Research Article

Simultaneous Quantitation of Lupeol and Beta-Amyrin Using Reverse Phased High Performance Liquid Chromatography from Plants Having Antidiabetic Activity

Shreeda s. Adhyapak*, Vidya dighe
Department of Chemistry, Ramnarain Ruia College, Matunga, Mumbai, Maharashtra, India.
***Corresponding author's E-mail:** shreeda_adhyapak@yahoo.com

Accepted on: 24-10-2013; **Finalized on:** 31-12-2013.

ABSTRACT

A reverse phase high performance liquid chromatography (HPLC) method has been developed and validated for simultaneous quantitative determination of two triterpenoids viz. lupeol and beta–amyrin from two different plants. The plants used are dried seed kernel powder of *Caesalpinia bonducella* Linn, and the plant parts of *Coccinia indica* Wight & Arn. are dried root powder and dried fruit powder. The chromatographic separation was performed on a Agilent Zorbax C_8 column (150 x 4.6 mm, 5 µm). The detection was carried out at 210 nm for simultaneous quantitation of lupeol and beta-amyrin. The detector response was linear for concentrations ranging from 0.5 µg/mL to 500.0 µg/mL for lupeol and 0.1 µg/mL to 500.0 µg/mL for beta–amyrin with correlation coefficient of 0.999 for both the components. The method was precise as the value of percent relative standard deviation was found to be less than 2. The accuracy of the developed HPLC method was checked by carrying out the recovery experiment at three different levels, by using standard addition method.

Keywords: Beta-amyrin, *Caesalpinia bonducella* Linn., *Coccinia indica* Wight & Arn., Lupeol, Reversed Phase High Performance Liquid Chromatography.

INTRODUCTION

Diabetes mellitus is caused due to deficiency in production of insulin by the pancreas, or by the ineffectiveness of the insulin produced. Insulin dependent diabetes mellitus (IDDM) is characterized by a relative or absolute insufficiency of insulin secretion and non-insulin dependent diabetes mellitus (NIDDM) is characterized by resistance of the metabolic action of insulin on target tissue.[1] Diabetes is the most common disease associated with carbohydrate metabolism and is a major cause of morbidity. In spite of introduction of synthetic hypoglycemic agents, diabetes and related complications continue to be major medical problem. Hence there has been a continuous search for safer and more effective drugs in treatment of diabetes. Medicinal plants have been used as a source of medicine since times immemorial. Herbal medicine is still the mainstay of health care in several developing countries.

Caesalpinia bonducella Linn. (Family: Fabeacea) commonly known as Fever nut and locally known as 'Sagargotha' is a shrub widely distributed throughout the coastal region of India. It is used as a folklore medicine for treatment of diabetes. The tribal people of India use it for controlling blood sugar. The seeds are reported to possess anti-diabetic or hypoglycemic activity.[2] The roasted seeds are made into coffee for treating diabetes.[3,4] The seeds are found to contain various chemical constituents like furanoditerpenes such as α-Caesalpin, β-Cesalpin and pentacyclic triterpenoid such as lupeol and beta-amyrin are reportedly present.[3,5]

Coccinia indica Wight & Arn. (Family: Cucurbitacea) commonly known as Ivy gourd and locally known as 'Tondali' grows abundantly and wildly all over India and is used as a vegetable. The fruits, leaves, roots of *Coccinia indica* Wight & Arn. have been widely used in treatment of diabetes milletus.[6] Roots are boiled to make decoction that is taken 2 to 3 times a day orally to treat diabetes.[7] The triterpenoids such as β-amyrin and Lupeol are reportedly present.[8]

In the present research work, two triterpenoids viz. lupeol and beta–amyrin are simultaneously quantitated from seed kernel powder of *Caesalpinia bonducella* Linn., fruit powder and root powder of *Coccinia indica* Wight & Arn.

Lupeol is reported to reduce blood glucose by reducing the activity of alpha-amylase. It also shows antiprotozoal, anticancer, chemopreventive and anti-inflammatory properties.[9]

Beta-amyrin is reported to have antihyperglycemic activity.[10] It has been reported to exhibit various other pharmacological activities *in vitro* and *in vivo* conditions against various health-related conditions, including conditions such as inflammation, microbial, fungal, and viral infections and cancer cells. β- amyrin is involved in the biosynthetic pathways of other biologically active compounds such as avenacine, centellosides, glycyrrhizin or ginsenosides.[11]

In the literature[12] determination of lupeol and beta-amyrin in epicuticular wax of cabbage (*Brassica oleracea* Linn.) by using HPLC has been reported. The separation was obtained in on Hypersil BDS C_{18} column (250 mm x 3 mm i.d., particle size 3µm).

A semi preparative HPLC method[13], for separating a mixture of triterpenoids viz. alpha-amyrin, beta-amyrin, lupeol and their corresponding acetates from the swallow roots (*Decalpis hamiltonii* Wight & Arn.) has been

reported. Analytical and semi-preparative HPLC analysis was carried out on C_{18} Shimadzu, CLC, ODS column (250 mm x 4.6 mm i.d., particle size 5 µm) with guard column. The analysis was carried out at 40° C.

A HPLC method[14] for identification of an isomer of lupeol i.e epi-lupeol and beta amyrin from resins and various valuable medicinal plants has been reported. Reverse phase high performance chromatographic separation was obtained in isocratic mode on Hypersil 5 µm ODS (250 mm x 4.6 mm) using methanol as mobile phase

However, no HPLC method is reported for the simultaneous quantification lupeol and beta–amyrin from seed kernel powder of *Caesalpinia bonducella* Linn., fruit and root powder of *Coccinia indica* Wight & Arn. has not yet been reported.

Thus, precise and accurate HPLC method has been developed and validated using International Conference on Harmonization (ICH) guidelines for simultaneous determination and quantification of lupeol and beta–amyrin from dried seed kernel powder of *Caesalpinia bonducella* Linn., dried fruit powder and dried root powder of *Coccinia indica* Wight & Arn.

EXPERIMENTAL METHODS

Materials

Standard, Reagents and Chemicals

Standard Lupeol (purity 99.9%) and Beta-amyrin (purity 99.8 %) HPLC grade were procured from Sigma-Aldrich Chemie GmbH (Aldrich Division; Steinheim, Federal Republic of Germany).

Acetonitrile (purity 99.70%) and n-propanol (purity 99.90%) used in present research work was of HPLC grade and were procured from Merck, India. Distilled Water for HPLC was procured from Lichrosolv Merck, India.

Plant Material

The seed kernels of *Ceaslpinnia bonducella* Linn, fruits and roots of *Coccinia indica* Wight & Arn. were collected from Keshav Srushti, Thane. Herbarium of the plants was prepared and authenticated from Botanical Survey of India (BSI), Pune, India.

The plant parts seed kernel powder of *Caesalpinia bonducella* L. and fruit and root powder of *Coccinia indica* W& A. were washed with water to remove soil particles, dried in the shade, and finely powered. The powder was passed through the 85 mesh sieve and stored in an airtight container at room temperature (28 ± 2^0 C) and used for further analysis.

Quantitative analysis

Preparation of stock solution of standard lupeol (1000.0 µg/mL)

The stock solution of lupeol was prepared by dissolving 10.0 mg of lupeol standard in 5.0mL of n-propanol in a 10.0 mL standard volumetric flask, and the contents were sonicated in an ultrasonic bath (Model: TRANS-O-SONIC, Frequency: 50 Hz) for 5.0 minutes for complete dissolution of lupeol. The contents were then diluted up to the mark with n-propanol to obtain a solution of 1000.0 µg/mL of lupeol.

Preparation of stock solution of standard beta-amyrin (1000.0 µg/mL)

The stock solution of beta-amyrin was prepared by dissolving 10.0 mg of beta-amyrin standard in 5.0mL of n-propanol in a 10.0 mL standard volumetric flask, and the contents were sonicated in an ultrasonic bath (Model: TRANS-O-SONIC, Frequency: 50 Hz) for 5.0 minutes for complete dissolution of beta-amyrin. The contents were then diluted up to the mark with n-propanol to obtain a solution of 1000.0 µg/mL of beta-amyrin.

Preparation of working standard solutions of lupeol and beta-amyrin

2.5 mL of stock solution of lupeol and beta-amyrin (1000.0 µg/mL) was taken in a two separate 25.0 mL volumetric flasks. The volume of both the flask was made up to 25.0mL, with n-propanol. The working standard solutions of lupeol and beta-amyrin each with concentration of 100.0 µg/mL were thus prepared.

5.0 mL of stock solution of lupeol and beta-amyrin (1000.0 µg/mL) was taken in a two separate 10.0 mL volumetric flasks. The volume of both the flask was made up to 10.0mL, with n-propanol. The working standard solutions of lupeol and beta-amyrin each with concentration of 500.0 µg/mL were thus prepared.

The aliquots (0.05 mL to 5.0 mL) of 100.0 µg/mL solution of lupeol were transferred to 10.0 mL volumetric flasks and the volume of each flask was made up to 10.0 mL, with n-propanol, to obtain the working standard solutions of lupeol, in the concentration range of 0.50 µg/mL to 50.0 µg/mL.

Similarly, the aliquots (0.01 mL to 5.0 mL) of 100.0 µg/mL solution of beta-amyrin were transferred to 10.0 mL volumetric flasks and the volume of each flask was made up to 10.0 mL, with n-propanol, to obtain the working standard solutions of beta-amyrin, in the concentration range of 0.10 µg/mL to 50.0 µg/mL.

Preparation of sample solution

About 500 mg of dried seed kernel powder of *Caesalpinia bonducella* Linn. was accurately weighed and transferred to 100.0 mL stoppered conical flask and 20.0 mL of n-propanol was added to it.

About 500 mg of dried fruit powder of *Coccinia indica* Wight & Arn. was accurately weighed and transferred to 100.0 mL stoppered conical flask and 20.0 mL of n-propanol was added to it.

About 500 mg of dried root powder of *Coccinia indica* Wight & Arn. was accurately weighed and transferred to 100.0 mL stoppered conical flask and 10.0 mL of n-propanol was added to it. All the three flasks were then

shaken at 80 rpm, on a conical flask shaker at room temperature (28°C ± 2°C). The contents of the each flask were filtered through Whatman No.41 filter paper (E. Merck, Mumbai, India) and the filtrates were further used as the sample solution for the assay experiment. The sample solutions were filtered through 0.45µm filter paper before analysis.

Preparation of mobile phase

The mobile phase used in the present research work for simultaneous quantification of lupeol and beta–amyrin from three different sample solutions viz. seed kernel powder of *Caesalpinia bonducella* Linn. and from fruit powder and root powder of *Coccinia indica* Wight & Arn. was Acetonitrile : Water in the volume ratio of 95:5. The mobile phase was then degassed in ultra-sonicator bath for 10 min.

HPLC conditions

Chromatographic separation was carried out with Shimadzu UFLC Prominence chromatograph, equipped with binary gradient pump (LC-20AD), fitted with auto sampler (SIL-20 AC HT) and oven (CTO-20 AC) having PDA detector (SPD-M20A). The chromatograms and data were recorded using LC solutions Software.

An Agilent Zorbax RP C_8 (150mm x 4.6mm, i.d. 5µm) column and mobile phase comprising of acetonitrile and distilled water in volume ratio of (95:5), was used for the analysis. The system was run at a flow rate of 0.7 ml/min, 10 µL of sample was injected in the chromatographic system and the detection was done at 210nm. The proposed HPLC method was validated and applied for the quantitative determination of lupeol and beta–amyrin from dried seed kernel powder of *Caesalpinia bonducella* Linn., dried root powder and dried fruit powder of *Coccinia indica* Wight & Arn.

Method Validation

Linearity

Linearity of standard lupeol was evaluated by injecting different concentrations in the range of 0.50 µg/mL to 500.0 µg/mL of lupeol. Each solution was injected three times and the values of peak areas of lupeol and mean peak area of lupeol for each concentration were recorded. Similarly, linearity of standard beta-amyrin was evaluated by injecting different concentrations in the range of 0.10 µg/mL to 500.0 µg/mL of beta- amyrin. Each solution was injected three times and the values of peak areas of beta-amyrin and mean peak area of beta-amyrin for each concentration were recorded. The calibration curve of lupeol and beta-amyrin was obtained by plotting a graph of mean peak areas against corresponding concentrations of both the standards.

Limit of Detection and Limit of Quantification

The limit of detection (LOD) and limit of quantification (LOQ) were determined at signal to noise ratios of 3:1 and 10:1, respectively.

System Suitability

The system suitability test was carried out to confirm that the chromatographic system used to carry out the analysis gives precise, accurate and reproducible results.

System suitability was determined by injecting 10 µL of mixture of standard solution of lupeol and beta-amyrin with concentration of 10.0 µg/mL each, six times into the chromatographic system, under the optimized chromatographic conditions. The chromatograms were recorded and the peak areas values and the retention times of lupeol and beta-amyrin were noted for each injected solution. The system suitability parameters like peak tailing, resolution between two peaks and column efficiency were evaluated and noted for injected concentration of both the standards lupeol and beta-amyrin. The results are given in Table 1.

Table 1: Results for system suitability

System suitability parameter	Lupeol	Beta-amyrin
Peak Tailing	1.043	1.061
Resolution between lupeol and beta-amyrin	2.719	
Column efficiency	9255.214	9945.268

Precision

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

The repeatability was evaluated by triplicate analysis of three sample solutions i.e. n-propanolic extract of the dried seed kernel powder of *Caesalpinia bonducella* Linn, was injected in the chromatographic system in triplicates on the same day in the same laboratory under the specified chromatographic conditions. The peak areas of lupeol and beta–amyrin were recorded. Similarly the repeatability was also carried out for n-propanolic extract dried fruit powder and dried root powder of *Coccinia indica* Wight & Arn.

The intermediate precision of the method was evaluated by analyzing the sample solutions in triplicate on three different days, in the chromatographic system, under the specified chromatographic conditions. The peak areas of lupeol and beta–amyrin were recorded.

Similarly the intermediate precision was also carried out for n-propanolic extract dried fruit powder and dried root powder of *Coccinia indica* Wight & Arn.

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

Solution Stability

The stabilities of standard lupeol and beta–amyrin solution were determined by comparing the peak areas of lupeol and beta–amyrin solution, of concentration 10

µg/mL, at different time intervals, for a period of minimum 48 hrs, at room temperature. The results showed that the peak areas of lupeol and beta–amyrin almost remained unchanged (values of percent relative standard deviation were less than 2) over a period of 48 hrs, and no significant degradation was observed within the given period, indicating the stability of standard solutions of lupeol and beta–amyrin for minimum 48 hrs.

Table 2: Method validation data for simultaneous quantification of lupeol and beta–amyrin

Parameters	Observations	
	Lupeol	Beta-amyrin
Linear Working Range (µg/mL)	0.5 – 500	0.1 – 500
Correlation coefficient (r)	0.999	0.999
Limit of Detection (LOD) (µg/mL)	0.165	0.33
Limit of Quantification (LOQ) (µg/mL)	0.5	0.1
Stability of standard solution	48 hrs.	48 hours
Repeatability % R.S.D. (n=3)		
Seed kernel powder of *Caesalpinia bonducella* Linn.	1.07	0.98
Fruit powder of *Coccinia indica* Wight & Arn.	0.87	0.89
Root powder of *Coccinia indica* Wight & Arn	0.87	0.90
Intermediate precision % R.S.D. (n=9)		
Seed kernel powder of *Caesalpinia bonducella* Linn.	1.06	0.99
Fruit powder of *Coccinia indica* Wight & Arn.	0.84	0.89
Root powder of *Coccinia indica* Wight & Arn	0.90	0.91

Table 3: Results for Assay and Recovery

Parameters	Observations	
	Lupeol	Beta-amyrin
Assay (µg/g)		
Seed kernel powder of *Caesalpinia bonducella* Linn.	110.91 ± 1.62	64.86 ± 0.80
Fruit powder of *Coccinia indica* Wight & Arn.	382.10 ± 4.20	316.75 ± 3.49
Root powder of *Coccinia indica* Wight & Arn.	30.00 ± 0.43	64.85 ± 0.67
Percent Recovery		
Seed kernel powder of *Caesalpinia bonducella* Linn.	98.21 %	99.07 %
Fruit powder of *Coccinia indica* Wight & Arn	99.45 %	98.69 %
Root powder of *Coccinia indica* Wight & Arn.	98.23 %	99.93 %

Specificity

Specificity is the ability of the method to measure the analyte response in the presence of its impurity. The specificity of the proposed HPLC method was ascertained by injecting 10.0 µL of blank solution (Figure 1) to observe for interference, if any, with the peaks of interest in the chromatogram of the sample solution (Figure 2). It was observed that there is no interference from the blank solution. N-propanol was taken as blank solution since standard and sample solutions were prepared in n-propanol.

Robustness

Robustness tests examine the effect of the operational parameters on the analysis results. Robustness of the method was determined by making small deliberate changes in the chromatographic conditions utilized in present method. By introducing small changes in the mobile phase composition (± 20% of aqueous phase) and flow rate (± 0.1 mL) the effects on the results were examined. The amounts of lupeol and beta-amyrin from dried seed kernel powder of *Caesalpinia bonducella* Linn., dried fruit powder and dried root powder of *Coccinia indica* Wight & Arn. obtained by altered method to that obtained by normal method were found to be similar. It was concluded that the method is robust as the above mentioned deliberate changes made the method did not affect the results.

Assay procedure

The developed and validated HPLC method was used for quantification of lupeol and beta–amyrin from the n-propanolic extract of dried seed kernel powder of *Caesalpinia bonducella* Linn., dried fruit powder and dried root powder of *Coccinia indica* Wight & Arn.10µL of n-propanolic extract of the dried seed kernel powder of *Caesalpinia bonducella* Linn, dried root powder and dried fruit powder of *Coccinia indica* Wight & Arn. (n=7) was

injected separately into the chromatographic system under the specified conditions. Amounts of lupeol and beta–amyrin present in the above mentioned sample solution were determined from the calibration curve, by using the peak area of lupeol and beta–amyrin in the sample. The results are tabulated in Table 3.

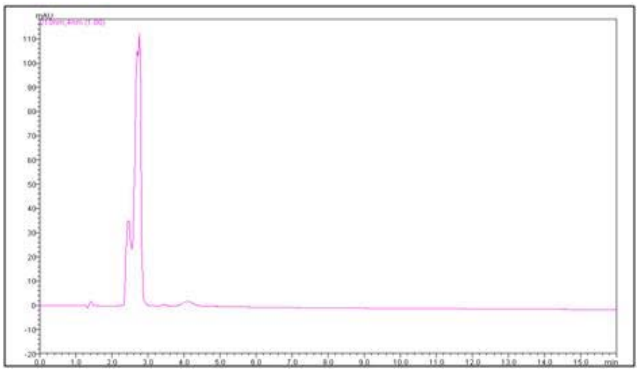

Figure 1: HPLC chromatogram obtained for blank (n-propanol)

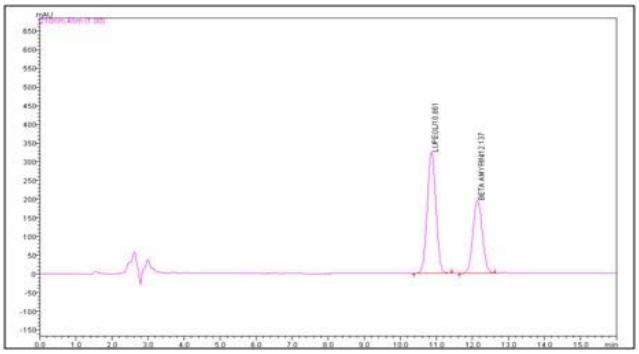

Figure 2: HPLC chromatogram obtained of Standard lupeol and beta-amyrin

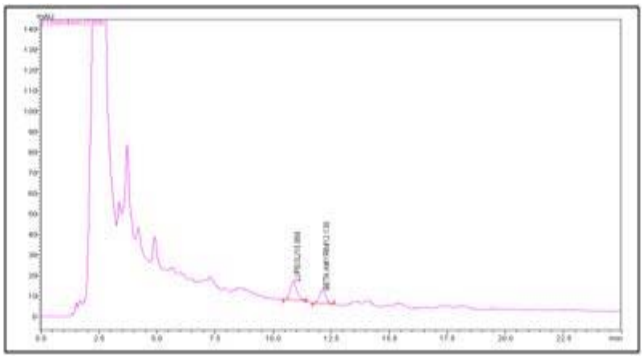

Figure 3: HPLC chromatogram of dried seed kernel powder of *Caesalpinia bonducella* Linn.

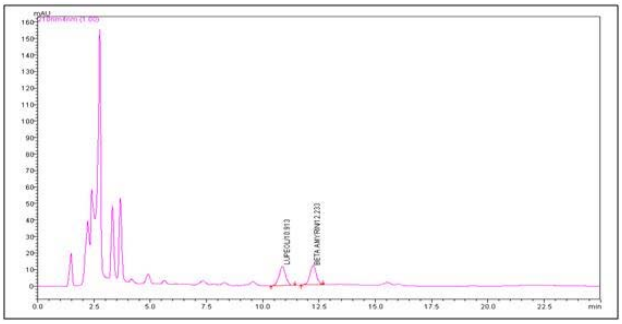

Figure 4: HPLC chromatogram of dried fruit powder of *Coccinia indica* Wight & Arn.

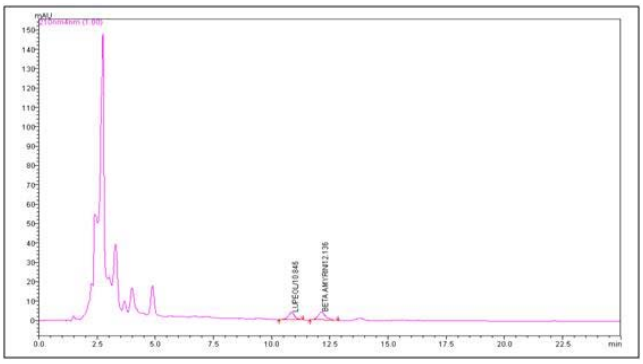

Figure 5: HPLC chromatogram of dried root powder of Coccinia indica Wight & Arn.

Accuracy

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

To accurately weighed about 500 mg of dried seed kernel powder of *Caesalpinia bonducella* Linn known amounts of standard lupeol (100µg, 200µg, 300µg) and beta-amyrin (60µg, 120µg, 180µg), were added, and extracted using n-propanol as described earlier.

To accurately weighed about 500 mg of dried fruit powder of *Coccinia indica* Wight & Arn. known amounts of standard lupeol (100µg, 150µg, 200µg) and beta-amyrin (100µg, 150µg, 200µg), were added, and extracted using n-propanol as described earlier.

To accurately weighed about 500 mg of dried root powder of *Coccinia indica* Wight & Arn. known amounts of standard lupeol (60µg, 90µg, 120µg) and beta-amyrin (80µg, 120µg, 160µg), were added, and extracted using n-propanol as described earlier.

Each of the three different levels containing sample solution and standard of all the three samples was injected in seven replicates; the samples were analyzed under the specified chromatographic conditions, as described above. The lupeol and beta-amyrin contents were quantified and the percentage recovery was calculated. The percent recovery values were then calculated. The results of accuracy are tabulated in Table 3.

RESULTS AND DISCUSSION

Different mobile phases were tried for simultaneous HPLC separation of lupeol and beta–amyrin from other components of the dried seed kernel powder of *Caesalpinia bonducella* Linn. and dried fruit and root powder of *Coccinia indica* Wight & Arn. and good separation was achieved by using acetonitrile : water (95:5 v/v) as mobile phase. Detection was carried out at λ = 210 nm as both lupeol and beta–amyrin showed maximum response at this wavelength. The identity of the bands of lupeol and beta–amyrin in the sample solutions was confirmed by comparing their retention times in sample with that of reference standards. The retention time for lupeol and beta–amyrin were 10.95

and 12.25 minutes respectively. Figure 2 shows typical HPLC chromatograms of standard lupeol, standard beta amyrin and Figure 3 shows chromatographic separation of lupeol and beta–amyrin in n-propanolic extract of dried seed kernel powder of *Caesalpinia bonducella* Linn. Figure 4 shows chromatographic separation of lupeol and beta–amyrin in n-propanolic extract of dried fruit powder of *Coccinia indica* Wight & Arn. and Figure 4 shows chromatographic separation of lupeol and beta–amyrin in n-propanolic extract of dried root powder of *Coccinia indica* Wight & Arn. The developed method provided a good separation of the phyto constituents with the resolution (Rs) of 2.719 whereas the tailing factor is 1.043 and 1.061 for lupeol and beta–amyrin respectively. The resolution and tailing factor values lies between the acceptable limits.

A good linear relationship was observed for lupeol and beta–amyrin in the concentration in the range of 0.5µg/mL to 500µg/mL and 0.1 µg/mL to 500 µg/mL respectively with correlation coefficient of 0.999 for both the components (Table 1). When the method was validated for instrumental precision, repeatability and intermediate precision, the values of percentage relative standard deviations were less than 2, indicating the proposed method is precise and repeatable (Table 1).

The mean amounts of lupeol and beta-amyrin found in seed kernel of *Caesalpinia bonducella* Linn. were 110.9 µg/g and 64.86 µg/g respectively with percent recoveries 98.21 and 99.07 for lupeol and beta-amyrin respectively. The mean amounts of lupeol and beta-amyrin found in fruits of Coccinia indica Wight & Arn. Linn. were 382.10 µg/g and 316.75 µg/g respectively with percent recoveries 99.45 and 98.69 for lupeol and beta-amyrin respectively. The mean amounts of lupeol and beta-amyrin found in seed kernel of *Caesalpinia bonducella* Linn. were 30.0 µg/g and 64.85 µg/g respectively with percent recoveries 98.23 and 99.93 for lupeol and beta-amyrin respectively.

Almost all reported methods use C_{18} analytical column of 250 mm length, whereas the present method uses a shorter C_8 analytical column of 150 mm length which gives a good separation of the phytoconstituents i.e lupeol and beta-amyrin with resolution (R_s) greater than 1.5.

As lupeol and beta-amyrin lack chromophores, the sensitivity of UV detection is limited and is dependent on mobile phase. Acetonitrile as the mobile phase enabled separation along with sensitive detection at 210 nm.

The HPLC method has been reported in literature[12] for simultaneous determination of lupeol and beta-amyrin in epicuticular wax of cabbage (*Brassica oleracea* Linn.) The column used for separation is obtained Hypersil BDS C_{18} column (250 mm x 3 mm I.D.; particle size 3µm). The analysis was done at room temperature and detection was carried out using diode-array detector using acetonitrile as mobile phase at flow rate of 0.8mL/min. The retention times obtained were 15.1 min for lupeol and 21.8 min for beta-amyrin.

A semi preparative HPLC method has been reported in literature[13], for separating a mixture of triterpenoids viz. alpha-amyrin, beta-amyrin, lupeol and their corresponding acetates from the swallow roots (*Decalpishamiltonii* Wight & Arn.). The semi-preparative HPLC analysis is carried out on C_{18} Shimadzu, CLC, ODS column (250 mm x 4.6 mm i.d., particle size 5 µm) with guard column. The analysis was carried out at 40° C using water: methanol (94:6 v/v ratio) as mobile phase with 1.0 mL/min flow rate and the retention times for lupeol and beta-amyrin obtained are 16.20 min and 19.10 min respectively.

The earlier mentioned litereture[14] describes HPLC method for determination of isomer of lupeol i.e. epi-lupeol and beta-amyrin from resins and various valuable medicinal plants using Hypersil 5 µm ODS (250 mm x 4.6 mm) with methanol as mobile phase at 0.9 mL/min flow rate.

The present method uses a mobile phase comprising of acetonitrile: water in the volume ratio of 95: 5 with flow rate of 0.7 mL/min. The column oven temperature was kept at 40° C and the detection was done at λ = 210 nm using a photo diode array detector. The retention times obtained by present method were 10.86 min for lupeol and 12.13 min for beta-amyrin.

Hence, in the present research work, the retention times of lupeol and beta amyrin are less compared to the reported methods and simultaneous quantitation of lupeol and beta-amyrin from three different samples viz. seed kernel of *Caesalpinia bonducella* Linn., fruit of *Coccinia indica* Wight & Arn. and root of *Coccinia indica* Wight & Arn. was carried out. The developed HPLC method was validated using ICH guidelines. The developed and validated HPLC method is simple and fast as compared to the HPLC methods reported in the literature.

CONCLUSION

The developed HPLC technique is precise, specific and accurate and can be used for the routine quality control analysis and simultaneous quantitative determination of lupeol and beta–amyrin from dried seed kernel powder of *Caesalpinia bonducella* Linn. and dried fruit and root powder of *Coccinia indica* Wight & Arn.

Acknowledgement: The authors would like to acknowledge Dr. P. S. Ramanathan Advance Instrumentation Centre for providing us with the instrumentation facility for our work.

REFERENCES

1. Garber A, Diabetes mellitus, In International medicine. Stein J. H. Ed, Mosby, St. Luios, 1998, 1850-1854.
2. Rahmatullah M, Jahan R, Khatun MA, Jahan F, Azad AK, Anwarul Bashar ABM, Miajee Z, Ahsan S, Nahar N, Ahamd I, Chowdhary MH. A Pharmacological evaluation of Medicinal Plants used by folk medicinal practitioners of station Purbo

Para village of jamalpur Sadar Upzila In Jamalpur district Bangladesh, American-Eurasian Journal Sustainable Agriculture, 4(2), 2010, 170-195.

3. Quality standards of Indian Medicinal Plants, Indian Council for Medicinal and Research, New Delhi, 2, 2003, 25-33.
4. Kannur DM, Hukkui VI, Akki KS, Antidiabetic activity of *Caesalpinia bonducella* seed extracts in rats, Fitoterapia, 77, 2006, 546-549.
5. Prabhakar P, Doble M, A target based Therapeutic Approach towards Diabetes mellitus using medicinal plants, Current Diabetes Reviews, 4, 2008, 291 – 308.
6. Gunjan M, Gautam K, Jha AK, Mishra U, Pharmacognostic and antihyperglycemic study of *Coccina indica*, International Journal of Phytomedicine, 2, 2010, 36-40.
7. Shaheen SZ, Bolla K, Vasu K, Charya M, Antimicrobial activity of fruit extract of *Coccinia indica*, African Journal of Biotechnology, 8 (24), 2009, 7073-7076.
8. Khare CP, Encyclopedia of Indian Medicinal plants, Springer-Verlag Heidelberg, 2004, 155 – 156.
9. Gallo M, Sarachine MJ, Biological activites of Lupeol, International Journal of Biomedical and Pharmaceutical Sciences, 3(1), 2009, 46-66.
10. Santos FA, Frota JT, Arruda BR, Antihyperglycemic and hypolipidemic effects of α, β-amyrin, a triterpenoid mixture from *Protium heptaphyllum*in mice, Lipids in Health and Disease, 11, 2012, 98.
11. Francisco AO, Mariana HC, Fernanda RC, Roberto CP, Regilane MS, Protective effect of α- and β-amyrin, a triterpene mixture from *Protium heptaphyllum* (Aubl.) March. trunk wood resin, against acetaminophen-induced liver injury in mice, Journal of Ethnopharmacology, 98, 2005, 103-108.
12. Martelanc M, Vovk I, Simonovska B, Determination of three major triperpenoids in epicuticular wax of cabbage (*Brassica oleraceae* L.) by high performance liquid chromatography with UV and mass spectrometric detection, Journal of Chromatography A, 1164, 2007, 145 – 152.
13. Nagarajan S, Rao JML, Triterpenoids from Swallow Roots – A Convenient HPLC method for separation, Journal of Chromatographic Science, 45, 2007, 189-194.
14. Hernandez-Vazquez L, Palazon J, Mangas S, Navarro-Ocaña A. Valuable medicinal plants and resins: Commercial phytochemicals with bioactive properties, Industrial Crops and Products, 31, 2010, 476-480.

Source of Support: Nil, **Conflict of Interest:** None.