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Development and Validation of RP-HPLC Method for Estimation of Corosolic Acid from Leaves of *Lagerstroemia speciosa*

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ABSTRACT

Banaba (*Lagerstroemia speciosa*, Lythraceae, Pride of India) is a medicinal plant, leaves are used to treat diabetes and hyperglycemia. This effect is attributed to its chemical constituents belonging to the groups of terpenoids, tannins, and flavanoids. The samples of leaves of *L. speciosa* were extracted with methanol followed by ethyl acetate. The yield of methanolic extract was found to be 14.49-15.92%w/w. The yield of ethyl acetate soluble extract was found to be 2.17-2.89%w/w. HPLC method was developed for quantification of marker compound corosolic acid from leaves of *L. speciosa*. This method was validated for linearity, precision, accuracy, limit of detection, limit of quantification, robustness and specificity. The content of corosolic acid by HPLC was found to be in range of 0.47-0.88 % w/w respectively. The objectives of this paper are to present a new method of identification of corosolic acid, a terpenoid, using HPLC.

INTRODUCTION

Lagerstroemia speciosa (Lythraceae, commonly known as Banaba, pride of India) is a medicinal plant that grows in the Philippines, China, India and Southeast Asia. The leaf is opposite, oblong to ovate, glaborus; flowers are large, pink to purple; fruit is woody, and the seeds are winged. Major constituents of *Lagerstroemia speciosa* leaf include lagertannin, corosolic acid, maslinic acid; ellagic acid, lagerstroemin, flosin B and reginin A, flosin A, lutein, phytol, sitosterol and sitosterol acetate, kaempferol, quercetin, and isoquercitrin, Traditionally, the whole plant and specifically leaves are used to treat **diabetes** and **hyperglycemia** (elevated blood sugar). The hypoglycemic (blood sugar lowering) effect of banaba extract is reported to be similar to that of insulin which induces glucose transport from the blood into body cells. This effect is attributed to the various active chemical constituents present like corosolic acid and lagertannins. Banaba extracts are also known to have antiobesity, anti-oxidant and anti-gout effects.

Corosolic acid is a naturally occurring pentacyclic triterpene which is chemically 2 alpha-hydroxy ursolic acid, 2R, 3β-dihydroxyurs-12-en-28-oic acid. It displays a potential anti-diabetic activity as well as anti-oxidant, anti-inflammation and antihypertension properties.

The purpose of our work was to identify and quantify corosolic acid content in plant *Lagerstroemia speciosa* by HPLC.

MATERIALS AND METHODS

Instrument

A Shimadzu model HPLC equipped with quaternary LC-2010 AHT VP pumps variable wavelength programmable UV/VIS detector SPD-10 AVP column oven (Shimadzu), SCL 10 AVP system controller (Shimadzu), rheodyne injector fitted with a 20 µl loop and class VP 5.032 software was used.

Chemicals and reagents

1. Methanol AR (Merck, India)
2. Ethyl acetate AR (Merck, India)
3. Acetonitrile HPLC (Merck, India)
4. Orthophosphoric acid HPLC (Merck, India)
5. Water HPLC (Merck, India)

Standard marker compound

Standard corosolic acid (97%) was gifted by Sami Laboratories Ltd., Bengaluru, India. It was used without any further purification.

Chromatographic conditions

The chromatographic separations were performed using Phenomenex Luna C_{18} (250 mm x 4.6 mm i.d, 5 µm particle size) column at ambient temperature. The optimized mobile phase was found to be acetonitrile: 0.1% aqueous orthophosphoric acid (85:15, v/v) and was pumped at the flow rate of 1.0 ml/min. The mobile phase was filtered through a 0.45 micron membrane filter and degassed before use. Autosampler with injection volume 20 µl was used and sample tray was kept at 15 ºC temperature.

Detection

Variable wavelength programmable UV/visible detector.

Preparation of standard solutions of corosolic acid

An accurately weighed 10 mg of pure powder of standard corosolic acid was transferred in 100 ml volumetric flask. About 80 ml of methanol was added to dissolve it. Volume was adjusted with methanol up to the mark to obtain a standard stock solution of (100 µg/ml). Further, 0.2, 0.25, 0.5, 1.0, 1.5 and 2.0 µg/ml of stock solutions were transferred separately to 10 ml volumetric flask and diluted up to the mark using methanol to produce working standard solutions of 2.0, 2.5,5.0,10.0,15.0 and 20.0 µg/ml.

Determination of wavelength of maximum absorbance

The standard solution of corosolic acid was scanned over wavelength of 200 to 400 nm by using UV-Visible spectrophotometer.

Preparation of calibration curve

Graded concentrations of standard corosolic acid solution 2.0, 2.5, 5.0, 10.0, 15.0 and 20.0 µg/ml volume were injected serially by rheodyne injector in triplicate. It was scanned at λ_{max} 210 nm by UV/ visible detector. Data of peak area was recorded for each injection of standard solution. Calibration curve of concentration of peak area versus standard corosolic

acid was plotted.

Preparation and analysis of sample solutions of corosolic acid from leaves of *L. speciosa*

Accurately weighed 5 g powder of leaves of *L. speciosa* was extracted in soxhlet extractor with methanol separately. The methanolic extract was concentrated under reduced pressure to dryness (15.25%w/w). The dried residue was dissolved in water and partitioned twice between ethyl acetate and water. The ethyl acetate soluble fractions were combined and concentrated under vacuum to yield ethyl acetate soluble extract (2.58%w/w). The ethyl acetate soluble extract was dissolved in about 45 ml methanol in 50 ml volumetric flask separately and sonicated for 20 min separately. The volume was adjusted with methanol up to the mark.

1 ml extract of leaves of *L. speciosa* prepared and was diluted by 100 fold with methanol and methanolic solutions of each sample was filtered through 0.45 µ Millipore membrane filter separately. 20 µl aliquots of finally prepared solution was analyzed in triplicate under specified chromatographic condition. The amount of corosolic acid was calculated by putting values of area response into regression equation of corosolic acid.

Method validation

Linearity and range

A calibration curve was plotted over a concentration range of 2.0-20.0 µg/ml for corosolic acid. Accurately measured standard working solution of corosolic acid (2.0, 2.5, 5.0, 10.0, 15.0 and 20.0 µg/ml) were transferred to the vials of autosampler rack. Aliquots (20 µl) of each solution were injected from autosampler under the specified chromatographic conditions. Calibration curve was constructed by plotting peak area versus concentration of standard corosolic acid and the regression equation was calculated.

Repeatability

Method precision

The precision of the method was checked by repeatedly injecting (n = 6) standard solution of corosolic acid (10 µg/ml). The deviations in result were reported in terms of % RSD.

Intermediate precision

Intermediate precision was evaluated in terms of intraday and interday precision. The intraday and interday precision of the proposed method were determined by estimating the corresponding responses three times on the same day and on three different days for three different concentrations (5, 10 and 15 µg/ml) of standard corosolic acid. The deviations in result were reported in terms of % RSD.

Accuracy (% Recovery)

The accuracy of the method was determined by calculating recoveries of corosolic acid by the standard addition method. Known amounts of standard solutions of corosolic acid (50, 100 and 150%) were added to preanalyzed sample solution of leaves of *L. speciosa*. Here, known amounts of standard solutions of corosolic acid (2.4, 4.8 and 7.2 µg/ml) were spiked to prequantified solution of *L. speciosa* and solution was analyzed by proposed method. The amount of corosolic acid was estimated by applying these values to the regression equation of the calibration curve.

Limit of detection

The limit of detection (LOD) was determined by visually evaluating the minimum level at which the corosolic acid could be reliably detected.

Limit of quantification

The limit of quantification was determined by analyzing the known concentration of corosolic acid at which it could be quantified with acceptable accuracy and precision.

Robustness

Robustness of the HPLC method was determined by applying small but deliberate changes in the flow rate, ratio of mobile phase, detection wavelength, column temperature. The study of robustness of the method was carried out at concentration of corosolic acid (5 µg/ ml). The results were expressed in terms of % RSD.

Specificity

The specificity of the method was ascertained by comparing the RT values of standard

corosolic acid with corosolic acid from extract of leaves of *L. speciosa* and spectrum of the standard corosolic acid with the spectrum obtained from leaves of *L. speciosa*.

RESULTS AND DISCUSSION

Determination of wavelength maxima

UV maxima for corosolic acid was found to be 210 nm.

Optimization of chromatographic condition

Analytical method development with C_{18} column (250 mm x 4.6 mm i.d., 5 µm particle size) was preferred over other columns. A Phenomenex C_{18} was preferred as it has high carbon loading with very closely packed material to give high resolution.

Acceptable resolution with reasonable peak shape were achieved by using mobile phase acetonitrile and 0.1% aqueous orthophosphoric acid (85:15 v/v) with flow rate of 1 ml/min. The method parameter was optimized to analyze corosolic acid in the extract of the leaves of *L. speciosa*.

Typical HPLC chromatogram of standard corosolic acid showing single peak is seen in figure 1.

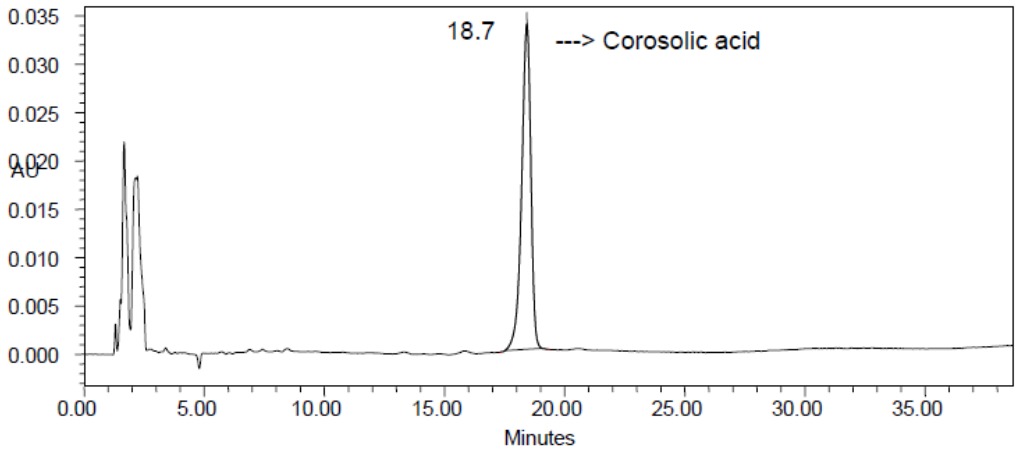

Figure No. 1: Chromatogram of standard corosolic acid (5 µg/ml) with corresponding retention time RT=18.7 min at 210 nm by RP-HPLC method

Calibration curve of standard corosolic acid

The calibration curve of mean peak area versus concentration of standard corosolic acid was

plotted over a concentration range of 2.0-20.0 µg/ml. The regression equation was found to be y=53828x+7782.5 with correlation coefficient R^2 = 0.9987 (figure 2). The calibration curve was found to be linear which indicate that an excellent correlation exits between concentrations of mean peak area versus standard corosolic acid.

Calibration curve of standard corosolic acid

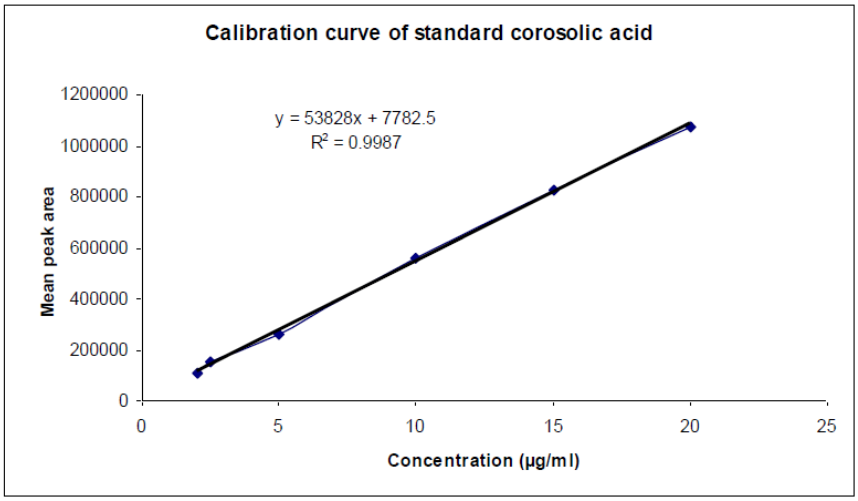

Figure No. 2: Calibration curve standard corosolic acid by HPLC method

Linear regression data is presented in table 1.

Table No. 1: Linear regression equation data for standard corosolic acid by RP-HPLC method

Sr. No.	Concentration of standard corosolic acid (µg/ml)	Mean peak area ± SD (n=3)	% RSD
1	2.0	109431.70 ± 833.59	0.7617
2	2.5	153699.00 ± 502.89	0.3271
3	5.0	275961.00 ± 576.79	0.8126
4	10.0	562275.70 ± 1207.59	0.2147
5	15.0	836152.00 ± 3852.40	0.4607
6	20.0	1072298.33 ± 1126.98	0.1051

Repeatability

Method precision

The results of method precision study for corosolic acid are shown in table 2. It was carried out by repeatedly injecting (n=6) corosolic acid (10 µg/ml) and corresponding response was measured in terms of peak area and retention time. The % RSD values for method precision study is found to be less than 1.0 %. It indicates that the given method is precise.

Table No. 2: Method precision data for standard corosolic acid (10 µg/ml) by RP-HPLC method

Corosolic acid (10 µg/ml)	Area
1	561025
2	560367
3	563435
4	560998
5	565425
6	562505
Mean	562292.5
SD[a]	1905.406
% RSD [b]	0.3388

SD[a] = Standard deviation, % RSD[b] = Relative standard deviation,

n[c] = Number of replicate

Intermediate precision

The results of intermediate precision are shown in table 3. Intraday and interlay precision were studied by analyzing (5.0, 10.0 and 15.0 µg/ml) of corosolic acid solution three times on the same day and on three consecutive days three times and response was measured in terms of mean peak area.

Table No. 3: Intermediate precision data for standard corosolic acid by RP-HPLC method

Concentration of standard corosolic acid	Intraday precision (n=3)		Interday precision (n=3)	
	Peak area (Mean ± SD)	% RSD	Peak area (Mean ± SD)	% RSD
5.0	275961 ± 576.79	0.2090	272294.3 ± 3178.21	1.1671
10.0	562275 ± 1207.59	0.2147	561359 ± 8656.09	1.5419
15.0	836152 ± 3852.40	0.4607	833160.7± 8127.49	0.9755

SD = Standard deviation, % RSD = Relative standard deviation, n =3 Number of replicate

The %RSD values for intraday and interday precision study was found in the range of 0.2090-0.4607 and 0.9755-1.5419 respectively. It indicates that the proposed method is found to be reproducible.

Accuracy (% Recovery)

The study of accuracy was determined by standard addition method. Standard corosolic acid was added at three levels; *i.e.* at 50%, 100% and 150%. The results of recovery studies are shown in table 4. Then % recovery of the spiked corosolic acid was found in the range of 98.14 ± 0.80 - 100.97 ± 1.05, which was within a limit of 98%-102%. The resultant % RSD was found between 0.8170-1.4960 i.e., indicating that proposed method is accurate.

Table No. 4. Results of accuracy (% Recovery) study of corosolic acid from leaf powder of *L. speciosa* from Punjab state by HPLC method

% Level	Amount of sample taken (mg)	Amount of standard spiked (mg)	Total amount recovered (mg)	% Recovery ± SD[a]	% RSD [b] (n^c=3)
50	4.8	2.4	7.21	100.41 ± 1.50	1.4960
100	4.8	4.8	9.65	100.97 ± 1.05	1.0380
150	4.8	7.2	11.87	98.14 ± 0.80	0.8170

SD^a= Standard deviation, % RSD^b = relative standard deviation, n^c = Number of replicate

Limit of detection

The limit of detection for corosolic acid was found to be 0.067 µg/ml.

Limit of quantification

The limit of quantification for corosolic acid was found to be 0.225 µg/ml.

Robustness

The results of robustness study for corosolic acid are shown in table 5. It was revealed that % RSD values of minor modification of experimental conditions like change in flow rate, mobile phase composition, column temperature and wavelength were found to be less than 2%. It indicates that the proposed method is robust. It can withstand minor alterations in experimental conditions.

Table No. 5. Robustness data for corosolic acid (5 µg/ml) by RP- HPLC method

Parameter (n^b=3)	Modification	Corosolic acid (5 µg/ml)			
		Mean RT ± SD, (n^b=3)	% RSD[a]	Mean Area ± SD, (n^b=3)	% RSD[a]
Flow rate (1 ml/min)		18.62 ± 0.2820	1.5146	276075 ± 2584	0.9361
Mobile phase composition Acetonitrile:0.1%aqueous orthophosphoric acid (85:15 v/v)	82:18	18.52 ± 0.2683	1.4492	275863 ± 1840	0.6672
	88:12	18.76 ± 0.3046	1.6238	276012 ± 2463	0.8925
Wavelength (210 nm)		18.56 ± 0.1397	0.7529	275914 ± 2379	0.8624
Column temperature (40^oC)		18.57 ± 0.1089	0.5869	275834 ± 2033	0.7372

SD= Standard deviation, % RSD[a]=Relative standard deviation, n^b= number of replicate =3

Specificity

The peak of standard corosolic acid matched with the peak of corosolic acid found in the extract of leaves of *L. speciosa.* The retention time of standard corosolic acid (RT=18.7 min) matches with the retention time (RT=18.7-18.9) of corosolic acid found in the extract of each of three samples of leaves of *L. speciosa.* The proposed method is specific as the peak

of other constituents did not interfere with peak of corosolic acid in each of three sample extracts of leaves of *L. speciosa.*

Analysis of corosolic acid in leaves of *L. speciosa*

Sample extract of leaves of *L. speciosa* was analyzed by proposed method in triplicate. HPLC chromatogram of the analysis of corosolic acid in leaves of *L. speciosa* is shown in figure 3 respectively.

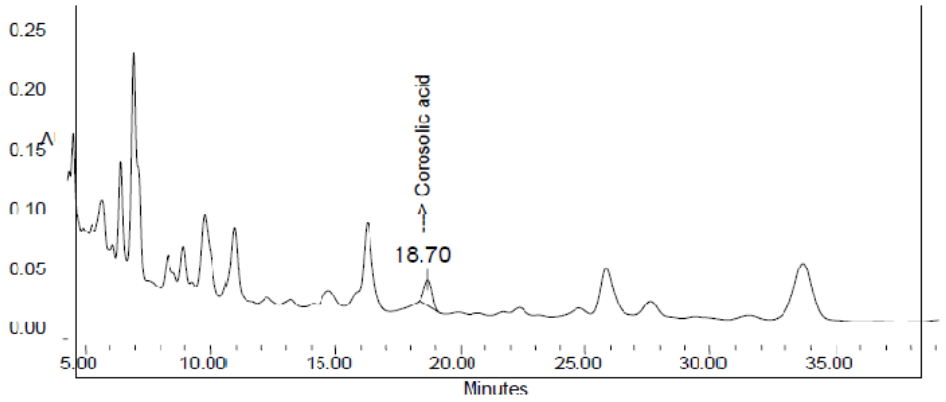

Figure No. 3: HPLC chromatogram of extract of *L. speciosa* obtained from Gujarat state showing peak of corosolic acid at RT 18.70 min.

It showed that the amount of corosolic acid found in the leaves of *L. speciosa* was 0.886%w/w with mean area 490020 ± 2639 respectively.

Table No. 6: Summary of validation parameters of RP-HPLC method for estimation of corosolic acid

Sr. No.	Parameters	Results
1	Correlation coefficient (R^2)	0.9987
2	Linearity and range (µg/ml)	2.0-20.0
3	Regression equation	y = 53828x + 7782.5
4	Precision (% RSD) Repeatability of retention time Repeatability of area Intraday	 0.4896 0.3388 0.2090-0.4607
5	% Recovery	98.14 ± 0.80 - 100.97± 1.048.
6	Limit of detection (µg/ml)	0.067
7	Limit of quantification (µg/ml)	0.225
8	Robustness	Robust
9	Specificity	Specific

CONCLUSION

The proposed method is found to be robust and able to withstand minor changes in the experimental conditions. As % RSD obtained for each parameter of robustness study for corosolic acid was found to be less than 2%.

The peaks of other constituents present in the extracts of *L. speciosa* did not interfere with the peak of corosolic acid. Hence, the proposed method is specific.

Analysis of leaves of *L. speciosa* shows that the content of corosolic acid varies from 0.5-0.87 %w/w.

Thus, the developed RP-HPLC method is simple, accurate, precise, and reproducible and has ability to separate corosolic acid from other constituents usually found in the extract of leaves of *L. speciosa*. Hence, the proposed RP-HPLC method is suitable for the quality control analysis of corosolic acid in the leaves of *L. speciosa.*

REFERENCES

1. Chen L, Wu Q, Wei W, Tang, L. (2006). Study on antioxidant activity of the extract of leaves of *Lagerstroemia speciosa*. *Shipin Yu Fajiao Gongye*, 32(3), 47-50.
2. Deocaris CC, Aguinaldo RR, Delaysla, JL,. Asencion AS, Elmer-Rico EM. (2005). Hypoglycemic activity of irradiated Banaba (*Lagerstroemia speciosa* Linn.) Leaves, *J Appli Sci Res*, 1(1), 95-98.
3. Yonaha, N, Miura T, Kaneko T, Nishi Y, Mitsui R, Fujita Y, Yamada Y, Seino Y. (2006). Effect of corosolic acid on post challenge plasma glucose levels. *Diabetes Res Clin Pract*, 73(2), 174-77.

4. Hosoyama H, Sugimoto A, Suzuki Y, Sakane I, Kakuda T. (2003). Isolation and quantitative analysis of the α-amylase inhibitor in *Lagerstroemia speciosa* (L.) Pers. (Banaba). *Yakugaku Zasshi*, 123(7), 599-605.
5. Hou W, Li Y, Zhang Q, Wei X, Peng A, Chen L, Wei Y. (2009). Triterpene acids isolated from *Lagerstroemia speciosa* leaves as α-glucosidase inhibitors. *Phytother Res*, 23(5), 614-18.
6. Mallavadhani U, Mohapatra S, Mahapatra A. (2008). Quantitative analysis of corosolic acid, type-II anti-diabetic agent in different parts of *Lagerstroemia speciosa* Linn. *J Planar Chromat*, 21(6), 461-64.
7. Manalo JB, Devera FV, Bonifacio TS, Unalivia FD, Arida VP. (1993). Phytochemical investigation of *Lagerstroemia speciosa* leaves (Banaba) Pers. *Philippine J Sci*, 122(1), 15-31.
8. Okada Y, Omae A, Okuyama T. (2003). A new triterpenoid isolated from *Lagerstroemia speciosa* (L.) Pers. *Chem Pharm Bull*, 51(4), 452-54.
9. Phung TH, Nguyen XT, Do NL, Nguyen XC, Phan VK. (2009). Isolation of corosolic acid and urosolic acid from the plant *Lagerstroemia speciosa* (L.) Pers. *Tap Chi Duoc Hoc*, 49(5), 32-35.
10. Ragasa CY, Ngo HT, Rideout JA. (2005). Terpenoids and sterols from *Lagerstroemia speciosa*. *J Asian Nat Pro Res*, 7(1), 7-12.
11. Schmandke H. (2005). *Lagerstroemia speciosa* L.: Hope for diabetics? 1,2,3,4,6-penta-O-galloyl-D-glucose (PGG) with insulin like effect. *Ernaehrungs-Umschau*, 52(12), 490.
12. Takahashi M, Osawa K, Ueda J, Yamamoto F, Tsai CT. (1976). The components of the plants of *Lagerstroemia g*enus. III. The structure of the new tannin "lagertannin" from the leaves of *Lagerstroemia speciosa* (L.) Pers. *Yakugaku Zasshi*, 96(8), 984-87.
13. Tanaka T, Tong HH, Xu Y, Ishimaru K, Nonaka G, Nishioka I. (1992). Tannins and related compounds. CXVII. Isolation and characterization of three new ellagitannins, lagerstannins A, B and C having a gluconic acid core, from *Lagerstroemia speciosa* (L.) Pers. *Chem Pharm Bull*, 40(11), 2975-80.
14. Unno T, Sakane I, Masumizu T, Kohno M, Kakuda T. (1997). Antioxidative activity of water extracts of *Lagerstroemia speciosa* leaves. *Biotechnol Biochem*, 61(10), 1772-74.
15. Vijaykumar K, Murthy PB, Kannababu S, Syamsundar B, Subbaraju B. (2006). Quantitative determination of corosolic acid in Lagerstroemia speciosa leaves, extracts and dosage forms. *Inter J Appl Sci Eng*, 4(2), 103-114.
16. Zong W, Xia W, Cui B. (2007). Determination of corosolic and maslinic acids in *Lagerstroemia speciosa* leaves by TLC/HPLC method *Pharm Chem J*, 41(4), 222-24.