Research Article

STANDARDIZATION OF ANTI-DIABETIC AYURVEDIC HERBO-MINERAL FORMULATION

Kruti Pandya[1*], Kunal Maniar[1], Hardik Soni[1], Dr Surendra Bhatt[1], Prashant Patel[2], Bhavna Solanki[1], Nilesh Gurav[1]
[1]Vasu Research Centre, A Division of Vasu Healthcare Pvt Ltd, Vadodara, India.
[2]ARIBAS, New VV Nagar, Vadodara, India.
***Corresponding author's E-mail:** krutishah85@gmail.com

Accepted on: 05-06-2011; **Finalized on:** 30-08-2011.

ABSTRACT

Standardization of Glucova Active tablet, a herbo mineral formulation is essential in order to assess the quality of drugs, based on the concentration of their active principles. The formulation has a very good effect on Diabetes and the protocol included different parameters for authentification of raw material as well as finished product. HPLC and HPTLC were also carried put to authenticate the drugs along with other parameters like Microbial analysis, heavy metal analysis etc. the data got thus suggests that all the ingredients are useful in the formulation and have good reported activity against diabetes.

Keywords: Herbo-mineral formulation, HPLC, HPTLC, Heavy metal analysis, Microbial analysis.

1. INTRODUCTION[1-12]

1.1 Herbal Medicine

Medicinal herbs have been known for centuries and are highly valued all over the world as a rich source of therapeutic agents for prevention of diseases and ailments. India is rightfully called the "Botanical Garden of the World". Use of plants as a source of medicine has been inherited and is an important component of the health care system in India. The use of herbal medicines is steadily growing. Approximately 60 % of the population use herbal medicines to treat medical illnesses[13].

1.2 Standardization

Standardization is an essential factor for polyherbal formulation in order to assess the quality of the drugs based on the concentration of their active principle. The World Health Organization (WHO) has appreciated the importance of medicinal plants for public health care[14]. The process of evaluating the quality and purity of crude drugs by means of various parameters like morphological, microscopical, physical, chemical and biological observation is called standardization.

1.3 Introduction to Diabetes

Diabetes is a disorder of metabolism-the way our body uses digested food for growth and energy. Most of the food we eat is broken down by the digestive juices into a simple sugar called glucose. Glucose is the main source of fuel for the body. After digestion, the glucose passes into bloodstream where it is available for body cells to use for growth and energy. For the glucose to get into the cells, insulin must be present. Insulin is a hormone produced by the pancreas, a large gland near the stomach. When we eat, the pancreas is supposed to automatically produce the right amount of insulin to move the glucose from our blood into our cells. If body doesn't make enough insulin or the insulin doesn't work right, the sugar cannot get into the cells. It stays in the blood. This makes high levels of glucose (or sugar) in the blood (hyperglycemia).

As a result, glucose builds up in the blood, overflows into the urine, and passes out of the body (glucosuria). Thus, the body loses its main source of fuel even though the blood contains large amounts of glucose. Thus Diabetes is a chronic condition that occurs when the pancreas does not produce enough insulin or when the body cannot effectively use the insulin[2].

There are two types of Diabetes,

Type I: Diabetes Mellitus

Type II: Diabetes Mellitus

a. Juvenile onset diabetes/insulin dependent diabetes mellitus (IDDM)

b. Adult onset diabetes/non-insulin dependent diabetes mellitus (NIDDM)

1.4 Causes of Diabetes

The main causes for diabetes are Heredity i.e. family history of late onset diabetes, Obesity i.e. over weight, Lack of physical activity i.e. sedentary life style, Women with prior gestational diabetes, Stress and Strain**.**

2.0 Pharmacological activity of Ingredients of Glucova Active tablet.

It consists of fifteen ingredients viz., extract of *Enicostemma littorale Blume, Pterocarpus marsupium Roxb, Eugenia jambolana, Tinospora cordifolia Willd, Momordica charantia Linn, Gymnema sylvestre R.Br, Syzygium cumini Linn, Azadirachta indica, Caesalpinia bonducella, Swertia chiraita Buch Ham, Curcuma longa, Emblica officinalis, Symplocos racemosa Roxb* and powder of Pramehahar kwath, Chandraprabha and Trikatu.

Literature survey of all these plants reveals that they all have scientific reports to be useful in diabetic

conditions.[15-17], Pramehahar Kwath[18] and Chandraprabha Powder[19]

2.1 Composition of Herbo-mineral formulation[20-23]

Table 1: Ingredients with its botanical name and part used

Sr No.	Common Name	Botanical Name	Part Used
1	Mammejak	*E. littorale*	Whole plant
2	Vijaysar	*P marsupium*	Heart wood
3	Karvella	*M. charantia*	Fruit
4	Guduchi	*T. cordifolia*	Stem
5	Madhunashini	*G. sylvestre*	Aerial
6	Jamun	*S. cuminii*	Seed
7	Neem	*A. indica*	Leaves
8	Latakaranj	*C.Bonducella*	Seed
9	Kiratatikta	*S. chiraita*	Aerial
10	Haridra	*C. longa*	Rhizome
11	Amalaki	*E. officinalis*	Fruit
12	Lodhra	*S. racemosa*	Stem bark
13	Pramehahar Kwath powder	-	Powder
14	Chandraprabha powder	-	Powder
15	Trikatu	-	Powder

3.0 MATERIAL AND METHODS

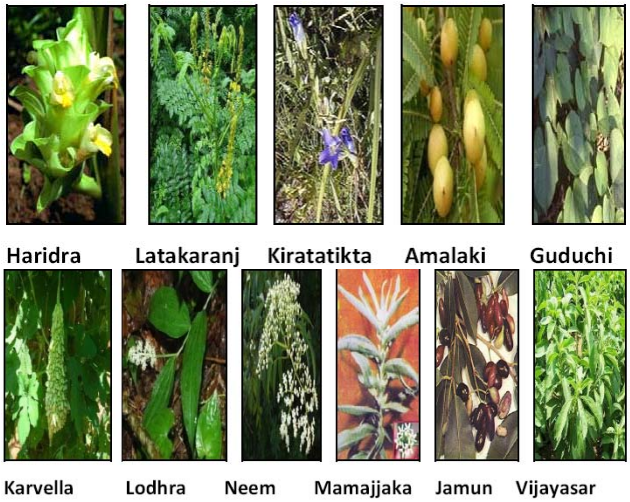
3.1 Collection of raw material and finished product

Glucova Active tablet consists of fifteen ingredients as listed above. The extracts were procurred from standard places in and around India while the powders of Chandraprabha, and Herbo-mineral formulation were formulated by Vasu Healthcare Pvt. Ltd. Vadodara, India. All the extracts used in Herbo-mineral formulation were vacuum dried powder form.

3.2 Evaluation of Quality Control Parameters for Raw Material

Organoleptic parameters: Organoleptic parameter like colour, odour and taste of all extracts were carried out. These parameters helped in visual identity of drug extract which were derived from plant by various extraction procedures.

3.3 Identification of plants



3.4 Organoleptic characteristics of ingredients used in the Herbo-mineral formulation

Organoleptic characteristics are provided in table 2.

3.5 Physicochemical parameters: The physiochemical Parameters (tables 3-8) includes tests like Moisture content / Loss on drying**,** Determination of ash, Water soluble ash**,** Determination of extractive matter**,** Alcohol soluble extractive**,** Water soluble extractive**,** Determination of pH**,** Bitter value, Tannin value and Saponin value.

3.6 Evaluation of Quality Control Parameters for Glucova Active Tablet

Description: The general appearance, its visual identity is essential and involves the measurement of a number of attributes such as size, shape, colour, type of coating in tablet etc.

Uniformity of weight, Measurement of diameter and thickness of the tablet, Measurement of hardness, Determination of pH, Disintegration & Dissolution test for tablet and determination of Moisture content. The results are as given in table 9.

3.7 Microbial Analysis: Microbial analysis was carried out for all the extracts as per procedure of Indian pharmacopoeia 2007 and WHO Guideline. It included total bacterial count, Total Fungal Count, Presence of *Escherichia coli, Salmonella ebony, Pseudomonas aeruginosa,* and *Staphylococcus aureus.* Pure culture of *Escherichia coli* (NCIM: 2065; ATCC: 8739), *Salmonella ebony* (NCIM: 2257 NCTC: 6017), *Pseudomonas aeruginosa* (ATCC 9027), *Staphylococcus aureus* (ATCC 6358) were obtained from NCIM Pune. The media used for the microbial limit test were of HiMedia Pvt. Ltd[24-26]. The results are as given in table 10.

3.8 Heavy Metal Analysis

Preparation of samples by Acid digestion method: 2 g of each sample of Herbo-mineral formulation tablet was taken in a Kjeldahl flask. An acid mixture of HNO3:HClO4 (4:1) was added in the flask and heated continuously till the solution is colorless. The sample was then transferred to a 25 ml volumetric flask and the volume was made-up with distilled water. Reagent blank was synchronously prepared according to the above procedure. The standards of Lead (Pb), cadmium (Cd) and arsenic (As) were prepared as per the protocol in the manual and the calibration curve was developed for each of them.[27] The samples were analyzed using Atomic absorbance spectrophotometer (AAS) 6300 (by SHIMADZU). The results are given in table 11.

Table 2: Organoleptic characters of ingredients of Herbo-mineral formulation

Sr. No.	Name of the ingredients	Part used	Description		
			Colour	Odour	Taste
1	Mamajjak	Whole plant	Cream	Characteristic	Bitter
2	Vijaysar	Heartwood	Brown	Characteristic	Bitter
3	Karela	Fruit	Dark brown	Characteristic	Bitter
4	Guduchi	Stem	Cream	Characteristic	Bitter
5	Madhunashini	Aerial	Brown	Characteristic	Bitter
6	Jamun	Seed	Chocolate brown	Characteristic	Bitter
7	Neem	Leaves	Light green	Characteristic	Bitter
8	Latakaranj	Seed	Light green	Characteristic	Bitter
9	Kiratatikta	Aerial	Light green	Characteristic	Bitter
10	Haridra	Rhizome	Yellow	Characteristic	Bitter
11	Amalaki	Fruit	Light Brown	Characteristic	Bitter
12	Lodhra	Stem bark	Light Brown	Characteristic	Bitter
13	Pramehahar Kwath	Formulation	Yellow	Characteristic	Bitter
14	Chandraprabha	Formulation	Reddish brown	Pleasant	Bitter
15	Trikatu	Formulation	Greyish brown	Pleasant	Bitter

Table 3: Moisture content and pH of ingredients present in Herbo-mineral formulation

Sr. No.	Name of Ingredients	Physicochemical Parameters	
		Moisture content % w/w	pH of 1% w/w sol^n
1	Mamejjak	2.15 ± 0.25	5.73 ± 0.26
2	Vijayasar	0.0 ± 0.17	4.47 ± 0.48
3	Karvellak	2.74 ± 0.26	4.98 ± 0.26
4	Guduchi	2.73 ± 0.15	5.49 ± 0.41
5	Madhunashini	3.07 ± 0.43	5.77 ± 0.59
6	Jamun	3.5 ± 0.28	4.38 ± 0.48
7	Neem	3.17 ± 1.12	5.0 ± 0.89
8	Latakaranj	1.58 ± 0.41	4.5 ± 1.23
9	Kiratatikta	0.58 ± 0.32	5.4 ± 0.65
10	Haridra	2.47 ± 0.16	3.77 ± 0.98
11	Amalaki	1.78 ± 0.26	2.85 ± 0.84
12	Lodhra	1.8 ± 0.48	4.4 ± 0.25
13	Pramehahar Kwath Powder	4.00 ± 0.22	3.92 ± 0.15
14	Chandraprabha powder	2.93 ± 0.46	4.81 ± 0.22
15	Trikatu	2.00 ± 0.26	3.9 ± 0.36

Table 4: Water and Alcohol Soluble Extractives of ingredients of Herbo-mineral formulation

Sr. No.	Name of the Ingredients	Physicochemical parameters	
		Extractive Values (%w/w)	
		Water Soluble Extractive Values	Alcohol Soluble Extractive Values
1	Mamejjak	55.0 ± 0.26	32.0 ±0.16
2	Vijayasar	45.0 ± 0.56	18.0 ± 0.89
3	Karvellak	52 .0 ± 0.48	13.0 ± 0.12
4	Guduchi	72.0 ± 0.75	13.36 ± 0.33
5	Madhunashini	58.0 ± 0.26	28.0 ± 0.13
6	Jamun	53.0 ± 0.15	45.0 ± 0.48
7	Neem	65.0 ± 0.22	72.88 ± 0.88
8	Latakaranj	46.24 ± 0.59	5.04 ± 0.12
9	Kiratatikta	85.7 ± 0.89	11.36 ± 0.36
10	Haridra	70.0 ± 0.78	40.00 ± 0.65
11	Amalaki	80.32 ± 0.26	54.4 ± 0.48
12	Lodhra	71 ± 0.26	53 ± 0.26
13	Pramehahar Kwath Powder	40 ± 0.45	21 ± 0.33
14	Chandraprabha powder	46 ± 0.89	18 ± 0.26
15	Trikatu	32 ± 0.56	20 ± 0.48

N = 3, Results ± SEM

Table 5: Ash values of Ingredients of Herbo-mineral formulation.

Sr. No.	Name of the ingredients (In the form of)	Physicochemical parameters		
		Ash value (%w/w)		
		Total ash	Acid insoluble ash	Water soluble ash
1	Mamajjak	4.75 %	0.15 %	3.00 %
2	Vijaysar	1.7 %	1.05 %	1.00 %
3	Karela	6.35 %	3.32 %	1.25 %
4	Guduchi	21.00 %	18.30 %	29.90 %
5	Madhunashini	27.00 %	4.10 %	5.50 %
6	Jamun	5.90 %	0.6 %	1.2 %
7	Neem	17.10 %	19.80 %	29.88 %
8	Latakaranj	2.7 %	9 %	16 %
9	Kiratatikta	3.4 %	5 %	1 %
10	Haridra	5.6 %	2 %	3.12 %
11	Amalaki	5.75 %	0.55 %	2.65 %
12	Lodhra	8 %	0.15 %	3 %
13	Pramehahar Kwath Powder	10.10 %	3.35 %	2.35 %
14	Chandraprabha powder	14.75 %	7.75 %	9.4 %
15	Trikatu	12.60 %	6.55 %	6.40 %

Table 6: Bitter value of ingredients present in Herbo-mineral formulation

Sr. No.	Name of the Ingredients	Bitter Value (%)
1	Mamajjak (*Enicostemma littorale*)	3.53
2	Vijaysar (*Pterocarpus marsupium*)	7.08
3	Karvellak (*Momordica charantia*)	2.5
4	Kiratatikta (*Swertia chiraita*)	5.03
5	Latakaranj (*Caesalpinia bonducella*)	2.43
6	Guduchi (*Tinospora cordifolia*)	21.40
7	Neem (*Azadirachta indica*)	19.13

Table 7: Tannin value of ingredients present in Herbo-mineral formulation

Sr. No.	Name of the Ingredients	Tannin Value (%)
1	Lodhra (*Symplocos racemosa*)	3.09
2	Vijaysar (*Pterocarpus marsupium*)	6.95
3	Amalaki (*Emblica officinalis*)	14.69

Table 8: Saponin value of ingredients present in Herbo-mineral formulation

Sr. No.	Name of the Ingredients	Saponin Value (%)
1	Jamun (*Syzgium cuminii*)	6.2

Table 9: Quality control parameters of Glucova Active Tablet

Parameters	1	2
Description		
Colour Shape Coating	White Round Film coating	White Round Film coating
Physiochemical parameters		
Average weight	600.6 ±.2.90 mg	601.6 ±1.82 mg
Diameter	11.6 ± 0.014 mm	11.67 ±0.11 mm
Thickness	6.07 ± 0.08 mm	6.11 ± 0.02 mm
Hardness	5.634 ± 0.102 kg/cm^3	5.611 ± 0.200 kg/cm^3
pH	6.99	7.00
Disintegration	39 ± 2min	42 ±2 min 10 sec
Dissolution	72.42 ± 0.25 %	71.82 ± 0.20 %
Moisture content	3.25 ± 0.1 %	4.10 ±0.1 %

Table 10: Microbial analysis

Sr. No	Name of the Sample	Microbial Analysis					
		TBC	TFC	*E.coli*	*Sal. sp*	*S. aureus*	*P. aeruginosa*
1	Mammejak	5 x10^2 cfu/g	4x10^2 cfu/g	Ab	Ab	Ab	Ab
2	Vijaysar	1x10^2 cfu/g	1x10^2 cfu/g	Ab	Ab	Ab	Ab
3	Karvellak	4x10^2 cfu/g	4x10^2 cfu/g	Ab	Ab	Ab	Ab
4	Guduchi	3x10^2 cfu/g	1x10^2 cfu/g	Ab	Ab	Ab	Ab
5	Madhunashini	3x10^2 cfu/g	4x10^2 cfu/g	Ab	Ab	Ab	Ab
6	Jamun	14x10^2 cfu/g	3x10^2 cfu/g	Ab	Ab	Ab	Ab
7	Neem	5x10^2 cfu/g	3x10^2 cfu/g	Ab	Ab	Ab	Ab
8	Latakaranj	2x10^2 cfu/g	Absent	Ab	Ab	Ab	Ab
9	Kiratatikta	5x10^2 cfu/g	1x10^2 cfu/g	Ab	Ab	Ab	Ab
10	Haridra	25x10^2 cfu/g	7x10^2 cfu/g	Ab	Ab	Ab	Ab
12	Amalaki	10x10^2 cfu/g	3x10^2 cfu/g	Ab	Ab	Ab	Ab
13	Lodhra	5x10^2 cfu/g	1x10^2 cfu/g	Ab	Ab	Ab	Ab
14	Pramehahar. Kwath	168 x 10^2 cfu/g	-	Ab	Ab	Ab	Ab
15	Chandraprabha	121 x 10^2 cfu/g	2x10^2 cfu/g	Ab	Ab	Ab	Ab
16	Herbo-mineral formulation	5 x 10^2 cfu/g	1x10^2 cfu/g	Ab	Ab	Ab	Ab

Table 11: Heavy Metal Analysis

Sr. No.	Name of the Sample	Heavy Metals		
		Lead (Limit-10 ppm)	Cadmium (Limit-0.3ppm)	Arsenic (Limit-10ppm)
1	Haridra	0.4168	0.1410	0.1510
2	Madhunashini	0.9648	0.0445	0.1827
3	Jamun	1.1881	0.0487	0.1915
4	Vijaysar	2.2238	0.0641	0.4243
5	Amalaki	1.1027	0.0572	0.1095
6	Latakaranj	2.9811	0.0376	0.1334
7	Kiratatikta	2.4216	0.0310	0.1108
8	Karvellak	0.8270	0.0509	0.1954
9	Lodhra	1.6540	0.1272	0.1746
10	Mammejak	0.2730	0.0567	0.7300
11	Neem	1.6269	0.0813	0.4351
12	Trikatu	1.1208	0.1034	0.1171
13	Herbo-mineral formulation	1.2300	0.1000	0.2800

Table 12: Solvent system for plant, extract, Herbo-mineral formulation

Sr. No.	Sample	Solvent system
1	*Enicostemma littorale*	Ethyl acetate: Methanol (8:2)
2	*Pterocarpus marsupium*	Ethyl acetate: Glacial acetic acid: Formic acid: Water (10:1.1:1.1:2.6)
3	*Momordica charantia*	Chloroform:Methanol (8.5:1.5)
4	*Tinospora cordifolia*	Chloroform:Methanol (9:1)
5	*Gymnema sylvestre*	Chloroform:Mehtanol:Water (6.5:3.5:1)
6	*Syzygium cuminii*	Toluene:Ethyl acetate (9:1)
7	*Azadirachta indica*	Ethyl acetate: Glacial acetic acid: Formic acid: Water (10:1.1:1.1:2.6)
8	*Caesalpinia bonducella*	N hexane:Ethyl acetate (6:4)
9	*Swertia chiraita*	Chloroform:Methanol (8.5:1.5)
10	*Curcuma longa*	Toluene:Ethyl acetate:Mehtanol (6:4:0.1)
11	*Symplocos racemosa*	Chloroform:Methanol (8.5:1.5)

3.9 High Performance Liquid Chromatography.

a) Estimation of curcumin in *Curcuma longa* extract

Sample Preparation: Dissolve 0.5 mg extract in 25 ml mobile phase. Filter the solution using 0.22 mm filter paper. Use filtrate as a sample solution.

Standard Preparation: Dissolve 0.5 mg extract in 25 ml mobile phase. Filter the solution using 0.22 mm filter paper. Use filtrate as a sample solution.

Chromatographic Conditions:

Analytical HPLC: Shimadzu LC20AT SPD 20A

Column: Luna 5µ C-18 (2), 250 X 4.6 mm

Mobile Phase: Acetonitrile:Tetrahydrofuran:2%GAA=5:3:2

Flow Rate: 1 ml/ min

Detector: UV 215nm

Temperature: Room temperature

Standard injected: 20µl

Sample injected: 20µl

Calculation:

$$\% \text{ Curcumin} = \frac{\text{wt of std}}{\text{Dil of std}} \times \frac{\text{dilution of sample}}{\text{wt of sample}} \times \frac{\text{peak area of sample}}{\text{peak area of std}} \times \frac{\text{purity of std}}{100\text{- LOD}} \times 100$$

HPLC for estimation of Active constituents

(a) Estimation of curcumin in *Curcuma longa* extract

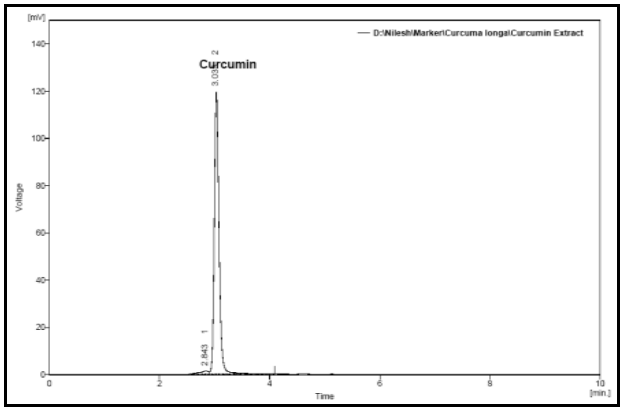


Figure 1: CURCUMIN STD

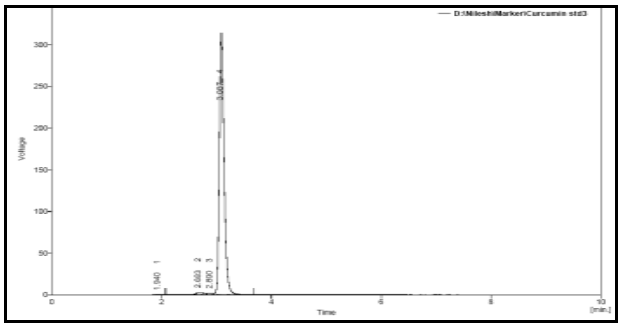


Figure 2: CURCUMIN EXTRACT

RESULT: Thus the % of Curcumin present in the given sample was 15.4 %

3.11 HPTLC finger-printing of raw materials

HPTLC is the most simple separation technique available today which gives better precision and accuracy with extreme flexibility for various steps (stationary phase, mobile phase, development technique and detection). The HPTLC was carried out using a Hemilton 100 µl HPTLC syringe, Camag Linomat V automatic spotting device, Camag twin trough chamber, Camag TLC Scanner-3, WINCAT integration software, aluminium sheet precoated with Silica Gel 0F254(Merck), 0.2 mm thickness. HPTLC finger printing technique is useful to identify and to check the purity of raw herbal extracts as well as finished product. Hence forth it is very useful tool in standardizing process of raw herbal extracts and finished products.

Steps involved in HPTLC analysis

- **Selection of plate and adsorbent:** Precoated aluminum plates with Silica Gel 60F254 (E. Merck, India) of 10 x 10 cm and 0.2 mm thickness, were used for the detection. The plates were pre-washed by methanol and activated at 60°C for 5 min prior to chromatography.

- **Sample solution:** Accurately weighed 50 mg of methanol extract of *Enicostemma littorale* whole plant was taken, dissolved in methanol and transferred to a 10 ml volumetric flask. The volume was made up to the mark with Petroleum ether. Preparation of sample solution was same for the raw herbs as well as for Herbo-mineral formulation tablet. This solution was further used for HPTLC finger-printing.

- **Application of sample:** Sample application is the most critical step for obtaining good resolution for quantification in HPTLC. The automatic application devices are preferable. The most recent automatic device "CAMAG LINOMAT V" was used to apply 1 band of 6 mm width with different concentration of *E. littorale* extract solution viz. 5 & 10 µl.

- **Development:** The plate was developed in CAMAG glass twin-through chamber (10-10 cm) previously saturated with the solvent for 60 min (temperature 25.2°C, relative humidity 40%). The development distance was 8 cm. Subsequently scanning was done. The mobile phase or solvent system for all the raw herbs, raw ingredients as well as Herbo-mineral formulation which is given in the Table 12.

3.12 HPTLC Finger Printing of the Raw Material

CAMAG TLC scanner 3 and LINOMAT-V densitometry evaluation system with WINCAT software was used for scanning of thin layer chromatogram objects in reflectance or transmission mode by absorbance or by fluorescence at 254 or 366 nm, respectively.

Rf value of sample was evaluated using following formula

$$Rf = \frac{\text{Distance travelled by sample from baseline}}{\text{Distance traveled by solvent from baseline}}$$

3.12.1 HPTLC finger printing of extract of *Pterocarpus marsupium* [28-29]

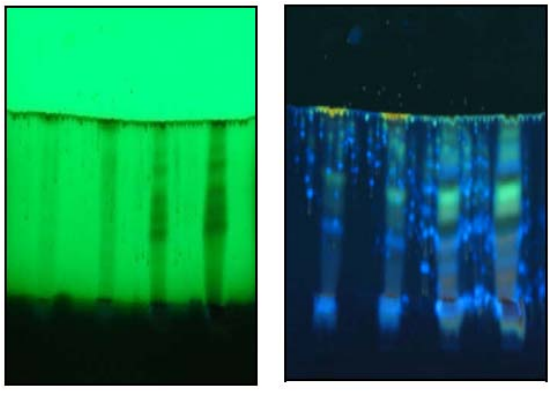


Figure 3: HPTLC plates of *Pterocarpus marsupium*

Track 1: 5 µg/ml of Methanol extract of Herbo-mineral formulation.

Track 2: 10 µg/ml of Methanol extract of Herbo-mineral formulation

Track 3: 5 µg/ml of Methanol extract of heart wood of herb *P. marsupium*.

Track 4: 10 µg/ml of Methanol extract of heart wood of herb *P. marsupium*.

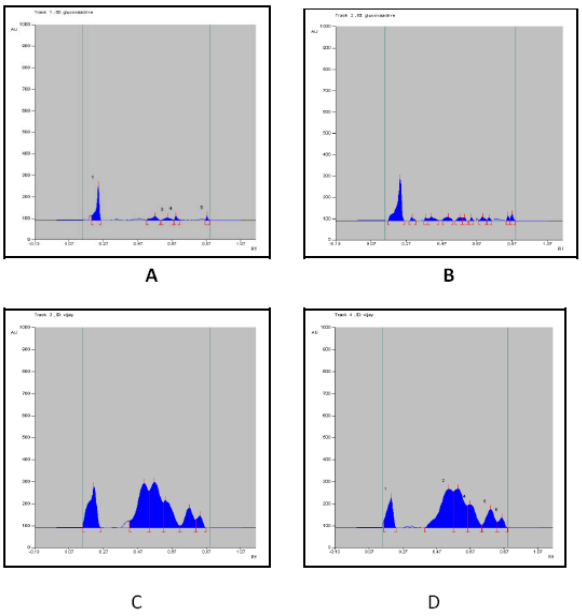


Figure 4: HPTLC finger printing chromatogram of *P. marsupium*

A-Track 1Chromatogram of methanol extract of Herbo-mineral formulation

B-Track 2: Chromatogram of methanol extract of Herbo-mineral formulation

C-Track 3: Chromatogram of methanol extract of herb of *P. marsupium*

D-Track 4: Chromatogram of methanol extract of herb of *P. marsupium*

3.12.2 HPTLC Finger printing of extract of *Syzygium cumini*

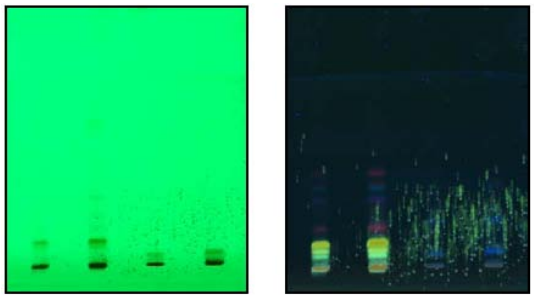


Figure 5: HPTLC plates of *S. cumini*

Track 1: 5 µg/ml of Methanol extract of Herbo-mineral formulation.

Track 2: 10 µg/ml of Methanol extract of Herbo-mineral formulation

Track 3: 5 µg/ml of Methanol extract of herb *S. cumini*.

Track 4: 10 µg/ml of Methanol extract of herb *S. cumini*.

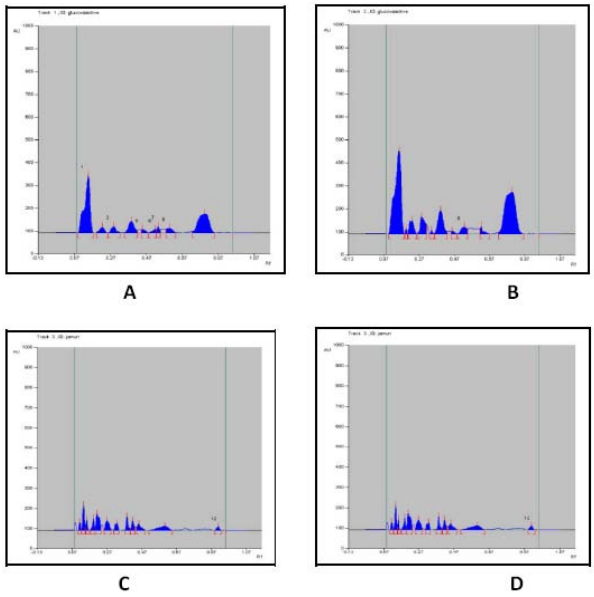


Figure 6: HPTLC finger printing chromatogram of *S. cumini*

A-Track 1: Chromatogram of methanol extract of Herbo-mineral formulation

B-Track 2: Chromatogram of methanol extract of Herbo-mineral formulation

C-Track 3: Chromatogram of methanol extract of herb of *S. cumini*.

D-Track 4: Chromatogram of standard extract of herb of *S. cumini*

3.12.3 HPTLC Finger printing of extract of *Tinospora cordifolia*[30-31]

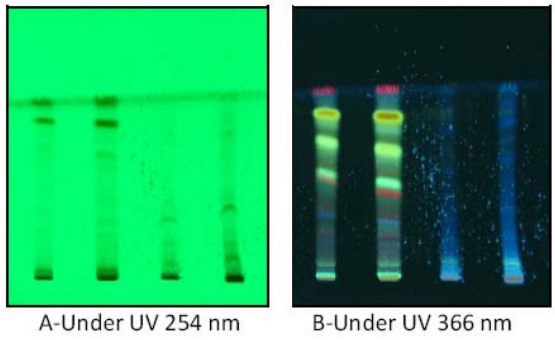


Figure 7: HPTLC plates of *T. cordifolia*

Track 1: 5 µg/ml of Methanol extract of Herbo-mineral formulation.

Track 2: 10 µg/ml of Methanol extract of Herbo-mineral formulation.

Track 3: 5 µg/ml of Methanol extract of herb *T. cordifolia*.

Track 4: 10 µg/ml of Methanol extract of herb *T. cordifolia*.

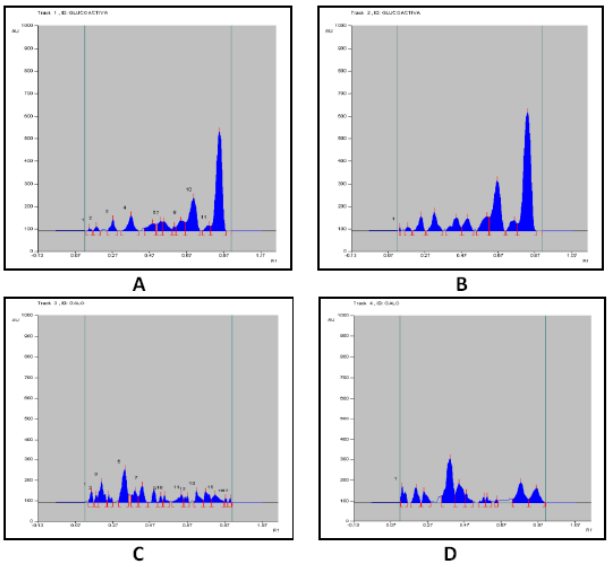


Figure 8: HPTLC finger printing chromatogram of *T. cordifolia*

A-Track 1: Chromatogram of methanol extract of Herbo-mineral formulation

B-Track 2: Chromatogram of methanol extract of Herbo-mineral formulationActive tablet

C-Track 3: Chromatogram of methanol extract of herb of *T. cordifolia*

D-Track 4: Chromatogram of standard extract of herb of *T. cordifolia*

3.12.4 HPTLC Finger printing of extract of *Gymnema sylvestre*[32]

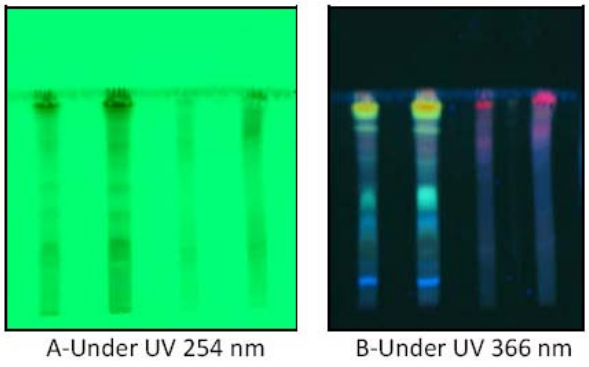


Figure 9: HPTLC plates of *G. sylvestre*

Track 1: 5 µg/ml of Methanol extract of Herbo-mineral formulation.

Track 2: 10 µg/ml of Methanol extract of Herbo-mineral formulation.

Track 3: 5 µg/ml of Methanol extract of herb *G. sylvestre*.

Track 4: 10 µg/ml of Methanol extract of herb *G. sylvestre.*

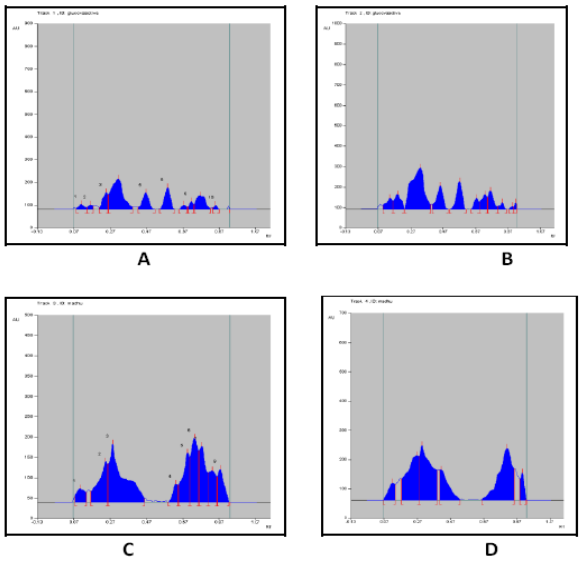


Figure 10: HPTLC finger printing chromatogram of *G. sylvestre*

A-Track 1: Chromatogram of methanol extract of Herbo-mineral formulation.

B-Track 2: Chromatogram of methanol extract of Herbo-mineral formulation.

C-Track 3: Chromatogram of methanol extract of herb of *G. sylvestre*

D-Track 4: Chromatogram of methanol extract of herb of *G. sylvestre*

3.12.5 HPTLC Finger printing of extract of *Momordica charantia*[33]

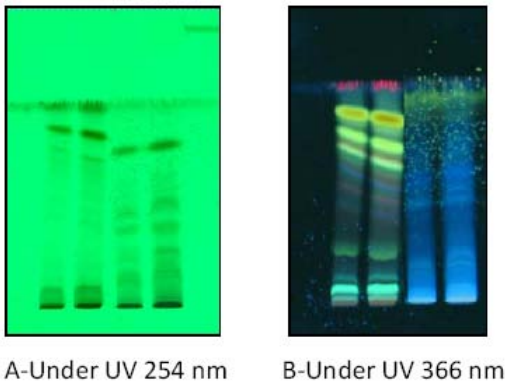


Figure 11: HPTLC plates of *M. charantia*

Track 1: 5 µg/ml of Methanol extract of Herbo-mineral formulation.

Track 2: 10 µg/ml of Methanol extract of Herbo-mineral formulation.

Track 3: 5 µg/ml of Methanol extract of herb *M. charantia.*

Track 4: 10 µg/ml of Methanol extract of herb *M. charantia*

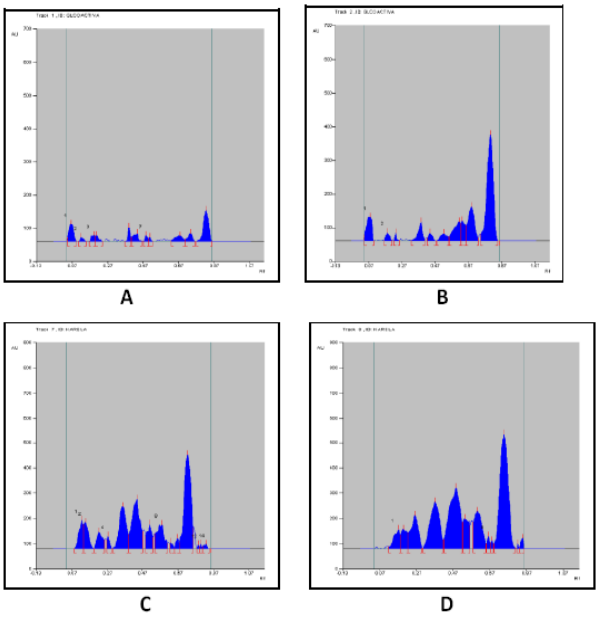


Figure 12: HPTLC finger printing chromatogram of *M. charantia*

A-Track 1: Chromatogram of methanol extract of Herbo-mineral formulation

B-Track 2: Chromatogram of methanol extract of Herbo-mineral formulation

C-Track 3: Chromatogram of methanol extract of herb of *M. charantia*

D-Track 4: Chromatogram of standard extract of herb of *M. charantia*

3.12.6 HPTLC finger printing of extract of Azadirachta indica.

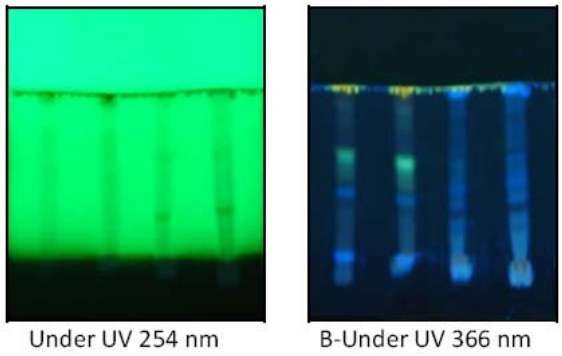


Figure 13: HPTLC plats of *A. indica*

Track 1: 5 µg/ml of Methanol extract of Herbo-mineral formulation.

Track 2: 10 µg/ml of Methanol extract of Herbo-mineral formulation.

Track 3: 5 µg/ml of Methanol extract of herb *A. indica.*

Track 4: 10 µg/ml of Methanol extract of herb A. indica

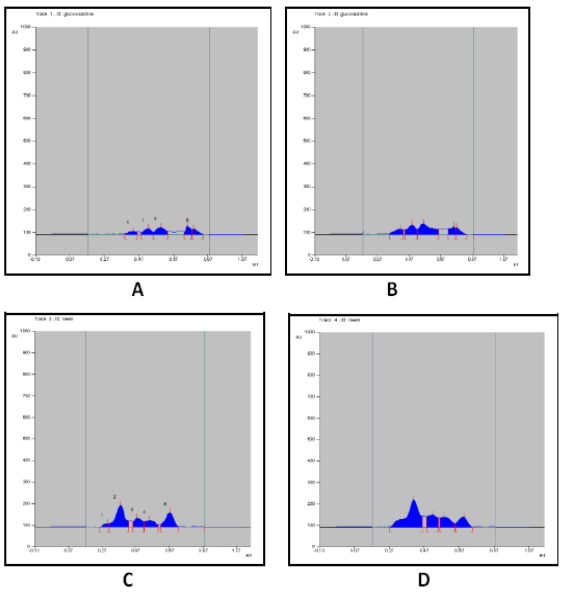


Figure 14: HPTLC finger printing chromatogram of *A. indica*

A-Track 1: Chromatogram of methanol extract of Herbo-mineral formulation

B-Track 2: Chromatogram of methanol extract of Herbo-mineral formulation

C-Track 3: Chromatogram of methanol extract of herb of *A. indica*

D-Track 4: Chromatogram of standard extract of herb of *A. indica*

3.12.7 HPTLC finger printing of extract of *Enicostemma littorale* [34-35]

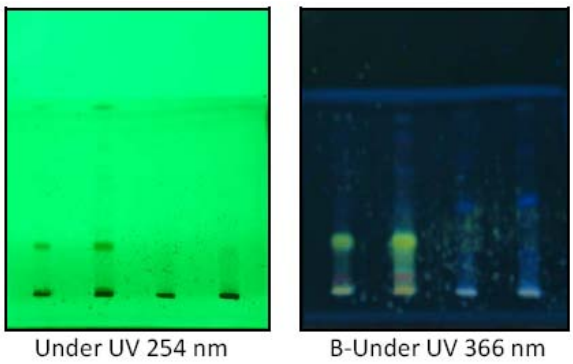


Figure 15: HPTLC plats of *E. littorale*

Track 1: 5 µg/ml of Methanol extract of Herbo-mineral formulation.

Track 2: 10 µg/ml of Methanol extract of Herbo-mineral formulation.

Track 3: 5 µg/ml of Methanol extract of herb *E. littorale.*

Track 4: 10 µg/ml of Methanol extract of herb *E. littorale.*

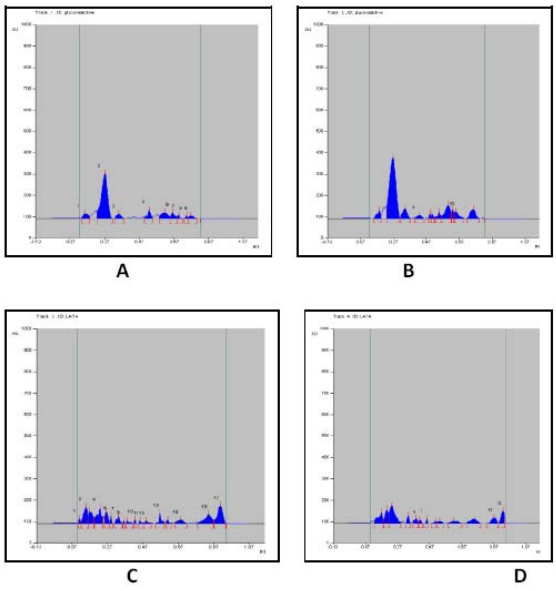


Figure 16: HPTLC finger printing chromatogram of *E. littorale*

A-Track 1: Chromatogram of methanol extract of Herbo-mineral formulation

B-Track 2: Chromatogram of methanol extract of Herbo-mineral formulation

C-Track 3: Chromatogram of methanol extract of herb of *Enicostemma littorale*

D-Track 4: Chromatogram of standard extract of herb of *Enicostemma littorale*

3.12.8 HPTLC finger printing of extract of *Caesalpinia bonducella*

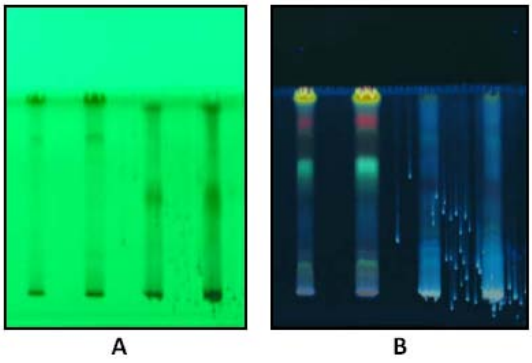


Figure 17: HPTLC plates of *C. bonducella*

Track 1: 5 µg/ml of Methanol extract of Herbo-mineral formulation.

Track 2: 10 µg/ml of Methanol extract of Herbo-mineral formulation.

Track 3: 5 µg/ml of Methanol extract of herb *C. bonducella.*

Track 4: 10 µg/ml of Methanol extract of herb *C. bonducella*

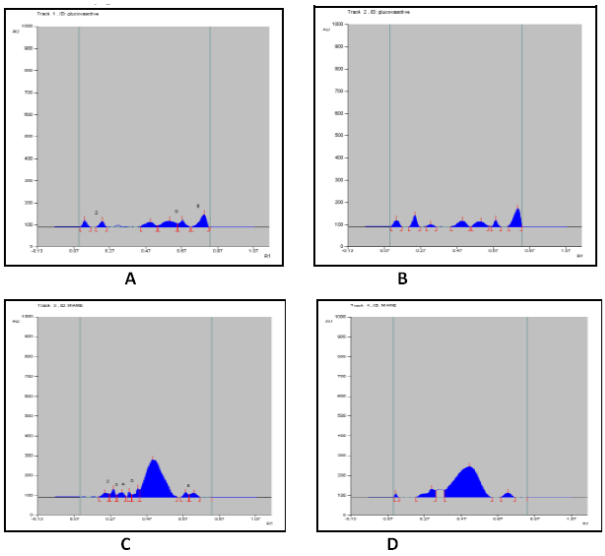


Figure 18: HPTLC finger printing chromatogram of *C. bonducella*

A-Track 1: Chromatogram of methanol extract of Herbo-mineral formulation

B-Track 2: Chromatogram of methanol extract of Herbo-mineral formulation

C-Track 3: Chromatogram of methanol extract of herb of *C.bonducella*

D-Track 4: Chromatogram of standard extract of herb of *C. bonducella*

3.12.9 HPTLC finger printing of extract of *Curcuma longa*

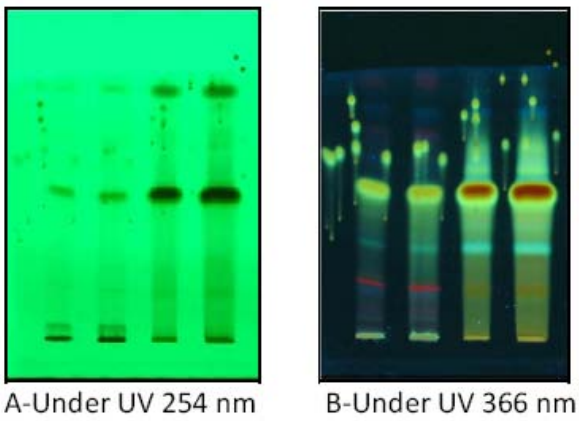


Figure 19: HPTLC plates of *C. longa*

Track 1: 5 µg/ml of Methanol extract of herb *C. longa.*

Track 2: 10 µg/ml of Methanol extract of herb *C. longa*

Track 3: 5 µg/ml of Methanol extract of Herbo-mineral formulation.

Track 4: 10 µg/ml of Methanol extract of Herbo-mineral formulation.

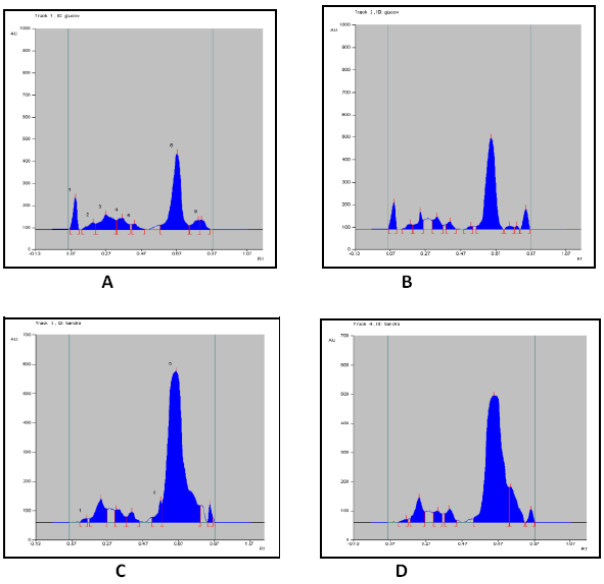


Figure 20: HPTLC finger printing chromatogram of *C. longa*

A-Track 3: Chromatogram of methanol extract of herb of *C. longa*

B-Track 4: Chromatogram of standard extract of herb of *C. longa*

C-Track 1: Chromatogram of methanol extract of Herbo-mineral formulation

D-Track 2: Chromatogram of methanol extract of Herbo-mineral formulation

3.12.10 HPTLC finger printing of extract of *Swertia chiraita*

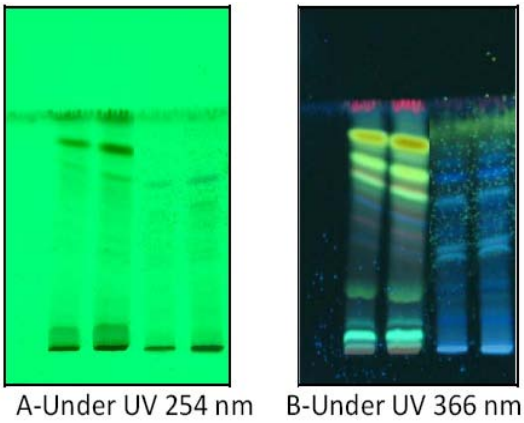


Figure 21: HPTLC plates of *S Chiraita.*

Track 1: 5 µg/ml of Methanol extract of Herbo-mineral formulation.

Track 2: 10 µg/ml of Methanol extract of Herbo-mineral formulation.

Track 3: 5 µg/ml of Methanol extract of herb *S. chiraita*

Track 4: 10 µg/ml of Methanol extract of herb *S. chiraita.*

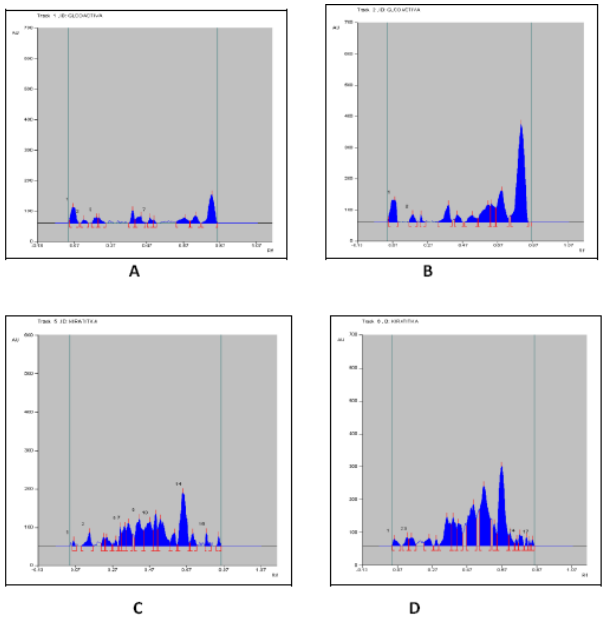


Figure 22: HPTLC finger printing chromatogram of *S. chiraita*

A-Track 1: Chromatogram of methanol extract of Herbo-mineral formulation

B-Track 2: Chromatogram of methanol extract of Herbo-mineral formulation

C-Track 3: Chromatogram of methanol extract of herb of *S. chiraita*

D-Track 4: Chromatogram of standard extract of herb of *S. chiraita*

3.12.11 HPTLC finger printing of extract of *Symplocos racemosa*

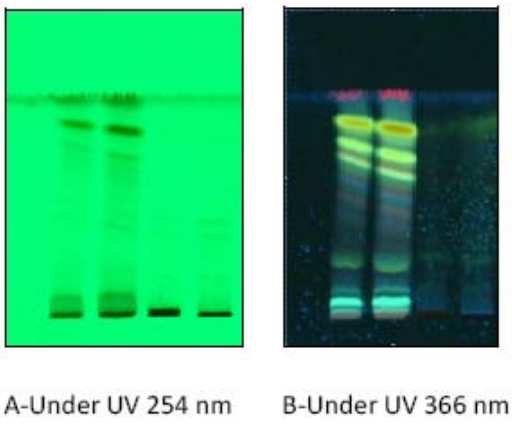


Figure 23: HPTLC plates of *S. racemosa*

Track 1: 5 µg/ml of Methanol extract of Herbo-mineral formulation.

Track 2: 10 µg/ml of Methanol extract of Herbo-mineral formulation.

Track 3: 5 µg/ml of Methanol extract of herb *S. racemosa*

Track 4: 10 µg/ml of Methanol extract of herb *S. racemosa*

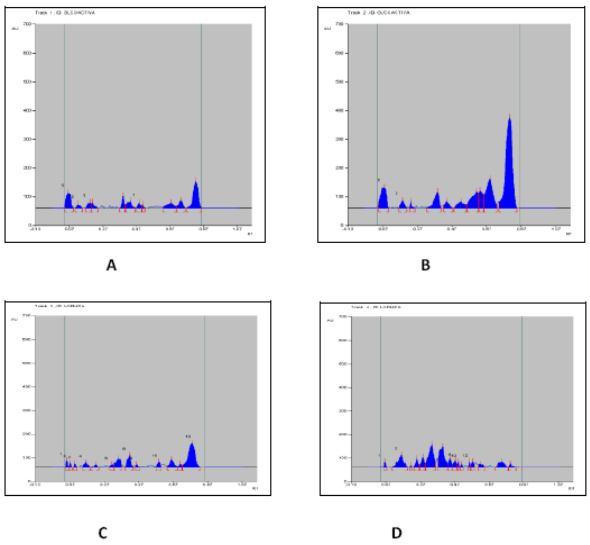


Figure 24: HPTLC finger printing chromatogram of *S. racemosa*

A-Track 1: Chromatogram of methanol extract of Herbo-mineral formulation

B-Track 2: Chromatogram of methanol extract of Herbo-mineral formulation

C-Track 3: Chromatogram of methanol extract of herb of *S. racemosa.*

D-Track 4: Chromatogram of standard extract of herb of *S. racemosa.*

4.0 SUMMARY AND CONCLUSION

All the extracts used in Herbo-mineral formulation shows their organoleptic characters. Moisture content of all the ingredients was less than 5%, so that it can prevent microbial growth and sticking problem in final processing of formulation. The pH of all ingredients was near about to neutral. The extracts used in Glucova Active Tablet were water soluble. Concentration of total ash and acid insoluble ash in each ingredient was less than the limit. The quality control parameters of the finished product were also under the limit. Also the data of HPTLC fingerprinting of all extracts indicates that all extracts were derived from genuine plants or parts of plant. Concentration of heavy metals in the extracts of Herbo-mineral formulation is also in limit. Thus in Conclusion, data suggested that tablets and all its extracts were consistent with various quality and purity parameters such as organoleptic characters, physico-chemical parameter, HPTLC fingerprinting, heavy metals and microbial analysis. So from above data, it can be said that Herbo-mineral formulation has all the necessary ingredients that are active against Diabetes.

5.0 Acknowledgement: We are highly thankful to the staff and management of **VASU HEALTHCARE PVT LTD** for providing us this opportunity in carrying out the research work

6.0 REFERENCES

1. Mohapatra P, Shirwaikara A, Aswatharam HN. Standardization of a Polyherbal Formulation. Pharmacognosy Magazine 2008;4(13):65-69.
2. Elsas JL, Longo N. Glucose Transporters. Annu Rev Med1992;43:377-393.
3. Tripathi KD. Essentials of Medical Pharmacology. Fifth Edition. Jaypee brothers, New Delhi 2003;242-243.
4. Pickup JC. Text Book of Diabetes. Second Edition. Black Well Scientific Publications, London 1991;Vol-2:4-9.
5. Rang HP, Dale MM, Ritter JM, Moor PK. Pharmacology. Fifth Edition. Elsevier, New Delhi 2003;385-387.
6. Mukherjee PK, Maiti K, Mukherjee K. Leads from Indian Medicinal Plants with Hypoglycemic potentials. Journal of Ethnopharmacology 2006;106:1
7. Hakin ZS. Potential antidiabetic agents from plant sources: Pharmacological Aspects. Indian Journal of Natural Product 1995;11(1):3.
8. Gupta AK, Tandon N, Sharma M. Quality Standards of Indian Medicinal Plants. Indian Council of Medicinal Research, New Delhi 2005;Vol-3:203- 211.
9. The Wealth of India, A dictionary of Indian Raw Materials and Industrial product. National Institute of Science communication & Council of Scientific and Industrial Research press, New Delhi 2002;Vol-3:174.
10. Nadkarani KM. Indian Materia Medica. Popular Prakashan Pvt. Ltd., Mumbai 1997;Vol-1:485.
11. Nadkarani KM. Indian Materia Medica. Popular Prakashan Pvt. Ltd., Mumbai 1997;Vol-1:485.
12. Sharma P, Mehta PM. Dravyaguna Vignyan. Chowkhamba Vidyabhawan, Varansi 1969;Vol-2:705.
13. Seth SD, Sharma B. Medicinal plants in India. Indian Journal of Medicinal Research 2004;120:9-11.
14. Elsas JL, Longo N. Glucose Transporters. Annu Rev Med1992;43:377-393.
15. The Ayurvedic Pharmacopoeia of India. First Edition. Government of India, Ministry of Health and Family Welfare Department of Indian System of Medicine and Homoeopathy, New Delhi 2006; Part-I:Vol-1:12.
16. Handa SS. Plants as drugs. The Eastern Pharmacist 1991;34:79-82.
17. Indian Herbal Pharmacopeia, Revised Edition-2002. Pg No. 218-220
18. Rastantrasar and Shiddhprayog Sangrah. Krushn Gopal Ayurved Bhavan, Ajmer 1680;Vol-1:711-712,619-621.
19. Pharmacopoeial Standard for Ayurvedic Formulations. Central Council for Research in Ayurveda and Siddha, New Delhi 2000;404.
20. Kirtikar KR, Basu BD. Indian Medicinal Plants. International book distributors, Dehradun, India 1999;Vol-1:828-829.
21. Kirtikar KR, Basu BD. Indian Medicinal Plants. International book distributors, Dehradun, India 1999;Vol-2:1052-1053.
22. Kirtikar KR, Basu BD. Indian Medicinal Plants. International book distributors, Dehradun, India 1999:Vol-1:77-80.

23. Kirtikar KR, Basu BD. Indian Medicinal Plants, International book distributors, Dehradun, India 1999;Vol-2:1130-1132.

24. Agrawal SS, Paridhavi M. Herbal Drug Technology. Universities Press (India) Private Limited 2007; 635-637.

25. Lohar DR. Protocol for testing of Ayurvedic, Siddha & Unani Medicines. Government of India, Department of AYUSH, Ministry of Health & Family Welfare, Pharmacopoeial Laboratory for Indian Medicines, Ghaziabad 2007; 91.

26. Indian Pharmacopoeia 2007, Volume 1,Published by Indian Pharmacopoeia Commision, Ghaziabad, Page 35.

27. Lohar DR. Protocol for testing of Ayurvedic, Siddha and Unani Medicines. Government of India, Department of AYUSH, Ministry of Health and Family Welfare, Pharmacopoeial Laboratory of Indian Medicines, Ghaziabad 2007; 47-52

28. Gayathri M, Kannabiran K. Ameliorative potential of aqueous extract of *Pterocarpus marsupium* Roxb bark on diabetes associated metabolic.

29. Manickam M, Ramanathan M, Jahromi MA, Chansouria JP. Anti hyperglycemic activity of phenolics from *Pterocarpus marsupium*. Journal of Natural Products 1997;60(6):609-610.

30. Bhatt RK, Sabata BK. A furanoid diterpene glucoside from *Tinospora cordifolia.* Phytochemistry 1989;28:2419-2422.

31. Wadood N, Wadood A, Shah SA. Effect of *Tinospora cordifolia* on blood glucose and total lipid levels of normal and alloxan-diabetic rabbits. Planta Med 1992;58(2):131-136

32. Persaud SJ, Al-Majed H, Raman A, Jones PM. *Gymnema sylvestre* stimulates insulin release *in vitro* by increased membrane permeability. J Endocrinol 1999;163(2):207-212.

33. Day C, Cartwright T, Provost J, Bailey CJ. Hypoglycaemic effect of *Momordica charantia* extracts. Planta Med 1990;56(5):426-429

34. Vijayvargia R, Kumar M, Gupta S. Hypoglycemic effect of aqueous extract of *Enicostemma littorale* Blume (Chhota chirayata) on alloxan induced diabetes mellitus in rats. Indian Journal of Experimental Biology 2000;38(8):781-784.

35. Maroo J, Ghosh A, Mathur R, Vasu VT, Gupta S. Antidiabetic efficacy of *Enicostemma littorale* methanol extract in alloxan-induced diabetic rats. Journal of Pharmaceutical Biology 2003;41(5):388-391.

****