Fast HPLC-DAD Method for Estimation of Catechin for Standardization of Extracts and Antiasthmatic Polyherbal Formulations Containing *Albizia lebbeck*

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ABSTRACT

Background: Many marketed polyherbal formulations containing *Albizia lebbeck* are available for treatment of allergic disorders such as asthma and bronchitis. But these formulations are not standardized in terms of markers. The present work discusses development and validation of a new, simple and rapid HPLC-DAD method for quantification of phytomarker- catechin in methanol extracts for standardization of extracts and polyherbal formulations containing *Albizia lebbeck*. **Materials and Methods:** HPLC analysis was carried out using C_{18} column (100 mm X 4.6 mm, 2.6 μm). 0.1% Phosphoric acid and acetonitrile in gradient mode was used as mobile phase. The column oven was equilibrated at 28°C. The injection volume was 2 μl and detection was carried out at 279 nm. **Results:** The developed HPLC method was found to be linear over the range of 3.0-60 μg/ml with correlation coefficient of 0.9998. The LOD and LOQ were found to be 1.0 μg/ml and 3.0 μg/ml, respectively. The recovery of the catechin was between 97.12 % -100.04 %. The low RSD values of intra and inter-day precision studies indicated good precision of the developed method. The method was found to be robust as small changes in method parameters did not significantly change peak symmetry and content of catechin. **Conclusion:** The developed method was sensitive, rapid and requires no special technique for sample preparation. The method was successfully applied for standardization of three bark extracts and marketed polyherbal formulations containing *Albizia lebbeck*.

Key words: *Albizia lebbeck*, Catechin, Fabaceae, HPLC, Shirish.

INTRODUCTION

Many medicinal plants are available in Ayurvedic and Unani system of medicines for the treatment of allergic disorders. *Albizia lebbeck*, belonging to Fabaceae family, is one of them.[1] It is commonly known as Shirish. Stem bark of this plant contains condensed tannins, catechin, isomer of leucocyanidin, melacacidin, leuco-anthracyanidin, lebbecacidin, friedelin, β-sitosterol, betulinic acid and its glycosides, albizzia saponins A, B and C. Bark also contains phenolic glycoside, albizinin and anthraquinone glycosides.[2] Catechin is phytomarker present in bark

of *Albizia lebbeck* and having antiallergic activity.[3,4] It is chemically, (2R,3S)-2-(3,4-dihydroxyphenyl)-3,4-dihydro-2H-chromene-3,5,7-triol belongs to the group of flavan-3-ols. Numbers of HPLC methods have also been reported for quantitative determination of catechin alone or in combination in different medicinal plants, biological fluids and Ayurvedic formulations.[5-14] But no HPLC method has been developed and validated for catechin present in bark extract of *Albizia lebbeck*. Thus, the present work aims to develop and validate an HPLC-DAD method for the

determination of catechin in stem bark extract of *Albizia lebbeck* for succeeding application in standardization of extracts and anti-asthamatic polyherbal formulations containing *Albizia lebbeck*.

MATERIALS AND METHODS

Chemicals and reagents

Reference standard of catechin (Purity 98% w/w) was purchased from Sigma-Aldrich Pvt. Ltd., Mumbai, India. Marketed polyherbal formulations containing *Albizia lebbeck* were purchased from local market of Mumbai, India. All the solvents and purified water were of HPLC grade from S. D. Fine chemicals, Mumbai, India. All the solutions were filtered through 0.2 μ PTFE filter (Goettingen, Germany). Three different samples of stem bark of *Albizia lebbeck* were collected from Mumbai, Maharashtra, Valsad district of Gujarat and Tirunelvelli district of Tamil Nadu in February 2012 and authenticated at Botanical Survey of India, Pune, India.

Instruments

HPLC system used was Dionex-UltiMate 3000 (Germany) consisting of auto-sampler, quaternary pumps, column oven and diode array detector. All data were analysed using Chromeleon software version 6.80.

Method development

Various organic solvents like methanol or acetonitrile were tried in different ratio with aqueous solvents such as water or aqueous acids in isocratic mode as mobile phase. Effects of these solvents on responses such as retention time (R_t), resolution (Rs), number of plates (N) and peak asymmetry (As) were evaluated. Mobile phase modifier TFA was used as ion pairing agent to produce sharper peaks. In all cases, no separation of components was observed in sample. Therefore it was decided to change mobile phase polarity with time to elute components of varying polarity. When gradient elution with methanol and aqueous acids was tried, a bulge in base line was observed. Hence, methanol was replaced by acetonitrile. Effect of acetonitrile in gradient mode with 0.1 % TFA, 0.1 % acetic acid or 0.1 % phosphoric acid on chromatographic parameters was checked. In all cases, a hump in the leading edge of the peak of standard catechin was observed. Hence, a specialized column involving core shell technology, Sunshell C$_{18}$ (100 mm X 4.6 mm I.D., 2.6 μm) from ChromaNik Technologies Ltd was selected for further analysis. Acetonitrile with 0.1 % phosphoric acid in different gradient elution modes was used as mobile phase. Sample volume was 2 μl.

Column oven was equilibrated at temperature of 28°C and mobile phase was pumped at flow rate of 2.0 ml/min leading to less elution time of catechin without loss of resolution of peaks.

Chromatographic conditions

HPLC analysis was carried out using Sunshell C$_{18}$ column (100 mm X 4.6 mm, 2.6 μm) (ChromaNik Technologies Ltd). The column oven was equilibrated at 28°C. The injection volume was 2.0 μl and detection was carried out at 279 nm. The mobile phase consisted of 0.1% Phosphoric acid (A) and acetonitrile (B) using a gradient elution of 10 % to 15 % B at 0 to 7 min and again 10 % B at 7 to 9 min.

Preparation of standard solution

Catechin (100 mg) was dissolved in 100 ml of methanol by sonication for 10 min to prepare a stock solution of 1000 μg/ml. From this, various aliquots were taken and diluted with appropriate volume of methanol to produce different concentrations. These concentrations were used for validation studies.

Preparation of sample solution

Albizia lebbeck dry stem bark powder (20 g)was defatted with 3 × 200 ml of petroleum ether (60-80°C) by maceration for 24 hr. The petroleum ether extract was discarded and remaining plant material was macerated with 3 × 200 ml of methanol. Methanol extracts thus obtained were pooled together. The methanol extract was dried under reduced pressure using rotary evaporator. Methanol extract (25 mg) thus obtained was dissolved in 10 ml of methanol, sonicated for 5 min and filtered through 0.2 μ PTFE filter.

Preparation of sample solutions for analysis of formulations

Granules

Accurately weighed 10.192 g of granules were macerated with 100 ml of methanol and stirred at room temperature for 24 hr. The resulting solution was filtered and evaporated to dryness. Accurately weighed 0.1034 g of methanol extract thus obtained was dissolved in 10 ml of methanol and sonicated for 10 min. The solution was filtered and injected in HPLC.

Herbal tea

Accurately weighed 10.348 g of herbal tea was macerated with 100 ml of methanol and stirred at room temperature for 24 hr. The resulting solution was filtered and evaporated to dryness. Accurately weighed 0.1012 g of methanol extract thus obtained was dissolved in 10 ml

of methanol and sonicated for 10 min. The solution was filtered and injected in HPLC.

Vati

Twenty *Vatis* were individually weighed; their mean weight was determined and the *Vatis* were triturated. Accurately weighed 8.765 g of *Vati* powder was macerated with 100 ml of methanol and stirred at room temperature for 24 hr. The resulting solution was filtered and evaporated to dryness. Accurately weighed 0.1020 g of methanol extract thus obtained was dissolved in 10 ml of methanol and sonicated for 10 min. The solution was filtered and injected in HPLC.

Syrup

Accurately measured 50 ml of syrup was extracted 3 × 100 ml of methanol. The methanol extract was concentrated to dryness. Accurately weighed 0.1018 g of methanol extract thus obtained was dissolved in 10 ml of methanol and sonicated for 10 min. The solution was filtered and injected in HPLC.

Method Validation

The developed method was validated for various parameters such as linearity, LOD, LOQ, accuracy, precision and system suitability as per ICH guidelines.[15] Linear relationship between concentration and area was evaluated over the range 3.0-60 μg/ml. Duplicate injections using 2.0 μl loop were applied and detection was carried out at 279 nm. Average area was determined. Graph of area Vs concentration was obtained and R^2 was calculated. The LOD and LOQ were calculated based on signal to noise ratio method. Catechin solutions in increasing concentrations were injected until signal to noise ratio of 3.0 and 10.0 were obtained for determination of LOD and LOQ, respectively. Known amount of sample was spiked with 32, 40 and 48 μg/ml of the standard solutions of catechin separately and % recovery was calculated. Repeatability (intraday) and intermediate precision (interday) were determined through triplicate injections of methanol extract at three levels that is 80 %, 100 % and 120 % (20 mg, 25 mg and 30 mg of methanol extract in 10 ml of methanol) and the % relative standard deviation (% RSD) at each level was determined. The robustness of the method was evaluated by deliberately varying the flow rate of mobile phase (1.9, 2.0 and 2.1 ml/ min), column oven temperature (27, 28 and 29°C) and detection wavelength (278, 279 and 280 nm) and their effects on retention time, RSD of area and % w/w of catechin in the sample were checked. For each condition, six injections of the standard solution (40 μg/ml) and two injections of sample (25 mg in 10 ml of methanol) were applied.

Stability studies of catechin solution

Stability of the sample solutions was evaluated at different time interval such as 24, 48 and 72 hr after preparation and storage at 4.0°C and 25.0°C separately. Stability was checked by comparing the results of the solutions after storage with that of freshly prepared solutions.

Quantification of catechin in extracts and polyherbal formulations by developed HPLC method

The developed method was applied for quantitative determination of catechin in three different extracts and antiasthmatic polyherbal formulations containing *Albizia lebbeck*. The three extracts were prepared from bark obtained from three different geographical sources of India (Gujarat, Tamil Nadu and Maharashtra) to identify content variation of catechin.

RESULTS AND DISCUSSION

Method validation

In this work, new, sensitive and time-saving HPLC-DAD method was developed for estimation of catechin in *Albizia lebbeck* and validated as per ICH guidelines. Acceptable separation of catechin from other components present in methanol extract was achieved (Figure 1 and Figure 2). Catechin eluted at mean retention time of 1.79±0.20 min and total run time was 9 min. Detection wavelength was selected at 279 nm which is λ_{max} of catechin. The developed method was also found to be suitable for analysis of catechin with resolution greater than 1.9, peak asymmetry 1.26, theoretical plates greater than 6600 and % RSD less than 2.0. The method was found to be linear over the proposed range 3.0-60 μg/ml with linear regression
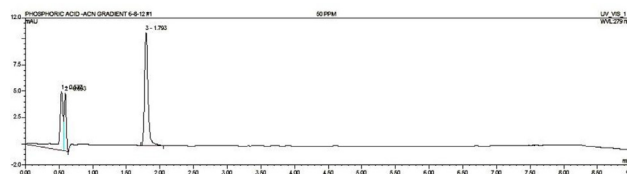

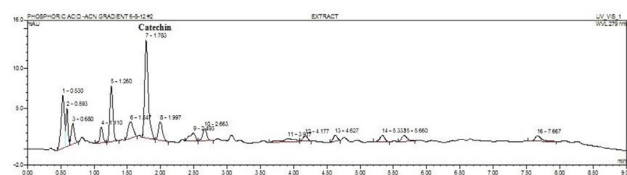**Figure 1: HPLC chromatogram of standard catechin.**

Figure 2: HPLC chromatogram of methanol extract of *Albizia lebbeck*.

Table 1: Percentage of recovery, to evaluate the accuracy of the method.

Component	Amount Present[a] (μg/ml)±S.D.	Amount Found[a] (μg/ml)±S.D.	% Recovery[a]±S.D.
Catechin	32.8225±0.26	32.0779±0.29	97.7335±0.92
	40.8709±0.44	39.6908±0.21	97.1240±1.57
	48.9220±0.62	48.9381±0.59	100.0417±1.58

[a] *n*=3, triplicate injections

Table 2: Validation results for intraday and interday precision for catechin.

Component	Amount level (mg/10 ml)	Intra day (% RSD)[b]		Inter day (% RSD)[b]
		Day 1	Day 1	Day 2
Catechin	20.0	0.9302	0.8226	0.6422
	25.0	0.9047	1.2849	0.9469
	30.0	0.6012	0.7272	1.0727

[b] *n*=3, triplicate injections

Table 3: Results of robustness studies for catechin.

Parameters	Retention time (Rt)	Area	% RSD[c]	% w/w of catechin[c]
Detection wavelength (nm)				
278	1.905	0.5049	1.2081	1.2459
279	1.905	0.5062	1.6788	1.2433
280	1.905	0.5019	1.7134	1.2406
Flow rate (ml/min)				
1.9	1.997	0.5422	1.9365	1.1907
2.0	1.905	0.5062	1.6788	1.2433
2.1	1.816	0.4933	1.9258	1.2481
Column oven temperature (oC)				
27	1.930	0.5129	1.1503	1.2204
28	1.905	0.5062	1.6788	1.2433
29	1.832	0.5092	0.6087	1.2302

[c] *n*=6, six injections

Table 4: % w/w of catechin in extracts and formulations.

Extracts / Formulations	% w/w of catechin[d] ± S.D.	mg of catechin[d] ± S.D.
Extract-I (Gujarat)	1.2575±0.00	12.5750±0.00/g of extract
Extract-II (Tamilnadu)	0.0939±0.00	0.9390±0.00/g of extract
Extract-III (Maharashtra)	0.0212±0.00	0.2120±0.00/g of extract
Granules	0.1723±0.02	1.0592±0.13/Teaspoon
Herbal Tea	0.3853±0.01	0.7018±0.02/Teaspoon
Vati	0.5628±0.00	0.0508±0.00/*Vati*
Syrup	0.0132±0.00	0.2382±0.02/Teaspoon

[d] *n*=3, triplicate injections

precision studies indicating that the method was precise (Table 2).

The robustness was estimated using the overall mean, standard deviation and % RSD for each variable. % RSD was lower than 2.0 for the variables such as detection wavelength, flow rate and column oven temperature. The present method was found to be robust for the % w/w of catechin present in the sample (Table 3).

Stability of catechin in the sample solutions was evaluated at 4.0°C and 25.0°C for 3 consecutive days to check for degradation if any. The results were evaluated as the percentage of non-degraded catechin at defined time intervals. Both samples showed less than 4 % degradation confirming that the samples were stable at 4.0°C and 25.0°C for 3 days.

Quantification of catechin in extracts and polyherbal formulations containing *Albizia lebbeck*

The developed method was applied for quantitative determination of catechin in three different methanol bark extracts and marketed polyherbal formulations containing *Albizia lebbeck* (Table 4). Amount of catechin was significantly higher in extract-I (Gujarat) as compared to extract-II and extract-III. In analysis of formulations, catechin content was highest in *Vati* as compared to other formulations. Thus, this method has justified its use in the quality control of polyherbal formulations prepared using bark of *Albizia lebbeck*.

CONCLUSION

Rapid, sensitive, selective, precise and accurate HPLC-DAD method has been developed and validated for quantifying catechin in *Albizia lebbeck* extracts without

coefficients (R^2) of 0.9998 for the compound studied, demonstrating an acceptable data fit to the regression line (y = 0.0124x+0.0066). The method was found be sensitive as it could detect (LOD=1.0 μg/ml) and quantitate (LOQ = 3.0 μg/ml) lower amount of the compound. The accuracy of the standard in the spiked sample was evaluated at low, medium and high levels with mean recoveries of 97.73 %, 97.12 % and 100.04 %, respectively. In all levels of accuracy study, % RSD was less than 2.0 (Table 1).

The values of % RSD are below 2.0 at all three levels of concentrations for both intraday and interday

requiring special sample preparation technique. The developed and validated method can be useful for standardization of anti-asthmatic polyherbal formulations containing *Albizia lebbeck*.

ACKNOWLEDGEMENT

CONFLICT OF INTEREST

Authors declare that there are no conflict of interest.

ABBREVIATIONS

DAD: Diode Array Detector; **LOD:** Limit of Detection; **LOQ:** Limit of Quantification; **OPA:** Ortho Phosphoric Acid; **HPLC:** High Performance Liquid Chromatography; **ICH:** International Conferences on Harmonization; **RSD:** Relative Standard Deviation; **SD:** Standard Deviation; **TFA:** Trifluoro Acetic Acid; **UV:** Ultra Violet.

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PICTORIAL ABSTRACT
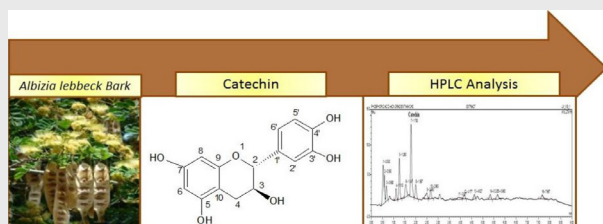
SUMMARY

Catechin was used as marker for marker-based standardization of extracts and polyherbal formulations containing *Albizia lebbeck* by HPLC.

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