IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Human Journals
**Research Article**
July 2022 Vol.:24, Issue:4


# HPLC Analysis and Quantification of Beta-Sitosterol Marker in Eye Drop and Ophthalmic Gel of *Boerhavia diffusa* Roots Aqueous Distillate

IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

**Pooja*, Zubair Ashfa, Kanoujia Jovita**

*Hygia Institute of Pharmacy, Hygia Institute of Pharmaceutical Sciences & Research Faizullahganj, Prabhandh Nagar, Ghaila Road, Lucknow-226020 India.*

*Amity Institute of Pharmacy, Amity University Gwalior-474005 Madhya Pradesh (U.P.) India.*

**Submitted:** 24 June 2022
**Accepted:** 29 June 2022
**Published:** 30 July 2022

**Keywords:** Beta-sitosterol, aqueous distillate, HPLC, *Boerhavia diffusa* Linn.

**ABSTRACT**

This work is based on a valid HPLC method evolved and quantification of test eye drop along with an ophthalmic gel of *Boerhavia diffusa* roots aqueous distillate was analyzed. The research method is based on the separation and quantification of beta-sitosterol in eye drop and ophthalmic gel in aqueous distillate of the plant. The method response of Beta-sitosterol was a straight-line function concentration range 1–10 µg mL–1 was found to be exact and valid. This HPLC method may be handed-down as a quality control tool for quantification of the marker contemporaneously in eye drop and ophthalmic gel of an aqueous distillate of *Boerhavia diffusa* roots as well as marketed formulation (Itone).

HUMAN JOURNALS
www.ijppr.humanjournals.com

## INTRODUCTION:

Notwithstanding scientific effectiveness, Acceptability of Ayurvedic and folk medicines in the world market is less. The affirmations of Ayurvedic formulations at worldwide level will only be increased by advancements of Ayurvedic research. This can be executed through the embodiment of advanced techniques. Here by HPLC analytical technique we can estimatethe major bioactive phytoconstituents.

Thus, here HPLC of *Boerhavia diffusa* Linn. (*B. diffusa*) 'Punarnava or Rakhtpunarnava will be analysed as it is primitive medicine and classified as "rasayana" on account of its therapeutic, chemical and ethnopharmacological benefits. Root of *B.diffusa*restrainssome Vital alkaloids, rotenoids, lignans which are named as punarnavine, boeravinones A-F, liriodendrons, respectively. Flavonoids, amino acids, beta-sitosterols andtetracosanoic, esacosanoic, stearic and ursolic acids, used in chronic ailment. The entire plant was used in treatment of enlargement of the spleen, cancer, jaundice, dyspepsia, inflammation, abdominal pain, anti-stress, tonic and carminative agent.

The present work is specific and consistent by HPLC method validated and enumerated for quantification of test eye drop and ophthalmic gel of the roots of *Boerhavia diffusa* aqueous distillate and marketed formulation Itone eye drop from Dey's Pharmaceutical, Calcutta. This trend method for accompanying separation and quantification of this marker Beta sitosterol from any plant matrix. Quality control and quantification of the marker, as well as marketed formulation, has been done by this HPLC method.

## PROCEDURAL DEVELOPMENT:

In the proposed method of validation for the determination of beta- sitosterol in a matrix environment required adequate resolution of target moiety in the chromatogram. The desired work was run by Acetonitrile (ACN): Phosphate buffer (pH 7.2) in a ratio of 95:5 v/v as mobile phase. The strapping and sharp peak was established. Detected at 280 nm and flow rate was maintained at 1.0 ml/min.

## PREPARATION OF MOBILE PHASE:

Acetonitrile and Phosphate buffer (pH 7.2) 95:5 v/v was used as mobile phase, filtered by Millipore filtration assembly of 0.45 mm diameter paper, it was degassed and sonicated in the ultrasonicated bath.

**PREPARATION OF SAMPLE SOLUTION:**

(1µg/ml) sample solution of both eye drops and the ophthalmic gel was diluted in methanol, Mixed well and degassed with the help of sonication, filtered using HPLC filter for HPLC analysis.

**PREPARATION OF STANDARD SOLUTION:**

Marker **beta-sitosterol** (1µg/ml) standard solution was diluted in methanol mixed well and degassed by sonication, filtered using HPLC filter for HPLC analysis.

The calibration curve of marker beta-sitosterol was prepared in methanol in the range of 1-10µg/ml concentration. The correlation coefficient was 0.997, it indicates good linearity and obeys Beer's Lambert law.

**METHOD VALIDATION:**

HPLC method was developed by using Eclipse plus C 18 3.5 µm diameter, with a mobile phase of Acetonitrile: Phosphate buffer (pH7.2) (95:5 v/v) at the flow rate 1.0 ml/min with 50µl injection volume and UV detection at 280 nm as par the objective. The retention time for **beta-sitosterol** was found 23.72 min. The peak obtained was set on in this method hence selected for the study. The HPLC method developed was validated in different matrix environments as per ICH guidelines. Following parameters were used.

**LINEARITY:**

Beta sitosterolin six concentrations were analysed, Plots of calibration curve were in the concentration range (1µg/ml) generated by replicating analysis (n = 3). The peak areas were plotted. The sample peak was identified by comparison of retention time (Rt) and UV absorption spectrum of standard.

**ACCURACY:**

By the recoveries of beta sitosterol the accuracy of the method was evaluated and the amount of the standard was evaluated by measuring the peak areas and by fitting these values to the straight-line equation of calibration curve.

## MATERIALS AND METHODS

### CHEMICAL AND REAGENTS

Beta-sitosterol was obtained as a gift sample from Natural Remedies Pvt Ltd., India. HPLC grade methanol, acetonitrile and water were obtained from E. Merck, Mumbai, India.

### COLLECTION OF PLANT MATERIALS:

Plant samples *Boerhavia diffusa* Linn. (Roots) were collected from natural habitats of Uttar Pradesh village Sheopura (Balrampur) India and local market of Lucknow Uttar Pradesh India. The samples were authenticated by *National Botanical Research Institute, Lucknow*. (SPECIFICATION No: NBRI-SOP-202). The plants were washed with water and air dried. Roots were collected in separate paper covers and dried in shade for 15 days. Powdered using Homogenizer and stored at room temperature in airtight containers.

### METHOD OF PREPARATION:

Coarsely powdered drug was soaked in water and kept over-night. This makes the drugs soft and boiled in (1:16) where 1 part is drug and 16 parts are distilled water poured into the distillation assembly and boiled. The condensed vapour was collected in a receiver. The aliquots collected contain aroma of the active ingredients A condensed aqueous distillate of roots, were stored in airtight container, and used for HPLC study.

### METHOD (S) STANDARD STOCK SOLUTIONS AND CALIBRATION CURVE

The pure drug (beta sitosterol) standard stock solutions were prepared by dissolving 10 mg of the drug in 10 ml of methanol to get concentration of 1000 µg/ml to 200 µg/ml.

### CALIBRATION AND QUALITY LEVELS

The calibration curve having a range of 1µg/ml to 10 µg/ml and regression coefficient is 0.997 was drawn up by serial dilutions for precision, accuracy and ruggedness studies.

### MARKETED FORMULATION

For HPLC analysis Itone eye drop 0.2 ml was extracted with 200 ml of Methanol in a Soxhlet apparatus for 14 hours, followed by filtration (5E syringe filter), concentrated to 5 ml, followed by transferring its contents to 10 ml standard volumetric flasks and volume made up to mark with methanol.

## CHROMATOGRAPHIC PROCEDURE

A C18 column (250 mm X 4.6 mm, 5 µm) HPLC column was used. The mobile phase was a Gradient mixture of Acetonitrile and water and filtered through 0.45 µm Millipore filter degassed by sonication for 30 min. The flow rate was adjusted to 1.0 ml/min. Injection volume was adjusted to 20 µl and detection was made at 270 nm. Instrumentation and Chromatographic conditions are presented in Table 1.

## RESULT AND DISCUSSION

## METHOD VALIDATION

ICH harmonized tripartate guidelines Q2 (R1) were followed for the validation of the developed analytical method (ICH Harmonised Tripartite Guideline, Validation of Analytical Procedures: Text and Methodology Q2 (R1), Nov. 2005.

## SELECTIVITY AND SPECIFICITY

During the UV scan no appreciable difference was found in the spectra of reference standards and the analysed samples. Hence, the method demonstrated a high degree of selectivity. Refer Figure 1 and Figure 2 for HPLC chromatograms of Plant sample and Marketed formulation of *Boerhavia diffusa* Linn. Aqueous distillate formulations (eye drop, ophthalmic gels) respectively.

## SYSTEM SUITABILITY

System suitability tests were used to verify whether the resolution and reproducibility of the chromatography system were acceptable for the analysis. For Beta sitosterol in eye drop and ophthalmic gel of *Boerhavia diffusa* aqueous distillate % CV values for area and retention time was found to be <2% indicating that the system was suitable to carry out further analysis.

## RECOVERY

Recovery of the method was evaluated and useful for all the three components within acceptable limits (85.0 to 115.0%). This indicated that the method was reliable and accurate.

## RUGGEDNESS

The proposed method was not governed by the factors considered for ruggedness study. Change in flow rate and mobile phase composition affected the retention time of the analytes, but the results were satisfactory since % CV was <2%.

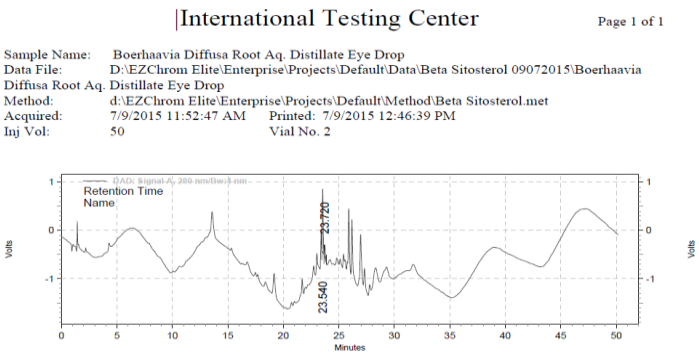
## STOCK SOLUTION STABILITY

The stability of the master stocks of all the standards was evaluated by storing the stocks in the refrigerator at 2-8°C for 72 hours. This was followed by contrasting concentrations of these stocks against freshly prepared stocks for each standard.

## CONCLUSION

An authentic and reproducible HPLC method is validated quantification of the test eye drop and ophthalmic gel, beta-sitosterol stock solution was made using methanol. The calibration curve had a range of 1µg/ml to 10 µg/ml and a regression coefficient of 0.997 figures6. The quantification of the marker in the plant root distillate was accomplished. The HPLC retention time for beta sitosterol was 23.72 min. Each formulation contained (1:16) of plant roots aqueous distillate. Test sample of eye drop formulation contained 0.86 % of beta sitosterol and Test sample of in-situ gel formulation contained 0.39 % of beta-sitosterol figure 1-5.

**Instrumentation and Chromatographic Conditions as Given Below—**



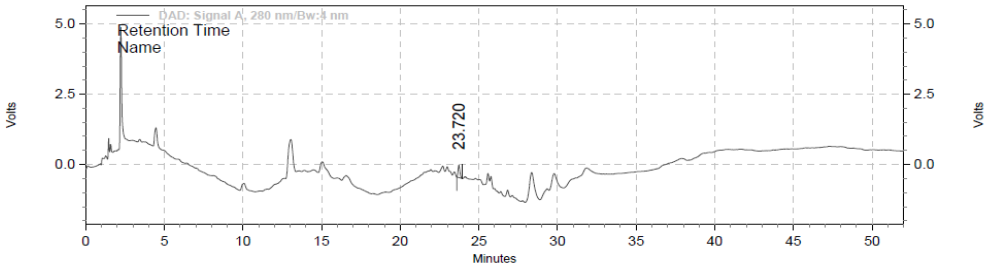

**DAD: Signal A, 280 nm/Bw:4 nm Results**

| Pk # | Name | RT | Area | Area % | Height | Height Percent |
|---|---|---|---|---|---|---|
| 1 | | 23.540 | 13618 | 80.61 | 2740 | 83.460 |
| 2 | | 23.720 | 3276 | 19.39 | 543 | 16.540 |
| Totals | | | 16894 | 100.00 | 3283 | 100.000 |

**Figure 1: HPLC profile of Eye Drop of *Boerhavia diffusa* roots.**

|International Testing Center Page 1 of 1

Sample Name: Boerhaavia Diffusa Root Aq. Distillate IN SITU Gel
Data File: D:\EZChrom Elite\Enterprise\Projects\Default\Data\Beta Sitosterol 09072015\Boerhaavia Diffusa Root Aq. Distillate IN SITU Gel
Method: d:\EZChrom Elite\Enterprise\Projects\Default\Method\Beta Sitosterol.met
Acquired: 7/9/2015 12:44:53 PM Printed: 7/9/2015 4:08:24 PM
Inj Vol: 50 Vial No. 3




**DAD: Signal A, 280 nm/Bw:4 nm Results**

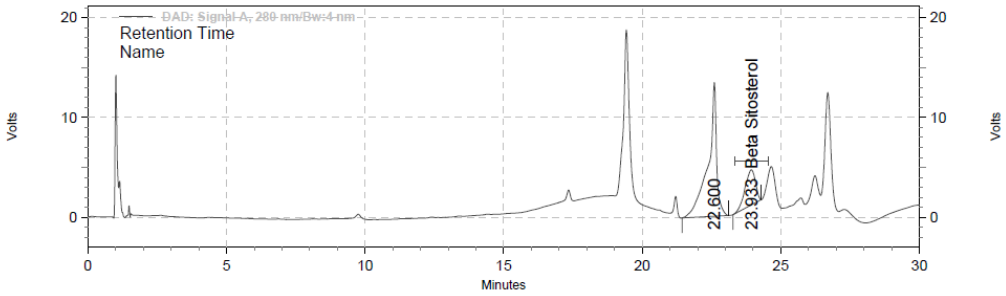
| Pk # | Name | RT | Area | Area % | Height | Height Percent |
|---|---|---|---|---|---|---|
| 1 | | 23.720 | 7277 | 100.00 | 919 | 100.000 |
| Totals | | | 7277 | 100.00 | 919 | 100.000 |



**Figure 2: HPLC profile of Ophthalmic Gel of *Boerhavia diffusa* roots.**

International Testing Center Page 1 of 1

Sample Name: Beta Sitosterol (Marker)
Data File: D:\EZChrom Elite\Enterprise\Projects\Default\Data\Beta Sitosterol 09072015-R2 Beta Sitosterol (Marker).dat
Method: d:\EZChrom Elite\Enterprise\Projects\Default\Method\Beta Sitosterol.met
Acquired: 7/9/2015 4:49:57 PM Printed: 7/9/2015 5:25:24 PM
Inj Vol: 50 Vial No. Vial 1




**DAD: Signal A, 280 nm/Bw:4 nm Results**

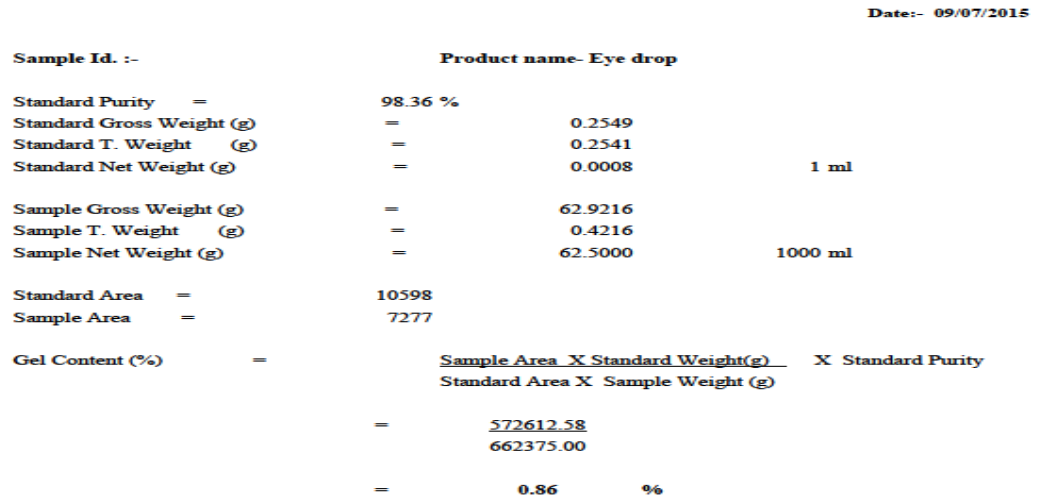
| Pk # | Name | RT | Area | Area % | Height | Height Percent |
|---|---|---|---|---|---|---|
| 1 | | 22.600 | 598171 | 75.66 | 28078 | 79.187 |
| 2 | Beta Sitosterol | 23.933 | 192426 | 24.34 | 7380 | 20.813 |
| Totals | | | 790597 | 100.00 | 35458 | 100.000 |

**Figure 3: HPLC profile of Marker Beta sitosterol.**

**Table 1: HPLC protocol of *Boerhavia diffusa* roots formulations.**

| | | |
|---|---|---|
| **DETECTOR** | 1260 DAD Visual- G1315D Serial No. DEAAX00519 | Agilent Technologies 1200 Infinity Services. |
| **AUTO SAMPLER** | 1260 ALS- G1329B, Serial No. DE | |
| **MANUFACTURING COMPANY** | | |
| **INSTRUMENT** | | Agilent Technologies |
| **COLUMN** | | Eclipse plus C 18 3.5 µm diameter |
| **FLOW RATE** | | 1ml/min. |
| **DETECTION WAVELENGTH** | | 280 nm |
| **MOBILE PHASE** | | Pump A Phosphate |

| | | | |
|---|---|---|---|
| | | | buffer pH 7.2 |
| | Pump B | | Acetonitrile |
| **METHOD NAME** | Beta sitosterol in *Boerhavia diffusa* roots aq. distillate | | |
| **GRADIENT TIME PROGRESS** | Time | Concentration A | Concentration B |
| | 0 | 95.0% | 5.0% |
| | 18 | 70% | 30% |
| | 25 | 45% | 55% |
| | 26 | 70% | 30% |
| | 27 | 95% | 5% |
| | 30 | 95% | 5% |
| **INJECTION VOLUME** | 50 µl | | |
| **DETECTOR** | 1260 DAD Visual-G1315D<br>Serial No. DEAAX00519 | | |
| **AUTOSAMPLER** | 1260 ALS- G1329B,<br>Serial No. DEABE00890 | | |



Date:- 09/07/2015

Sample Id. :- Product name- Eye drop

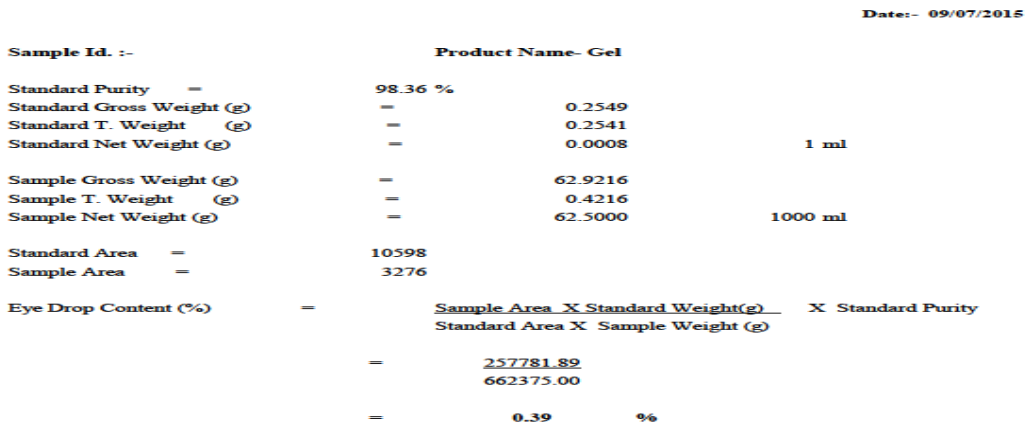
| | | | |
|---|---|---|---|
| Standard Purity = | 98.36 % | | |
| Standard Gross Weight (g) | = | 0.2549 | |
| Standard T. Weight (g) | = | 0.2541 | |
| Standard Net Weight (g) | = | 0.0008 | 1 ml |
| Sample Gross Weight (g) | = | 62.9216 | |
| Sample T. Weight (g) | = | 0.4216 | |
| Sample Net Weight (g) | = | 62.5000 | 1000 ml |
| Standard Area = | 10598 | | |
| Sample Area = | 7277 | | |

$$\text{Gel Content (\%)} = \frac{\text{Sample Area X Standard Weight(g)}}{\text{Standard Area X Sample Weight (g)}} \times \text{Standard Purity}$$

$$= \frac{572612.58}{662375.00}$$

$$= 0.86 \ \%$$

**Figure 4: Eye drop formulation contained 0.86 % of beta-sitosterol Detected by HPLC**



Date:- 09/07/2015

Sample Id. :- Product Name- Gel

| | | | |
|---|---|---|---|
| Standard Purity = | 98.36 % | | |
| Standard Gross Weight (g) | = | 0.2549 | |
| Standard T. Weight (g) | = | 0.2541 | |
| Standard Net Weight (g) | = | 0.0008 | 1 ml |
| Sample Gross Weight (g) | = | 62.9216 | |
| Sample T. Weight (g) | = | 0.4216 | |
| Sample Net Weight (g) | = | 62.5000 | 1000 ml |
| Standard Area = | 10598 | | |
| Sample Area = | 3276 | | |

$$\text{Eye Drop Content (\%)} = \frac{\text{Sample Area X Standard Weight(g)}}{\text{Standard Area X Sample Weight (g)}} \times \text{Standard Purity}$$

$$= \frac{257781.89}{662375.00}$$

$$= 0.39 \ \%$$

**Figure 5: Ophthalmic gel formulation contained 0.39 % of beta sitosterol Detected.**

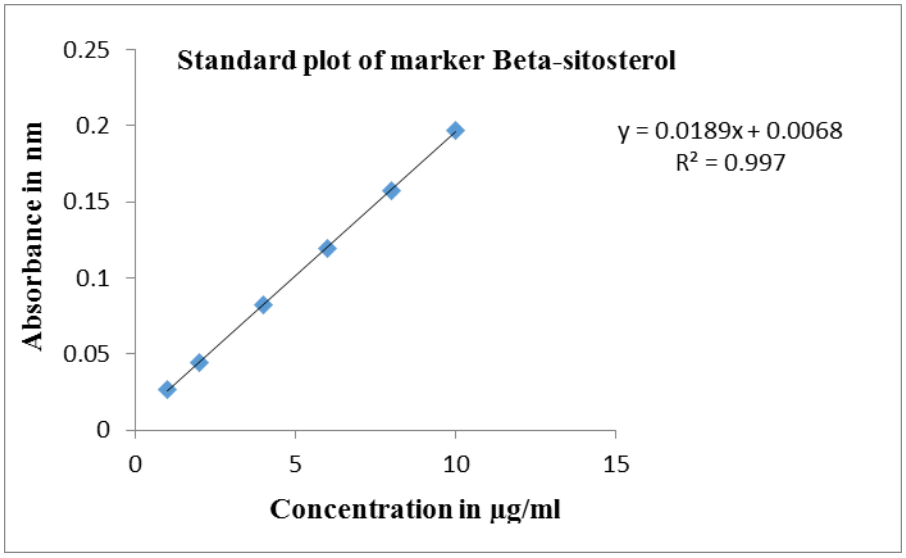



**Figure 6: Regression curve of marker beta-sitosterol in PBS (7.2) at 273.6 nm.**

## REFERENCES

1. Ahmad B. & Chung-Ping Y., Boravine a dihydrosiofuraxanthone from *Boerhavia diffusa*, *Phytochemistry*. 1992, 31 (12): 4382-84.
2. Semwal DK, Mishra SP, Semwal RB. Ayurvedicresearch and methodology: Present status and futurestrategies. *AYU*. 2015, 36(4):364-9.
3. Lad V. The complete book of ayurvedic home remedies.New York: Three Rivers Press; 1999.Chopra GL. Angiosperms. Systematic and life cycle.Jalandhar, Punjab, India: S. Nagin and Co; 1969, 361-5.
4. Hindawi Publishing Corporation BioMed ResearchInternational; 2014. Article ID 808302:1-19.
5. Wahi AK, Agrawal VK, Gupta RC. Phytochemical andpharmacological studies on Boerhavia diffusa Linn (punarnava) alkaloids. National Academy of ScienceLetters. 1997; 20.
6. Kirtikar KR, Basu BD. Boerhavia diffusa. Indian MedicinalPlants. Allahabad, India: *Lalit Mohan Basu Publications*; 1956, 2045-8.
7. Chakraborti KK, Handa SS. Antihepatotoxic activity ofBoerhavia diffusa. *Indian Drugs*. 1989; 27(13):161-6.
8. Aslam M. Asian medicine and its practice in Britain. In:Evans WC, editor. Pharmacognosy. London UK: SaundersCompany Ltd; 1996:499-500.
9. Rawat AK, Mehrotra S, Tripathi SC, Shome U.Hepatoprotective activity of Boerhavia diffusa L.roots-a popular Indian ethnomedicin. Journal ofEthnopharmacology. 1997; 56(1):61-6.
10. Indian Pharmacopoeia. Indian PharmacopoeiaCommittee. New Delhi: Ministry of Health and FamilyWelfare, Government of India; 2007; 1423-4.
11. Ayurvedic Pharmacopoeia of India. Dept of AYUSH.New Delhi: Ministry of Health and Family Welfare,Government of India; 1990;126-8.
12. Singh A , Sharma H, Singh R, Pant P, Srikant N, Dhiman KS. Identification and Quantification of Boeravinone-B in Whole Plant Extract of *Boerhavia diffusa* Linn and in its Polyherbal Formulation. Journal of Natural Remedies. 2017; 17 (3):88-95