Research Paper

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High Performance Liquid Chromatographic method for simultaneous estimation of α-amyrin, β-sitosterol and lupeol from dried whole plant powder of Leptadenia reticulata (Retz.) Wight & Arn. and Pluchea lanceolata (DC.) CB. Clarke

Chemistry

KEYWORDS : Leptadenia reticulata (Retz.) Wight & Arn. Pluchea lanceolata (DC.) CB. Clarke. alpha-amyrin beta-sitosterol lupeol method validation

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ABSTRACT

A simple reversed phase high performance liquid chromatographic (RP-HPLC) method was established for simultaneous determination of alpha-amyrin, beta-sitosterol and lupeol in the two medicinally important plants, Leptadenia reticulata Wight & Arn. and Pluchea lanceolata (DC.) CB. Clarke. respectively. A good linear regression relationship between peak areas and concentrations was obtained over the range of 0.1µg/mL to 500µg/mL for three phytochemicals with correlation coefficient of more than 0.998 for all three phytochemicals. Precision, specificity and robustness studies were carried out, values less than two were observed. Recovery values from 98.15 to 98.94 indicated accuracy of the method was good.

1. INTRODUCTION

Leptadenia reticulata (Retz.) Wight & Arn. belonging to family Asclepiadaceae, commonly known as 'Jivanti' is rich source of biologically active cardiac[1] and pregnane glycosides[2] which are known to possess anti-tumor and anticancer activity[3,4]. Previous chemical studies of the plant showed the presence of flavonoids, triterpenes and steroids[5,6].

Pluchea lanceolata (DC.) CB. Clarke. belonging to family Asteraceae, commonly known as 'Rasna' is an important xerophytic medicinal herb. It is a succulent, erect plant, traditionally used for dyspepsia, bronchitis and rheumatoid arthritis[7,8]. In Ayurveda, the plant is used as antipyretic, bitter, laxative and nervine tonic[9]. All parts of the plant are extensively used in indigenous system of medicine. The plant contains high amounts of medicinally important secondary metabolites *viz.* quercetin, beta-sitosterol, triterpenol, etc., which give it anti-inflammatory and analgesic properties[10,11].

Beta-sitosterol is a main phyto-sterol found in many plants including rice, wheat, corn, nut, peanut and more. Beta-sitosterol is reported to show activity as hepatoprotective, antioxidant and antipyretic, inflammatory disorders and is used for treatment of rheumatoid arthritis, colon cancer, benign prostatic hypertrophy and breast cancer[12].

Triterpenoids are among commonly present secondary metabolites in plants. Alpha-amyrin has been reported to show anti-inflammatory properties. Lupeol has been reported to show anticarcinogenic and anit-tumor activity[13].

A RP-HPLC method was reported[14] for separating and determining alpha-amyrin, beta-amyrin and lupeol from *Brassica oleracea* L. leaf surface extract. However, no HPLC method is reported for the simultaneous quantification of alpha-amyrin, beta-sitosterol and lupeol from dried whole plant powder of *Leptadenia reticulata* (Retz.) Wight & Arn. and *Pluchea lanceolata* (DC.) CB. Clarke.

Thus, in present research work, a simple, rapid, precise and accurate HPLC method has been developed and validated using International Conference on Harmonization (ICH) guidelines for simultaneous determination and quantification of alpha-amyrin, beta-sitosterol and lupeol from dried whole plant powder of *Leptadenia reticulata* (Retz.) Wight & Arn. and *Pluchea lanceolata* (DC.) CB. Clarke.

2. EXPERIMENTAL METHODS

2.1. Materials

2.1.1. Standard and reagents:

Reference standards alpha-amyrin (purity 99.3%), beta-sitosterol (purity 99.8%) and lupeol (purity 99.7%) were procured from Sigma-Aldrich Chemie GmbH (Aldrich Division, Steinbeim, Germany).

The solvents used in the analysis were of HPLC grade. Acetonitrile (purity 99.0%), distilled water (HPLC grade) and methanol (purity 99.0%) were obtained from LiChrosolv Merck, India.

2.1.2.. Plant material

Whole plants of *Leptadenia reticulata* (Retz.) Wight & Arn. and *Pluchea lanceolata* (DC.) CB. Clarke. were collected from Bhumel village, Dist- Nadiad, Gujarat and Sonmukhi Nagar, Jodhpur respectively. The sample voucher of *Leptadenia reticulata* (Retz.) Wight & Arn. and herbarium of *Pluchea lanceolata* (DC.) CB. Clarke. were submitted to and authenticated by Agarkar Research Institute, Pune, India (Voucher No. WP-090) and Botanical Survey of India, Pune, India (Certificate No. BSI/WRC/Tech./2012/79.) respectively. Whole plants of *Leptadenia reticulata* (Retz.) Wight & Arn. and *Pluchea lanceolata* (DC.) CB. Clarke. were washed with water to remove soil particles. The washed plant material of *Leptadenia reticulata* (Retz.) Wight & Arn. and *Pluchea lanceolata* (DC.) CB. Clarke. were dried in shade, finely powdered, sieved through BSS mesh size no.85 and stored in an airtight container at room temperature (25°C ± 2°C).

2.1.3. Preparation of standard stock solution of alpha-amyrin, beta-sitosterol & lupeol (10000 µg/mL*)*

About 100.0 mg of alpha-amyrin was accurately weighed and transferred to 10.0 mL volumetric flask. 5.0 mL of methanol solution was added into the volumetric flask and sonicated in an ultrasonic bath (Model: TRANS-O-SONIC, Frequency: 50 Hz) for 5 minutes for complete dissolution of alpha-amyrin. The volume was then made up to the mark with methanol. A stock solution of alpha-amyrin with concentration of 10000.0 µg/mL was thus, prepared.

A similar procedure was followed for preparation of standard stock solution of beta-sitosterol & lupeol

2.1.4. Preparation of sample solutions

About 2.0 g of dried whole plant powder of *Leptadenia reticulata* (Retz.) Wight and Arn. was accurately weighed and transferred to a 100 mL stoppered conical flask. 75.0 mL of methanol was added to it and the flask was sonicated in an ultrasonic bath for 15 minutes. The flask was then shaken at 50 rpm, on a conical flask shaker overnight at room temperature (28 ± 2°C). Sample was filtered through Whatman filter paper no.1. The filtrate was collected in a beaker and then evaporated on a hot water bath. The final volume was then made up to 50 mL with methanol in a 50 mL volumetric flask. The obtained extract was then filtered with 0.45µm nylon filters (Millipore) before analysis. The same procedure was followed for preparation of extract of whole plant of *Pluchea lanceolata* (DC.) CB. Clarke.

2.2. Instrumentation

2.2.1. Chromatographic conditions

HPLC Absorption spectra was recorded with a shimadzu prominence diode array detector SPD-M20A. PC with LC software version 1.24 (Shimadzu, Kyoto, Japan) consisted of Shimadzu Prominence LC - 20AD with Prominence solvent delivery module, a Prominence Autosampler SIL-20AC HT and a SPD-M20A Prominence Diode array detector. The separation was performed on Purespher® Star RP-18e Hibar® (250 mm X 4.6 mm, 5.0µm particle size) with the column temperature at 40°C. Chromatographic data was recorded and processed using a LC software version 1.24 SP2.

3. Method validation

3.1. Linearity

Each standard solution of alpha-amyrin, beta-sitosterol, lupeol in the concentration range of 0.1 µg/mL to 500.0 µg/mL was injected in triplicates into the chromatographic system, under optimized chromatographic conditions. The peak areas were recorded for each injected concentration of standards. The calibration curves of each of the three standards alpha-amyrin, beta-sitosterol & lupeol were obtained by plotting graphs of mean peak areas vs. corresponding concentrations. The results are listed in table.1

3.2. Limit of detection (LOD) and Limit of quantitation (LOQ)

The Limit of Detection (LOD) and Limit of Quantification (LOQ) were determined at signal to noise ratios of 3:1 and 10:1 respectively. The LOD and LOQ values obtained for all components are listed in Table 1.

3.3. Precision

The method was validated in terms of repeatability and intermediate precision.

Repeatabilty was carried out in the same laboratory, on the same day, by analyzing sample solution of dried whole plant powder of *Leptadenia reticulata* (Retz.) Wight & Arn. and *Pluchea lanceolata* (DC.) CB. Clarke. in triplicate.

The intermediate precision was carried out in same laboratory, but on three successive days.

The values of mean peak areas of alpha-amyrin, beta-sitosterol and lupeol, standard deviation (S.D.) & percent relative standard deviation (% R.S.D.) for both the plants were calculated.

The results obtained are depicted in Table 2

The results indicate that the method is precise and reproducible.

Table 1
Method validation parameters for the estimation of α-amyrin, β-sitosterol & Lupeol by the proposed HPLC method

Plant Name	Leptadenia reticulata (Retz.) Wight & Arn.			Pluchea lanceolata (DC.) CB. Clarke.		
Standard	α-amyrin	β-sitosterol	lupeol	α-amyrin	β-sitosterol	lupeol
Repeatability (% RSD) (n=3) on the same day	0.93	0.97	0.98	0.97	0.94	0.97
Intermediate precision (%RSD) (n=9) for three successive days	0.98	0.95	0.97	0.96	0.98	0.95

Table-2
% RSD for of dried whole plant powder of *Leptadenia reticulata* (Retz.) Wight & Arn. and *Pluchea lanceolata* (DC.) CB. Clarke. is as follows:

Standards	α-amyrin	β-sitosterol	Lupeol
Linear range (µg/mL) (n=6)	0.100 to 500.00	0.100 to 500.00	0.100 to 500.00
Correlation coefficient (r)	0.9998	0.998	0.9998
LOD (µg/mL)	0.80	0.41	0.43
LOQ (µg/mL)	2.43	1.24	1.29

3.4. System suitability

System suitability was carried out to verify that resolution and reproducibility of the system were acceptable for the analysis.

System suitability was determined by injecting, mixture of working standard solution of alpha-amyrin, beta-sitosterol and lupeol, each of concentration 100 µg/mL, six times, into the chromatographic system, under the optimized chromatographic conditions.

The chromatograms were recorded for each injected solution and the peak area values and the retention times of alpha-amyrin, beta-sitosterol and lupeol were noted. The system suitability parameters like retention time, reproducibility, instrument response, peak tailing factor, peak resolution and column efficiency were evaluated and noted for each injected concentration of the standards alpha-amyrin, beta-sitosterol and lupeol. The results of the system suitability are presented in Table-3.

Table-3
Results of system suitability of standard solution of alpha-amyrin, beta-sitosterol and lupeol

System suitability parameter	Beta-sitosterol	Alpha-amyrin	Lupeol
Peak Tailing	1.02	1.13	1.14
Resolution between lupeol and alpha-amyrin	----	9.69	
Resolution between alpha-amyrin and beta-sitosterol	2.06		----
Column efficiency	195531.80	297977.34	269491.65
Mean peak area	160778.3	63702	304889
Retention factor	18.04	19.56	13.02

3.5 Specificity

The specificity of the proposed HPLC method was ascertained by injecting 10.0µL of blank solution to observe for interference, if any, with the peaks of interest in the chromatogram of the sample solution. It was observed that there is no interference from the blank solution. Methanol was taken as blank solution since standard and sample solutions were prepared in methanol.

The chromatograms were compared by overlay. Good correlation was observed for chromatograms obtained from alpha-amyrin, beta-sitosterol and lupeol standards and samples at all R_t positions. The peaks of alpha-amyrin, beta-sitosterol and lupeol standard were not masked by any peaks arising due to other components present in the sample solution (Figure 1,2,3)

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Figure 1
High Performance Liquid Chromatographic method for simultaneous estimation of α-amyrin, β-sitosterol and lupeol from dried whole plant powder of *Leptadenia reticulata* **(Retz.) Wight & Arn. and** *Pluchea lanceolata* **(DC.) CB. Clarke. HPLC chromatogram obtained for blank methanol**

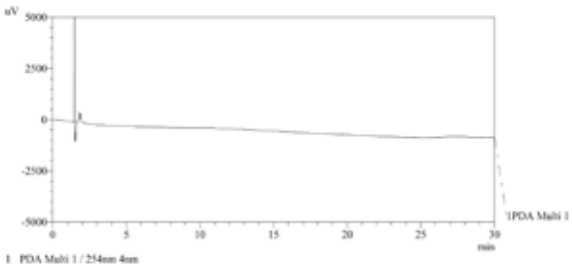


Figure 2
High Performance Liquid Chromatographic method for simultaneous estimation of α-amyrin, β-sitosterol and lupeol from dried whole plant powder of *Leptadenia reticulata* **(Retz.) Wight & Arn. and** *Pluchea lanceolata* **(DC.) CB. Clarke. HPLC chromatogram obtained for mobile phase**

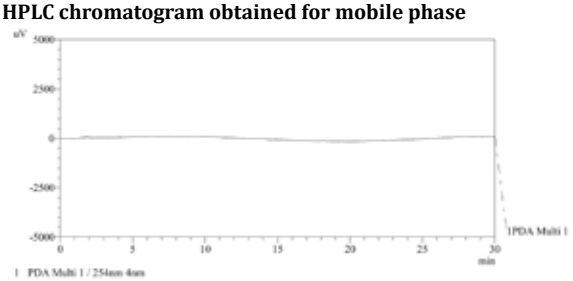
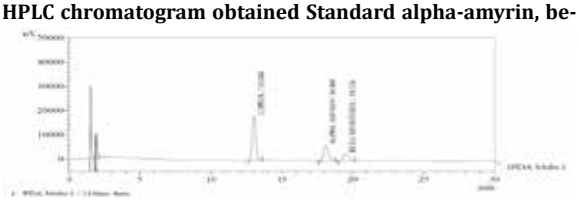


Figure 3
High Performance Liquid Chromatographic method for simultaneous estimation of α-amyrin, β-sitosterol and lupeol from dried whole plant powder of *Leptadenia reticulata* **(Retz.) Wight & Arn. and** *Pluchea lanceolata* **(DC.) CB. Clarke. HPLC chromatogram obtained Standard alpha-amyrin, beta-sitosterol and lupeol from dried whole plant powder of** *Leptadenia reticulata* **(Retz.) Wight & Arn. and** *Pluchea lanceolata* **(DC.) CB. Clarke.**



3.6 Robustness
The robustness of the method is a measure of the methods capacity to remain unaffected by small deliberate variations in the method parameters and provides an indication of reliability of the method during normal usage. The mobile phase composition was altered by ± 10% and flow rate of mobile phase was changed from 1.0 mL/min to ± 0.1 mL/min. The resolution between alpha-amyrin, beta-sitosterol , and lupeol and tailing factors from all three components from sample solution did not change much due to alteration in the methods. The amounts of alpha-amyrin, beta-sitosterol , and lupeol from whole plant powder of

Leptadenia reticulata (Retz.) Wight &Arn. and *Pluchea lanceolata* (DC.) CB. Clarke obtained by altered method and that obtained by normal method was found to be similar. The modifications did not affect system suitabilitycriteria. However, slight variations in the retention time were observed, which was due to changes made in the mobile phase composition and flow rate. From the observations it was concluded that the method is robust as the above mentioned deliberate changes made in the method did not affect the results.

3.7.Application of validated method for the simultaneous quantitation of α-amyrin, β-sitosterol and lupeol from dried whole plant powder of *Leptadenia reticulata* (Retz.) Wight & Arn. and *Pluchea lanceolata* (DC.) CB. Clarke.

The developed and validated HPLC method was used for quantitation of alpha-amyrin, beta-sitosterol and lupeol from whole plant powder of *Leptadenia reticulata* (Retz.) Wight &Arn. and *Pluchea lanceolata* (DC.) CB. Clarke. 10µL of the sample solution was injected into the chromatographic system. The identities of peaks of alpha-amyrin, beta-sitosterol and lupeol in the sample solution were confirmed by comparing the chromatogram of the sample (Figure 4 and Figure 5) with that of the standard solution of alpha-amyrin, beta-sitosterol and lupeol (Figure 6). Retention times of standards of alpha-amyrin, beta-sitosterol and lupeol standards were 18.101, 19.524 and 13.048 minutes respectively. The retention times of alpha-amyrin, beta-sitosterol and lupeol in *Leptadenia reticulata* (Retz.) Wight &Arn. sample solution were found to be 18.001, 19.391 and 12.978 minutes respectively. The retention times of alpha-amyrin , beta-sitosterol and lupeol in *Pluchea lanceolata* (DC.) CB. Clarke. sample solution were found to be 18.016, 19.207 and 12.982 minutes respectively.

To ascertain the repeatability of the method, the assay experiment was repeated seven times. Amount of alpha-amyrin, beta-sitosterol & lupeol found in 2000 mg of whole plant powder of Leptadenia reticulata (Retz.) Wight and Arn. were 0.96mg, 1.11 mg, 0.67 mg respectively and in Pluchea lanceolata (DC.) CB. Clarke were 0.73mg, 1.62mg 1.17mg respectively.

Figure-4
A typical chromatogram of standard solution of alpha-amyrin, beta-sitosterol and lupeol with concentration of 100µg/mL each

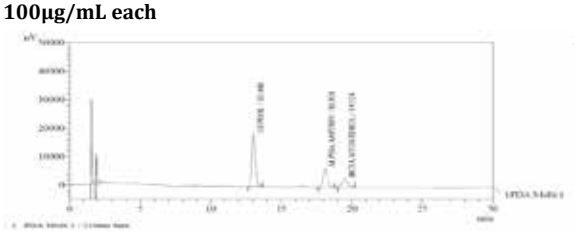


Figure-5
A TYPICAL CHROMATOGRAM FOR QUANTITATION OF ALPHA-AMYRIN, BETA-SITOSTEROL AND LUPEOL FROM THE EXTRACT OF DRIED WHOLE PLANT POWDER OF *LEPTADENIA RETICULATA* **(RETZ.) WIGHT &ARN.**

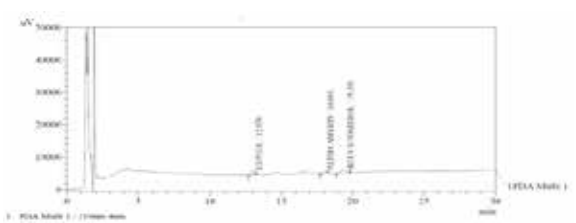
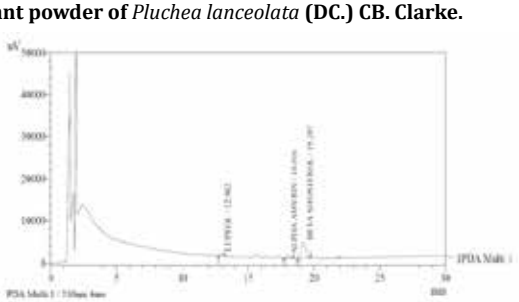


Figure-6
A typical chromatogram for quantitation of alpha-amyrin, beta-sitosterol and lupeol from the extract of dried whole plant powder of *Pluchea lanceolata* **(DC.) CB. Clarke.**



3.8. Recovery

The accuracy of the method was established by performing recovery experiment by using standard addition method at three different levels as shown in Table 4 & 5 . The Results of the accuracy are listed in Table. 6

Table 4

Results of recovery experiment of alpha-amyrin, beta-sitosterol & lupeol from dried whole plant powder of *Leptadenia reticulata* (Retz.) Wight &Arn.

Level	Amount of sample* (g)	Amount of alpha-amyrin added to sample (mg)	Mean amount of alpha-amyrin found (mg)	Amount of beta-sitosterol added to sample (mg)	Mean amount of beta-sitosterol found (mg)	Amount of lupeol added to sample (mg)	Mean amount of lupeol found (mg)
0	2.013	0.000	0.960	0.000	1.110	0.000	0.6700
1	2.009	0.024	0.983	0.040	1.149	0.008	0.6780
2	2.010	0.030	0.989	0.050	1.159	0.010	0.6799
3	2.011	0.036	0.996	0.060	1.169	0.012	0.6819

*** Sample: Whole plant powder of *Leptadenia reticulata* (Retz.) Wight and Arn.**

Table 5

Results of recovery experiment of alpha-amyrin, beta-sitosterol & lupeol from dried whole plant powder of *Pluchea lanceolata* (DC.) CB. Clarke.

Level	Amount of sample* (g)	Amount of beta-sitosterol added to sample (mg)	Mean amount of alpha-amyrin found (mg)	Amount of beta-sitosterol added to sample (mg)	Mean amount of beta-sitosterol found (mg)	Amount of lupeol added to sample (mg)	Mean amount of lupeol found (mg)
0	2.012	0.000	0.736	0.000	1.62	0.000	0.170
1	2.008	0.024	0.759	0.040	1.66	0.008	0.178
2	2.013	0.030	0.765	0.050	1.67	0.010	0.180
3	2.010	0.036	0.772	0.060	1.68	0.012	0.182

*** Sample: Whole plant powder of *Pluchea lanceolata* C.B. Clarke.**

4. RESULTS

The method developed to quantify α-amyrin, β-sitosterol and lupeol from dried whole plant powders of *Leptadenia reticulata* (Retz.) Wight & Arn. and *Pluchea lanceolata* (DC.) CB. Clarke. was validated and the results are tabulated in Table 6.

Table 6

High Performance Liquid Chromatographic method for simultaneous estimation of α-amyrin, β-sitosterol and lupeol from dried whole plant powder of *Leptadenia reticulata* (Retz.) Wight & Arn. and *Pluchea lanceolata* (DC.) CB. Clarke.

Parameters	Results		
	Alpha-amyrin	Beta-sitosterol	Lupeol
Linear Dynamic Range (µg/mL)	0.1-1000.0	0.1-1000.0	0.1-1000.0
Linear range (µg/mL)	0.1-500.0	1.0-500.0	0.1-500.0
Correlation coefficient (r)	0.999	0.998	0.999
LOD (µg/ mL)	0.80	0.41	0.43
LOQ (µg/ mL)	2.43	1.24	1.29
System suitability (% R.S.D.)	Less than 2	Less than 2	Less than 2
Leptadenia reticulata (Retz.) Wight & Arn.			
Precision	Less than 2		
Assay (mg/2g)	0.96	1.11	0.67
Percent Recovery	98.80	98.19	98.86
Pluchea lanceolata (DC.) CB. Clarke.			
Precision	Less than 2		
Assay (mg/2g)	0.73	1.62	0.17
Percent Recovery	98.15	98.94	98.23

5. DISCUSSION

A RP-HPLC method was reported[14] for separating and determining alpha-amyrin, beta-amyrin and lupeol from *Brassica oleracea* L. leaf surface extract. The separation was done on Thermo Fisher Scientifics Hypersil BDS C18 column (250 mm x 3 mm, 3 µm) with the mobile phase as acetonitrile, at a flow rate of 0.8 mL/min. The detection here was carried out at λ=200nm.

In a reported method[15], chromatographic separation of twelve compounds including alpha-amyrin, lupeol and beta-sitosterol from *Brassica oleracea* L., *Solanum lycopersicum* L., *Rosmarinus officinalis* L., *Salvia officinalis* L . and *Quercus robur* L. was studied. The study describes RP-HPTLC and RP-HPLC methods for a qualitative determination of twelve compounds (alpha-amyrin, beta-amyrin, delta-amyrin, lupeol, lupenon, lupeol acetate, cycloartenol, cycloartenol acetate, ursolic acid, oleanolic acid, stigmasterol, beta-sitosterol) and evaluation of their presence in different plant extracts. In the study, a reverse phase HPLC method has been reported. The experiment was performed on Thermo Fisher Scientifics Hypersil BDS C18 column (250 mm x 3 mm, 3 µm), using mobile phase comprising 6.5 % water in acetonitrile at a flow rate of 0.8 mL/min to achieve separation. The detection was done at λ=200 nm.

However, no HPLC method is reported for the simultaneous quantification of alpha-amyrin, beta-sitosterol and lupeol from dried whole plant powder of *Leptadenia reticulata* (Retz.) Wight & Arn. and *Pluchea lanceolata* (DC.) CB. Clarke.

Thus, in present research work, a simple, rapid, precise and accurate HPLC method has been developed and validated using International Conference on Harmonization (ICH) guidelines for simultaneous determination and quantification of alpha-amyrin, beta-sitosterol and lupeol form dried whole plant powder of *Leptadenia reticulata* (Retz.) Wight & Arn. and *Pluchea lanceolata* (DC.) CB. Clarke.

6. CONCLUSION

A new HPLC method has been developed, validated and used for the simultaneous estimation of three compounds alpha-amyrin, beta-sitosterol and lupeol in dried whole plant powder of *Leptadenia reticulata* (Retz.) Wight &Arn. and *Pluchea lanceolata* (DC.) C.B. Clarke. The HPLC method was validated in terms of linearity, precision, accuracy & system suitability. The developed HPLC technique is precise, specific and accurate. It can be used for the routine quality control analysis and quantitative determination of alpha-amyrin, beta-sitosterol and lupeol in dried whole plant powder of *Leptadenia reticulata* (Retz.) Wight &Arn. and *Pluchea lanceolata* (DC.) C.B. Clarke.

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REFERENCE

1. Kaneda, N.; Chai, H.; Perzuto, J.M.; Kinghom, A.D.; Farnsworth, N.R.; Tuchinda, P.; Udchachon, J.; Santisuk, T.; Rentrakul, V. Planta Med. 1992,58,429-431. | 2. Mitsuhashi, H.; Hayashi, K. Shoyab.Zasshi 1985,39, l-27. | 3. Deepak, D.; Khare, A.; Khare, MI Phytochemistry 1989,28,3255-3263. | 4. Satyavati, G.V.; Gupta, A.K.; Tandon, N.; Seth, S.D. Eds. Medicinal Plants of India (Vol.2), Indian Council of Medical Research: N. Delhi 1987, pp. 151-160. | 5. Sastry, B.S.; Vijaylakshmi, T.; Rao, D.V.; Rao, E.V. Indian Drugs 1985,22,611-612. | 6. Krishna, P.V.G.; Rao, E.V.; Rao, D.V. Planta Med. 1975,27,395-400 | 7. Chawla AS, Kaith BS, Handa SS, Kulshreshtha DK, Srimal RC. Chemical investigation and anti-inflammatory activity of Pluchea lanceolata. Fitoterapia 1991;62:441-4. | 8. Dixit GS, Tewari RP. Chemical constituents of Pluchea lanceolata. Sachitra Ayurved 1991;43:841 | 9. Kaith B. S., Neolupeol and anti-inflammatory activity of Pluchea lanceolata, Int J Pharmacognosy, 34 (1995) 73-75. | 10. Ali M, Siddiqui N A & Ramachandran R,. Phytochemical investigation of aerial parts of Pluchea lanceolata C.B. Clarke. Indian J Chem B, 40 (2001) 689-706. | 11. Anonymous 1969. The Wealth of India Raw Materials Publication & Information Directorate, CSIR, New Delhi, p. 169. | 12. Chopra RN, Chopra IC, Handa KL, Kapur LD 1958. In: The Use of Drugs in Arthritis and Bronchitis Indigenous Drugs of India. UN Dhur and Sons Ltd, Calcutta, p. 20. | 13. Kaith B. S., Neolupeol and anti-inflammatory activity of Pluchea lanceolata, Int J Pharmacognosy, 34 (1995) 73-75. | 14. Martelanc M., Vovk I. and Simonovska B. Determination of three major triterpenoids in epicuticular wax of cabbage (Brassica oleracea L.) by high-performance liquid chromatography with UV and mass spectrometric detection. Journal of Chromatography A. 1164: 145–152 (2007). | 15. Martelanc M., Vovk I. and Simonovska B. Separation and identification of some common isomeric plant triterpenoids by thin-layer chromatography and high-performance liquid chromatography. Journal of Chromatography A. 1216: 6662-6670 (2009). |