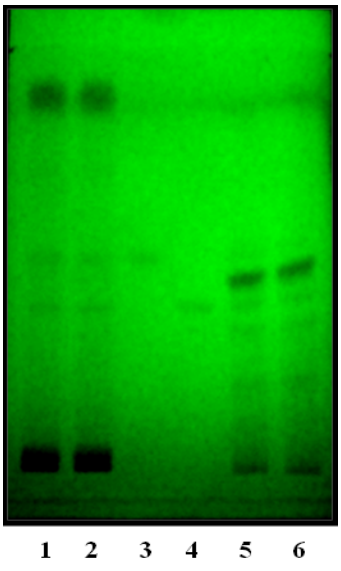

Fig. 1: TLC plate showing separation of standard apigenin (3), standard luteolin (4) and methanolic extracts of dried leaf powder of *Cardiospermum halicacabum* Linn. (1 and 2) and dried seed hull powder of *Hydnocarpus pentandra* (Buch.-Ham.)Oken (5 and 6)

Track 1 and 2 show separation of methanolic extract of dried leaf powder of *Cardiospermum halicacabum* Linn. Track 3 represent standard apigenin and track 4 represents standard luteolin. Track 5 and 6 show separation of methanolic extract of dried seed hull powder of *Hydnocarpus pentandra* (Buch.-Ham.)Oken.

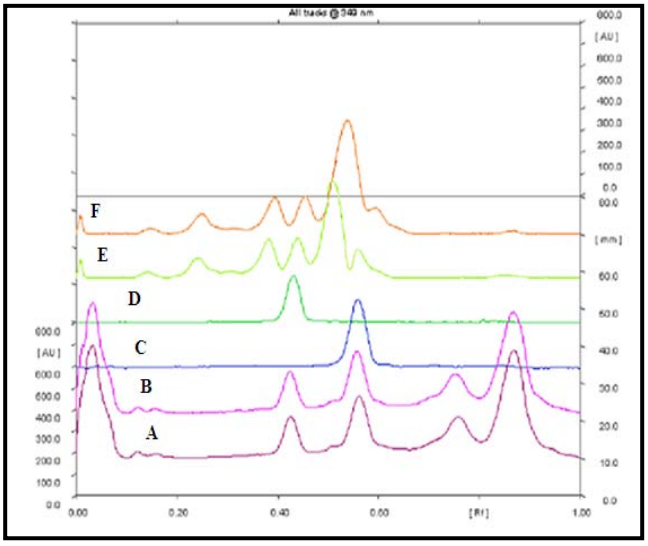

Fig. 2: Overlay chromatogram obtained for standard luteolin, standard apigenin and two plant samples at λ=349nm.

Figure 2 shows overlay chromatogram of methanolic extract of leaf powder of *Cardiospermum halicacabum* Linn. (A and B); standard apigenin (C); standard luteolin (D) and methanolic extract of seed hull powder of *Hydnocarpus pentandra* (Buch.-Ham.) Oken (E and F).

DISCUSSION

HPTLC due to its simplicity and minimum sample clean-up requirement, has been widely used as a quality control tool for the phytochemical evaluation of herbal drugs. Literature survey revealed that very little work using HPTLC has been reported on the plants selected for present research work. Therefore, in the present work, a simple and precise high performance thin layer chromatographic method was developed for simultaneous separation of luteolin and apigenin from methanolic extracts of dried leaf powder of *Cardiospermum halicacabum* Linn. and dried seed hull powder of *Hydnocarpus pentandra* (Buch.-Ham.) Oken. The developed method was further validated and used for the quantitation of these compounds.

A normal phase HPTLC method has been reported in the literature for simultaneous separation of flavonoids such as apigenin, luteolin, apigenin-7-O-glucoside and luteolin-7-O-glucoside from methanolic extract of aerial parts of *Platycodon grandiflorum* A. DC [9]. The reported HPTLC method was not used for quantitation of these flavonoids.

However, in the present research work, the developed HPTLC method has been validated in accordance with ICH guidelines for quantitation of luteolin and apigenin from leaves powder of *Cardiospermum halicacabum* Linn. and seed hull powder of *Hydnocarpus pentandra* (Buch.-Ham.) Oken.

In HPTLC method reported for quantitation of flavonoids including luteolin and apigenin from various medicinal plants, chromatographic analysis was carried out using Silica gel TLC plate with mobile phase comprising of water: acetic acid: butanol (5.0: 1.0: 4.0, v/v/v). After development, the plate was sprayed with 1% ethanolic 2-aminoethyl diphenyl borinate solution followed by ethanolic solution of polyethylene glycol- 400 and visualized under UV (254 nm and 365 nm) light [10].

In present developed HPTLC method, simultaneous quantitation of luteolin and apigenin from leaves powder of *Cardiospermum halicacabum* Linn. and seed hull powder of *Hydnocarpus pentandra* (Buch.-Ham.) Oken was achieved on TLC plates, precoated with Silica gel $60F_{254}$ using chloroform: methanol: formic acid (8.0: 2.0: 1.0, v/v/v) as mobile phase. The developed HPTLC method is advantageous compared to the reported method because in the developed HPTLC method, use of water as one of the mobile phase components is avoided since, normal phase chromatographic technique is used. If the mobile phase contains water as one of the components, it takes longer time for the mobile phase to ascend the polar stationary phase and drying of the plate also takes a longer time. Therefore, it makes the development process time consuming.

A reverse phase HPTLC method reported for simultaneous quantification of flavonoids apigenin, luteolin, quercetin and quercitrin from *Bauhinia variegata, Bacopa monnieri, Centella asiatica, Ginkgo biloba, Lonicera japonica, Rosa bourboniana, Rosa brunonii*, and *Rosa damascena* uses RP-18 F_{254} TLC plates with dual run, water (5% formic acid)/methanol (70:30) and water (5% formic acid)/methanol (50:50) as mobile phases [11]. However, in the present research work, the separation of luteolin and apigenin was achieved using normal phase TLC Silica gel 60 F_{254} plate in single run thus, making the developed HPTLC method, cost effective and less time consuming compared to the above reported method. Therefore, in the present research work, a simple normal phase High Performance Thin Layer Chromatographic method has been developed for simultaneous quantitation of luteolin and apigenin from leaves of *Cardiospermum halicacabum* Linn. and seed hulls of *Hydnocarpus pentandra* (Buch.-Ham.) Oken. The present developed method is the only HPTLC method used for simultaneous quantitation of apigenin and luteolin from methanolic extracts of dried leaf powder of *Cardiospermum halicacabum* Linn. and dried seed hull powder of *Hydnocarpus pentandra* (Buch.-Ham.) Oken.

CONCLUSION

A High Performance Thin Layer Chromatographic method has been developed and validated for simultaneous quantitation of flavone aglycones, luteolin and apigenin from methanolic extracts of dried leaf powder of *Cardiospermum halicacabum* Linn. and dried seed hull powder of *Hydnocarpus pentandra* (Buch.-Ham.) Oken.

The developed method is simple, precise and accurate and can also be used for routine quality control analysis and for the quantitation of luteolin and apigenin in herbal raw materials as well as in their formulations.

CONFLICT OF INTEREST

The authors declare no conflict of interest

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