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A DETAIL PHYTO-CHEMICAL EVALUATION OF AYURVEDIC TABLET FORMULATION USED FOR HAIR CARE

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ABSTRACT

Standardization of Ayurvedic formulations is an important step for the establishment of a consistent biological activity, chemical profile, or simply a quality assurance in herbal drugs. The WHO specifies guidelines for the assessment of the safety and quality of herbal medicines as a prerequisite for global harmonization. The present study was focused on an exhaustive evaluation of standardization parameters of a herbo-mineral Ayurvedic tablet preparation "Trichup Tablet". This was carried out employing the basic organoleptic test, physico-chemical tests, and bio-assays by sophisticated instruments like HPLC and HPTLC. HPTLC fingerprinting, assays of marker compounds were carried out to confirm the presence of the raw materials in the finished product along with their quality and potency. The study results revealed that the Tablet formulation was well standardized at various levels such as Physical consistency, Chemical profile, Microbial and Heavy metal limits.

KEYWORDS: Standardization, Herbo-mineral Ayurvedic Tablet, HPLC, HPTLC, Trichup Tablet.

INTRODUCTION

Standardization is a system that ensures a predefined amount of quantity, quality and therapeutic effect of ingredients in each dose. [1] It is very important that a system of standardization is established for every plant medicine in the market because the scope for

variation in different batches of medicine is enormous. Plant material may vary in its phytochemical content and therefore in its therapeutic effect according to different places of collection, with different times in a year for collection, with collection at the same time and places but in different years and with different environmental factors surrounding the cultivation of a particular medicinal plant. Adding to this variability is the fact that in herbal medicine several plants may be used together in the same preparation. This means that there should be a series of quality control tests which ensures the quality of the end product throughout the manufacturing process. [2]

World Health Organization (WHO) encourages, recommends and promotes traditional / herbal remedies in national health care programs because these drugs are easily available at low cost, and are generally regarded as safe. The WHO assembly in number of resolutions has emphasized the need to ensure quality control of medicinal plant products by using modern techniques and applying suitable standards. [3, 4]

Herbal product cannot be considered scientifically valid if the drug tested has not been authenticated and characterized in order to ensure reproducibility in the manufacturing of the product. Hence standardization is a very important process for the authentication of the drug.

In the present study, the herbo-mineral Ayurvedic Tablet preparation (Trichup Tablet) has been selected to establish its standardization status. The key ingredients used in the formulation are extracts of *Eclipta alba* (Bhringraj) whole plant, [5] *Glycyrrhiza glabra* (Yashtimadhu) Root, [6] *Emblica officinalis* (Amalaki) Fruit, [6] *Centella asiatica* (Mandukparni) whole plant, [5] *Hibiscus rosa-sinensis* (Japa) Flower, [6] *Tinospora cordifolia* (Guduchi) Stem, [6] *Tribulus terrestris* (Gokshur) Fruit[6] and powder of Triphala Churna (combination of three myrobalans), [6] Shukti Bhasma (calcined conch) [7] and the required excipients.

MATERIAL AND METHODS

Organoleptic parameters: Organoleptic parameter like appearance, colour and odour were used to confirm uniformity in visual identity of raw materials and finished product. The results are as tabulated in **Table 2**

Physicochemical parameters for extracts: [8] The physicochemical parameters include tests like pH, Loss on drying, Water soluble extractive and Determination of total ash of the relevant raw materials. The results are as tabulated in **Table 3 & 4**

Estimation of Actives: [9] Assay analysis includes estimation of Norwedalactone, Tannin (Titrimetric), Glycyrrhizin (gravimetric), Triterpene, Bitter and Saponin in respective ingredients. The results are as tabulated in **Table 5**

Evaluation of Standardization Parameters selected for Finished Product: The finished product were analyzed at three different stages. First the lubricated granules were analyzed for their description and moisture, at the second stage the core tablet was analyzed for its description, average net weight, disintegration time, hardness and friability. Finally, the finished product was analyzed for its description, average net weight, disintegration time and hardness. The results are as tabulated in **Table 6**

Microbial Analysis: [10] Bio-burden analysis consists of parameters like Total Bacterial Count, Total Fungal Count, and presence of pathogens like *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Salmonella enterica.* The results are as given in **Table 7**

Heavy Metal Analysis: [11] Sample preparation for heavy metal analysis was done by MARS Express microwave digestive system. The standard solutions of Pb, Cd, As and Hg were prepared. Then samples were analyzed for the presence of Pb, Cd, As, Hg using Atomic absorbance spectrophotometer AA 6300, SHIMADZU and HVG-1 by using a calibration curve of standard. The results are as given in **Table 7**

HPLC analysis for estimation of active components:

Apparatus, equipment and reagents: HPLC system's pump was from Shimadzu LC 20ATVP, Japan with 20mL Rheodyne injector, Phenomenex (Torrance, CA) Luna C_{18} (250cm x 4.6mm id) column and SPD-20 AT UV-Visible and spinchrom LC solution software were used. All the reagents used were of HPLC grade.

Estimation of Glycyrrhizin in *Glycyrrhiza glabra* (Yashtimadhu) Extract

Chromatographic Condition

Stationary phase: Phenomenex C_{18} column (250mm x 4.6mm i.d., 5µm particle size) was used at ambient temperature

Mobile Phase: Buffer: acetonitrile (60:40, v/v)

Flow rate: 1mL/min.

Injection volume: 20µL

Detection: At 254nm with UV detector

Preparation of solutions

Preparation of standard solution: Dissolve 1mg of the standard Glycyrrhizin in 10mL of the Mobile phase solution. Filter the solution using a 0.22mm filter paper and use the filtrate as the standard solution.

Preparation of sample solutions: Dissolve 50mg of Yashtimadhu extract in 25mL of the Mobile phase solution. Filter the solution using a 0.22mm filter paper and use the filtrate as the sample solution.

Estimation of Diosgenin in *Tribulus terrestris* (Gokshur) Extract

Chromatographic Condition

Stationary phase: Phenomenex C_{18} column (250mm x 4.6mm i.d., 5µm particle size) was used at ambient temperature

Mobile Phase: Acetonitrile: Water (80: 20)

Flow rate: 1mL/min.

Injection volume: 20µL

Detection: At 210nm with UV detector

Preparation of solutions

Preparation of standard solution: Dissolve 2.4mg of Diosgenin in 10mL of the mobile phase. Filter the solution using a 0.22mm filter paper and use the filtrate as the standard solution.

Preparation of sample solutions: Reflux 5.0g of Gokshur extract with 50mL of sulphuric acid (10%) for 4 hours. Cool and transfer to a separating funnel. Extract the solution with 50mL of ethyl acetate solution. Repeat the extraction 3 times. Pass the ethyl acetate layer through sodium sulphate and evaporate. Dissolve the residue in 50mL of methanol. Filter the solution using 0.22mm filter paper and use filtrate as a sample solution.

Estimation of HCA (Hydroxy Citric Acid) in *Hibiscus rosa-sinensis* (Japa) Extract

Chromatographic Condition

Stationary phase: Phenomenex C_{18} column (250mm x 4.6mm i.d., 5µm particle size) was used at ambient temperature

Mobile Phase: 100% Potassium dihydrogen phosphate

Flow rate: 1mL/min.

Injection volume: 20µL

Detection: At 215nm with UV detector

Preparation of solutions

Standard Preparation: Dissolve 1mg of standard HCA in 10mL solvent mixture (1mL of 30% H_3PO_4 + 9mL water). Filter the solution using 0.22mm filter paper and use the filtrate as the standard solution.

Sample Preparation: Dissolve 10mg of Japa extract in 10mL of solvent mixture (1mL of 30% H_3PO_4 + 9mL water). Filter the solution using a 0.22mm filter paper and use the filtrate as the sample solution.

HPTLC analysis for Trichup Tablet and its raw materials

HPTLC is one of the most advanced separation technique available today which gives better precision and accuracy with extreme flexibility for various steps (stationary phase, mobile phase, development technique and detection). HPTLC analysis was carried out using a Hemilton 100µl HPTLC syringe, Camag Linomat V automatic spotting device, Camag twin trough chamber, Camag TLC Scanner-4, WINCAT integration software and aluminum sheet precoated with Silica Gel F254 (Merck) 0.2mm thickness.

Application Mode	CAMAG Linomat 5 – Applicator
Filtering System	Whatman filter paper No.41
Stationary Phase	MERCK - TLC Silica gel 60 F_{254} on Aluminum sheets
Application (Y axis) Start Position	10mm
Development (Y axis) End Position	90mm from plate base
Band length	8mm
Development Mode	CAMAG TLC Twin Trough Chamber
Chamber Saturation Time	30 minutes

Visualization	@254nm, @366nm, @Visible (after spray of Anisaldehyde Sulphuric acid reagent)
Derivatization mode	CAMAG – Dip tank for about 1 minute
Drying Mode, Temp. & time	TLC Plate Heater Preheated at 100 ± 5°C for 3 minutes

Steps involved in HPTLC analysis

Selection of plate and adsorbent: Precoated aluminum plates with Silica Gel F254 of 20 x 20cm and 0.2mm thickness, was used for detection. The plates were pre washed by methanol and activated at 60°C for 5 min prior to chromatography.

Sample solution

Extract: Extract 1.0g of the sample raw material (Reference Standard / Test Drug) with 10mL of Methanol with constant shaking for 5minutes. Heat on a water bath at 90 to 100°C for 5 minutes. Filter it through a Whatman filter paper No.41 and use the filtrate for HPTLC Profiling.

Preparation of solution for Finished Product: Extract 2.0g of Trichup Tablet with 20mL of Methanol & reflux it on water bath at 90 to 100°C for 15minutes. Filter and evaporate up to 5mL in a porcelain dish and use this solution for HPTLC Profiling.

Preparation of Spray reagent (Anisaldehyde sulphuric acid reagent): 0.5mL of Anisaldehyde EP is mixed with 10mL of Glacial acetic acid AR, followed by 85mL Methanol AR and 5mL Sulphuric acid 98% GR.

Track 1: 8µl/mL methanol extract of the reference standard of the raw material

Track 2: 8µl/mL methanol extract of test drug under observation

Track 3: 8µl/mL methanol extract of Trichup Tablet.

Table 1: **Solvent System used for the Raw materials of Trichup Tablet for HPTLC Analysis**

Ingredients	**Solvent System**
Eclipta alba (Bhringraj) Ext	Toluene: Acetone: Formic Acid (9:6:1)
Glycyrrhiza glabra (Yashtimadhu) Ext	Toluene: EA: GAA (12.5:7.5:0.5)
Emblica officinalis (Amalaki) Ext	Toluene: EA: GAA: Formic Acid (2:4.5:2:0.5)
Centella asiatica (Mandukparni) Ext	Chloroform: GAA: Methanol: Water (6:3.2:1.2:0.8)

Hibiscus rosa-sinensis (Japa) Ext	Toluene: EA: Methanol (4.4:5:0.6)
Tinospora cordifolia (Guduchi) Ext	Chloroform: Methanol (9:1)
Tribulus terrestris (Gokshur) Ext	Toluene: EA (8:2)
Triphala Churna	EA: Dichloromethane: GAA: Formic Acid (5:5:2:2)

GAA: Glacial Acetic Acid; **EA**: Ethyl Acetate

RESULTS

Table 2: **Organoleptic parameters and ingredient's part used**

Ingredient	**Parts used**	**Organoleptic characters**		
		Colour	**Odour**	**Taste**
BH	Whole plant	Dark Brown	Characteristic	Bitter
YA	Root	Brown	Peculiar	Sweet
AM	Fruit	Brown	Characteristic	Sour and astringent
MP	Whole plant	Brown	Characteristic	Slightly bitter
JP	Flower	Dark Brown	Characteristic	Sour
GU	Stem	Dark Brown	Characteristic	Bitter
GO	Fruit	Brown	Characteristic	Bitter
TC	Formulation	Light Brown	Aromatic	Pungent
SB	Formulation	White	Odourless	Acrid

BH: Bhringraj Ext; **YA:** Yashtimadhu Ext; **AM:** Amalaki Ext; **MP:** Mandukparni Ext; **JP:** Japa Ext; **GU:** Guduchi Ext; **GO:** Gokshur Ext; **TC:** Triphala Churna; **SB:** Shukti Bhasma

Table 3: Physicochemical parameters

Ingredients	**Physicochemical parameter**	
	pH	**M/S (by LOD) %**
BH	6.58 ± 0.02	3.45 ± 0.12
YA	4.50 ± 0.14	2.87 ± 0.16
AM	3.22 ± 0.06	3.79 ± 0.25
MP	4.71 ± 0.15	2.57 ± 0.36
JP	4.22 ± 0.13	5.50 ± 0.18

GU	5.93 ± 0.25	3.65 ± 0.25
GO	6.12 ± 0.12	1.59 ± 0.36
TC	4.75 ± 0.35	0.57 ± 0.10
SB	11.41 ± 0.15	NA

BH: Bhringraj Ext; **YA:** Yashtimadhu Ext; **AM:** Amalaki Ext; **MP:** Mandukparni Ext; **JP:** Japa Ext; **GU:** Guduchi Ext; **GO:** Gokshur Ext; **TC:** Triphala Churna; **SB:** Shukti Bhasma; **NA**: Not Applicable; **M/S:** Moisture; **LOD**: Loss on Drying

Table 4: Extractive values and Ash value of Ingredients of Trichup Tablet

Ingredients	WSE (%)	TA (%)
BH	95.68 ± 0.12	13.83 ± 0.41
YA	92.48 ± 0.21	3.92 ± 0.21
AM	90.00 ± 0.45	9.10 ± 0.22
MP	91.60 ± 0.23	6.52 ± 0.36
JP	77.44 ± 0.24	6.50 ± 0.19
GU	73.36 ± 0.21	9.85 ± 0.28
GO	97.52 ± 0.32	8.34 ± 0.15
TC	88.80 ± 0.33	5.29 ± 0.21
SB	NA	1.76 ± 0.22

BH: Bhringraj Ext; **YA:** Yashtimadhu Ext; **AM:** Amalaki Ext; **MP:** Mandukparni Ext; **JP:** Japa Ext; **GU:** Guduchi Ext; **GO:** Gokshur Ext; **TC:** Triphala Churna; **SB:** Shukti Bhasma; **NA**: Not Applicable; **WSE**: Water Soluble Extractive; **TA**: Total Ash

Table 5: Assay estimation in extract raw material of Trichup Tablet

Sr No.	Ingredients	NW	GY	TN	TP	BT	SP
1	BH	3.68±0.02	NA	NA	NA	NA	NA
2	YA	NA	35.67±0.01	NA	NA	NA	NA
3	AM	NA	NA	26.18±0.04	NA	NA	NA
5	JP	NA	NA	NA	33.17±0.12	NA	NA
6	GU	NA	NA	NA	NA	4.46±0.05	NA

7	GO	NA	NA	NA	NA	NA	18.92±0.11
8	TC	NA	NA	41.46±0.22	NA	NA	NA

BH: Bhringraj Ext; **YA:** Yashtimadhu Ext; **AM:** Amalaki Ext; **JP:** Japa Ext; **GU:** Guduchi Ext; **GO:** Gokshur Ext; **TC:** Triphala Churna; **NW:** Assay of Norwedalactone; **GY**: Assay of Glycyrrhizin; **TN**: Assay of Tannin; **TP**: Assay of Triterpene; **BT**: Assay of Bitter**; SP:** Assay of Saponin; **NA**: Not Applicable

Table 6: **Standardization parameters for the finished product Trichup Tablet**

Parameter	**Limits**	**Results**		
		Batch 1	**Batch 2**	**Batch 3**
Stage 1: Lubricated Granules				
Description	Light brown to brown coloured granules powder	Brown coloured granules powder	Brown coloured granules powder	Brown coloured granules powder
Moisture (By KF)	NMT 3.8%	1.66	1.71	1.84
Stage 2: Core Tablet				
Description	Brown coloured biconvex round tablet	Brown coloured biconvex round tablets	Brown coloured biconvex round tablets	Brown coloured biconvex round tablets
Average Weight	500 ± 25 mg	507.6mg	508.2mg	508.6mg
Disintegration time	NMT 50 mins	38 min 58 sec	39 min 02 sec	39 min 11 sec
Hardness	NLT 2.0 Kg/cm^2	3.0 Kg/cm^2	3.3 Kg/cm^2	3.4 Kg/cm^2
Friability	NMT 1.0%	0.157 %	0.11 %	0.05 %
Stage 3: Finished Product				
Description	Orange coloured biconvex round film coated tablet	Orange coloured biconvex round film coated tablet	Orange coloured biconvex round film coated tablet	Orange coloured biconvex round film coated tablet

Average net Weight	530mg ± 26.5mg	531.45mg	531.51mg	532.06mg
Hardness	NLT 2.0kg/cm^2	5.0 kg/cm^2	5.0 kg/cm^2	5.1 kg/cm^2
Disintegration time	NMT 60 min	47 min 10 sec	47 min 12 sec	47 min 16 sec

Table 7: Results of Heavy metal content and Bio-burden in raw material of Trichup Tablet

Ingre-dients	**Heavy metal content**				**Bio-burden**					
	Pb 10ppm	**Cd** 0.3ppm	**As** 3.0ppm	**Hg** 1.0ppm	**TBC** NMT 10^7 cfu/g	**TFC** NMT 10^5 cfu/g	***E. coli*** Ab	***P.a*** Ab	***S.e*** Ab	***S.a*** Ab
BH	0.431	0.074	1.252	Absent	3 x 10^2	Absent	Absent	Absent	Absent	Absent
YA	0.525	0.065	0.152	Absent	4 x 10^2	Absent	Absent	Absent	Absent	Absent
AM	0.788	0.058	0.121	Absent	6 x 10^2	Absent	Absent	Absent	Absent	Absent
MP	0.648	0.054	0.528	Absent	7 x 10^2	Absent	Absent	Absent	Absent	Absent
JP	1.252	0.034	0.658	Absent	11 x 10^2	Absent	Absent	Absent	Absent	Absent
GU	1.748	0.028	1.256	Absent	9 x 10^2	Absent	Absent	Absent	Absent	Absent
GO	0.125	0.075	0.054	Absent	3 x 10^2	Absent	Absent	Absent	Absent	Absent
TC	0.857	0.054	0.658	Absent	8 x 10^2	Absent	Absent	Absent	Absent	Absent
SB	0.021	0.025	1.286	Absent	NA	NA	NA	NA	NA	NA
TT	1.985	0.094	1.958	Absent	13 x 10^2	Absent	Absent	Absent	Absent	Absent

BH: Bhringraj Ext; **YA:** Yashtimadhu Ext; **AM:** Amalaki Ext; **JP:** Japa Ext; **GU:** Guduchi Ext; **GO:** Gokshur Ext; **TC:** Triphala Churna; **SB**: Shukti Bhasma; **TT**: Trichup Tablet; **NA**: Not Applicable; **ppm**: parts per million, **cfu/g-** colony forming unit per gram, **Pb**: Lead, **Cd**: Cadmium, **As**: Arsenic, **Hg**: Mercury, **TBC**: Total bacterial count, **TFC**: Total fungal count, ***E. coil***: *Escherichia coli*, ***P.a***.: *Pseudomonas aeruginosa*, ***S.e***: *Salmonella enterica.*, ***S.a***: *Staphylococcus aureus;* **Ab**: Absent

HPLC Analysis

Estimation of *Glycyrrhiza glabra* (Yashtimadhu) Root Ext

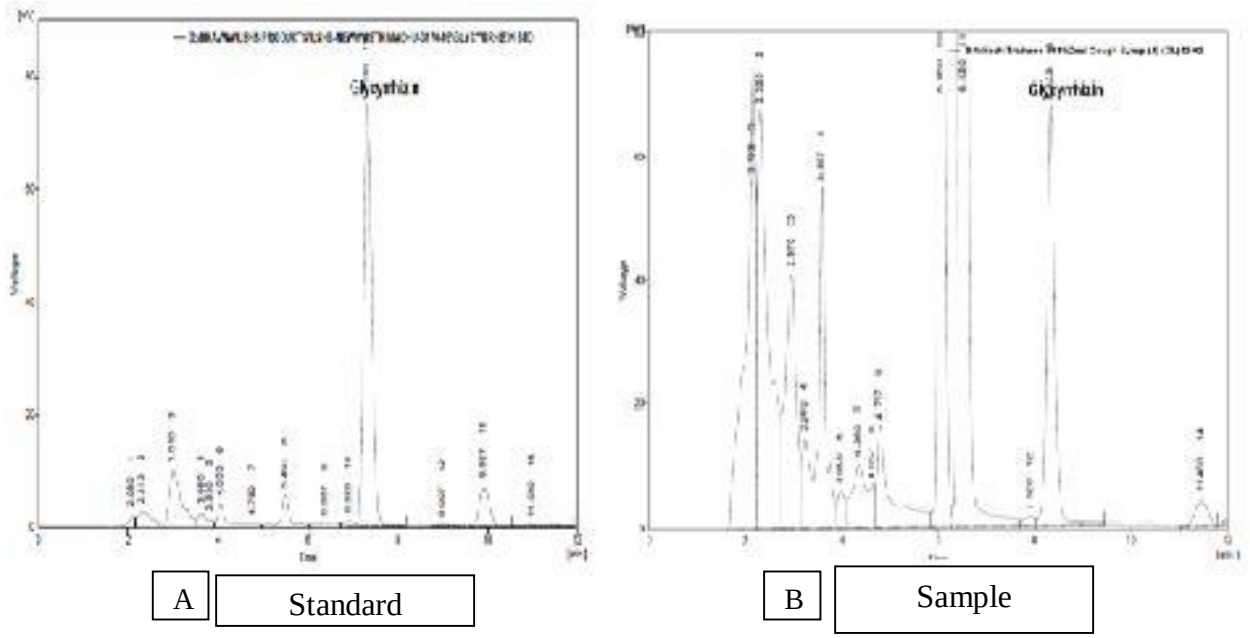


Figure 1: [A] HPLC chromatogram of standard glycyrrhizin & **[B]** *Glycyrrhiza glabra* extract. The result indicated 27.32% of glycyrrhizin in *Glycyrrhiza glabra* Root extract

Estimation of Diosgenin in *Tribulus terrestris* (Gokshur) Fruit Extract

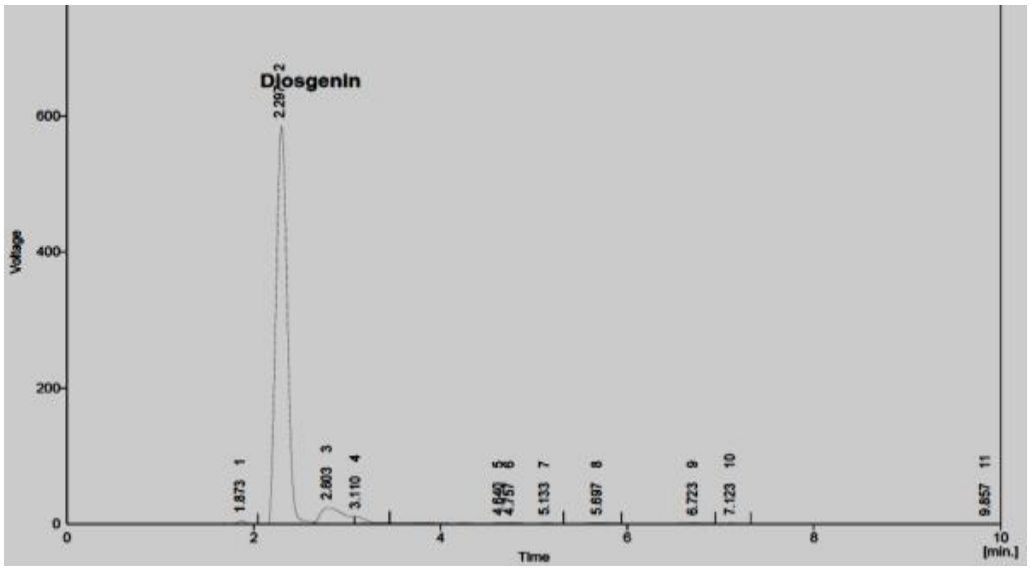


Figure 2: HPLC chromatogram of standard Diosgenin

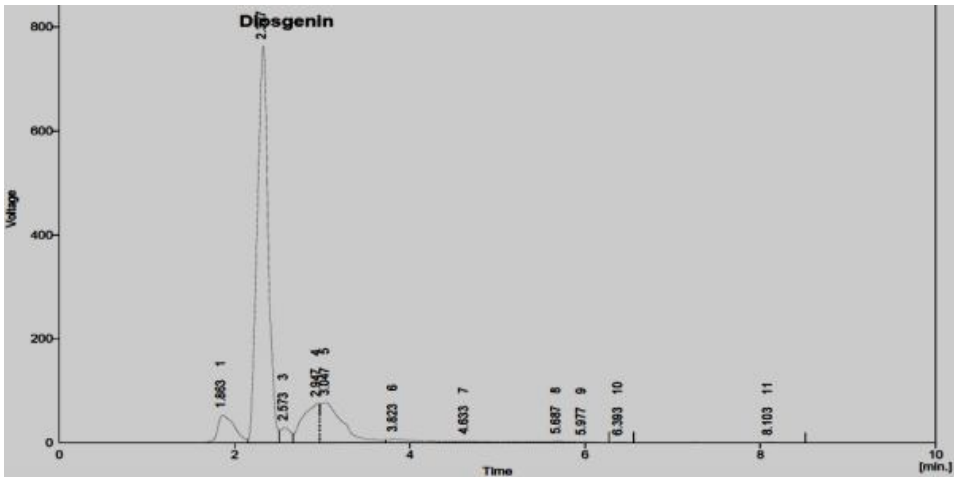


Figure 3: HPLC chromatogram of *Tribulus terrestris* extract

The result indicated 1.77% of Diosgenin in *Tribulus terrestris* Fruit Extract

Estimation of HCA in *Hibiscus rosa-sinensis* (Japa) Flower Extract

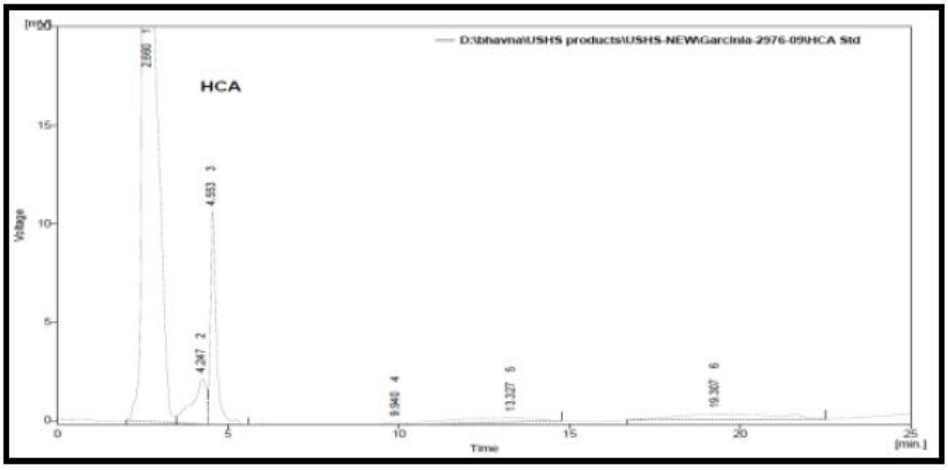


Figure 4: HPLC chromatogram of standard HCA

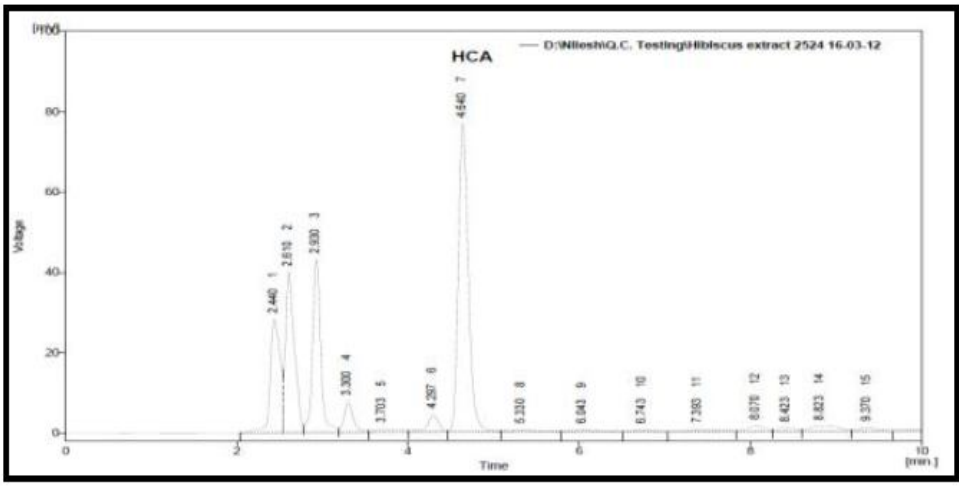


Figure 5: HPLC chromatogram of *Hibiscus rosa-sinensis* Extract

The result indicated 33.26% of HCA in *Hibiscus rosa-sinensis* Flower Extract

HPTLC Analysis

During HPTLC analysis, the sample shows comparison of individual extract with the finished product. The visualization of TLC plates was carried out in all 3 different wavelengths i.e. 254nm, 366nm and 540nm. From this only the best visualization result was selected and included in our study along with its 3D image. The R_f values found during this study indicates the prominent presences of that particular raw material in the finished product which is used to establish its qualitative presence.

Tracks of HPTLC fingerprinting plates were spotted in following way:

Track 1: 8µl/mL methanol extract of the reference standard of the Extract

Track 2: 8µl/mL methanol extract of test drug under observation

Track 3: 8µl/mL methanol extract of Trichup Tablet.

***Eclipta alba* (Bhringraj) Whole plant Extract (BH)**

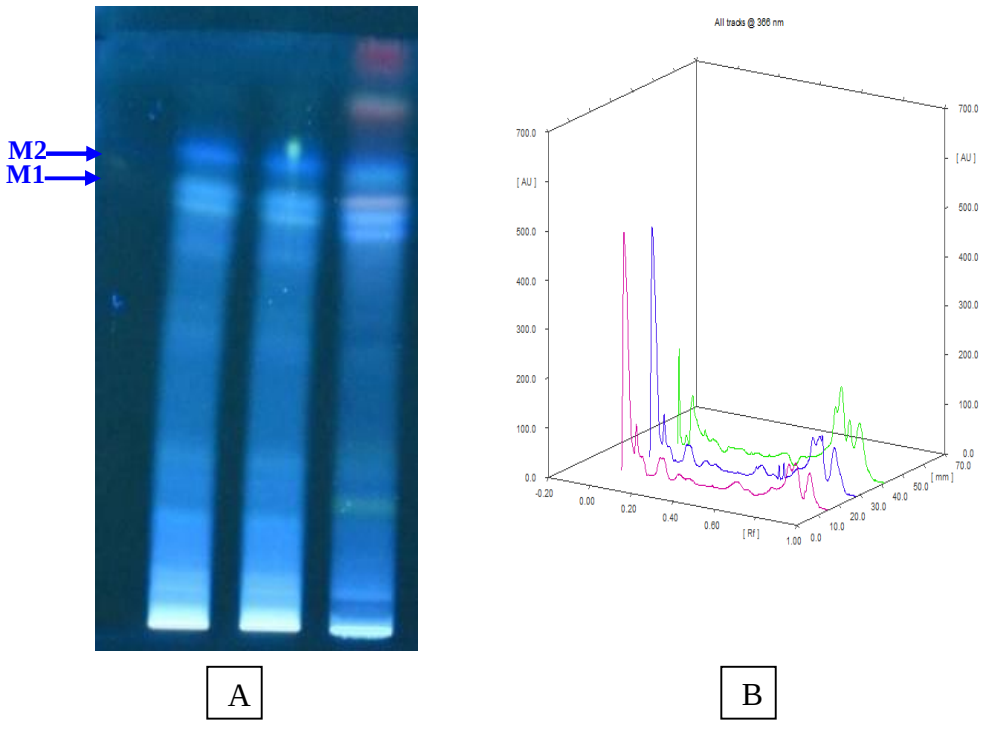


Figure 6: It shows the HPTLC Chromatogram of BH.

Track 1: reference standard **Track 2:** test drug **Track 3:** Trichup Tablet
A: HPTLC Plate of BH at 366nm under UV.
B: 3D image of the Fingerprinting of BH and finished product (366nm). The results indicate that HPTLC Chromatogram of BH and finished product has shown the similar R_f value of 0.73 and 0.80 at 366nm

***Glycyrrhiza glabra* (Yashtimadhu) Root Extract (YA)**

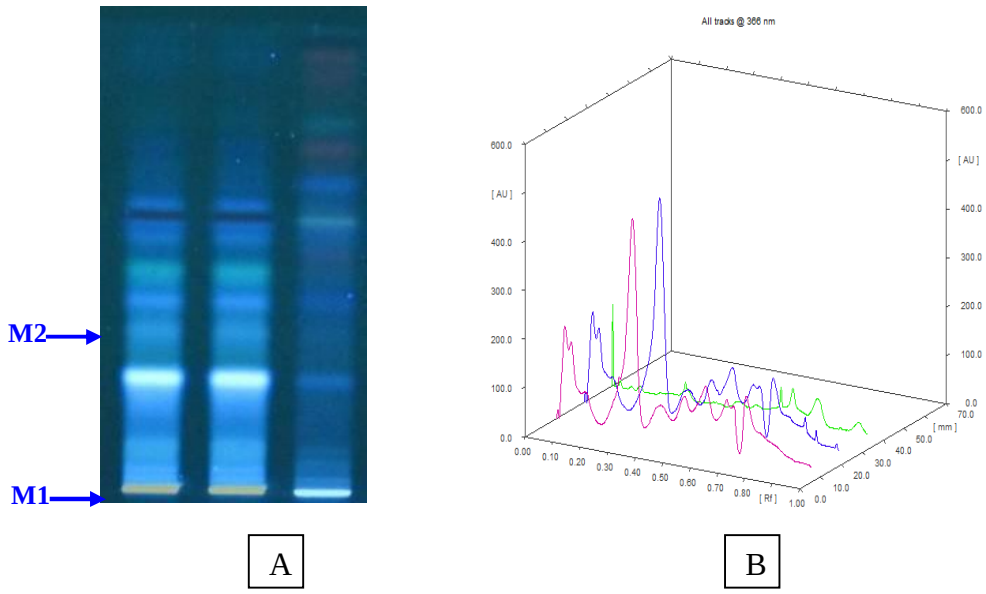


Figure 7: It shows the HPTLC Chromatogram of YA.

Track 1: reference standard **Track 2:** test drug **Track 3:** Trichup Tablet

A: HPTLC Plate of YA at 366nm under UV.

B: 3D image of the Fingerprinting of YA and finished product (366nm). The results indicate that HPTLC Chromatogram of YA and finished product has shown the similar R_f value of 0.25 and 0.51 at 366nm

***Emblica officinalis* (Amalaki) Fruit Extract (AM)**

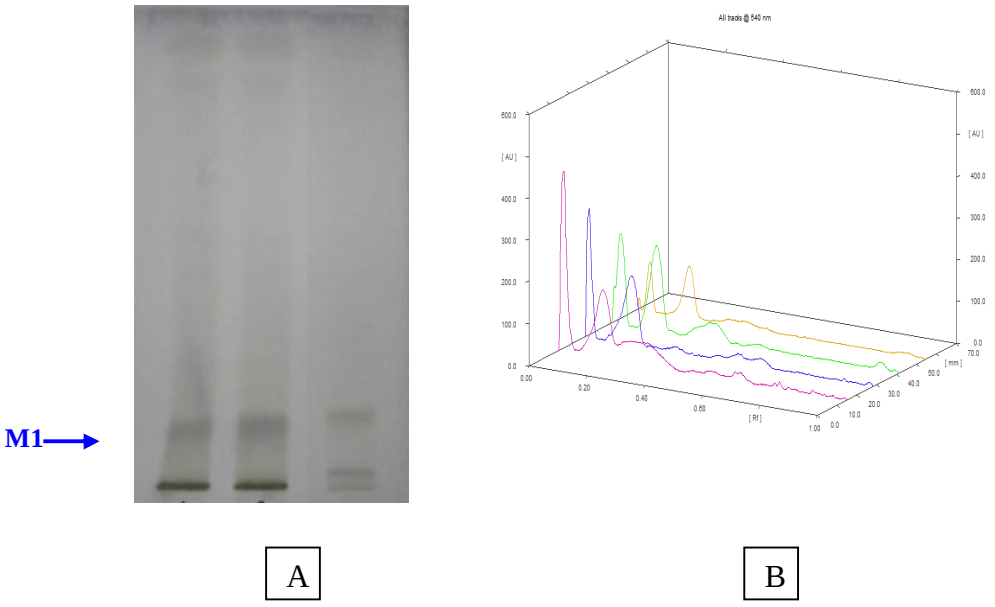


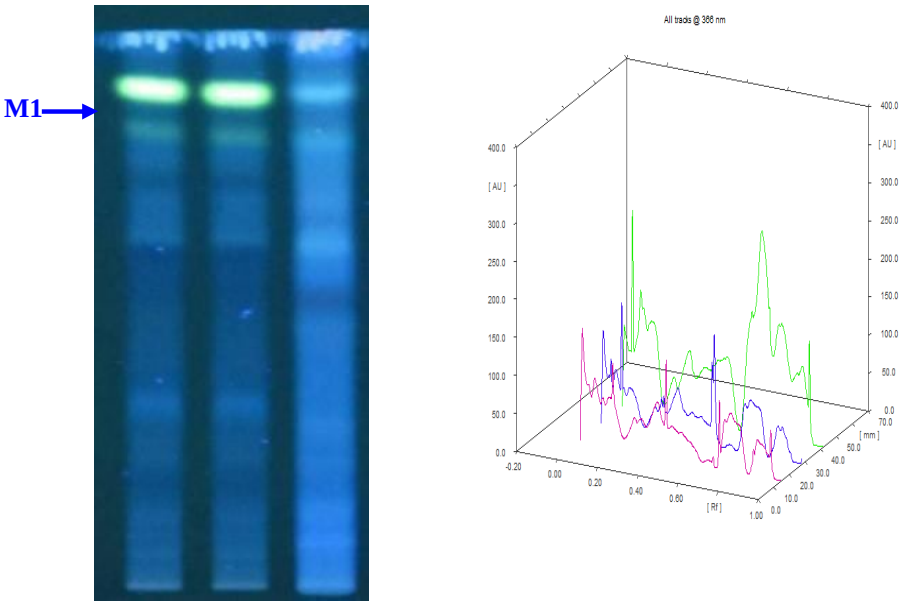
Figure 8: It shows the HPTLC Chromatogram of AM.

Track 1: reference standard **Track 2:** test drug **Track 3:** Trichup Tablet

A: HPTLC Plate of AM at 540nm under UV.

B: 3D image of the Fingerprinting of AM and finished product (540nm). The results indicate that HPTLC Chromatogram of AM and finished product has shown the similar R_f value of 0.12 at 540nm

***Centella asiatica* (Mandukparni) Whole plant Extract (MP)**



A B

Figure 9: It shows the HPTLC Chromatogram of MP.

Track 1: reference standard **Track 2:** test drug **Track 3:** Trichup Tablet

A: HPTLC Plate of MP at 366nm under UV.

B: 3D image of the Fingerprinting of MP and finished product (366nm). The results indicate that HPTLC Chromatogram of MP and finished product has shown the similar R_f value of 0.88 at 366nm

***Hibiscus rosa-sinensis* (Japa) Flower Extract (JP)**

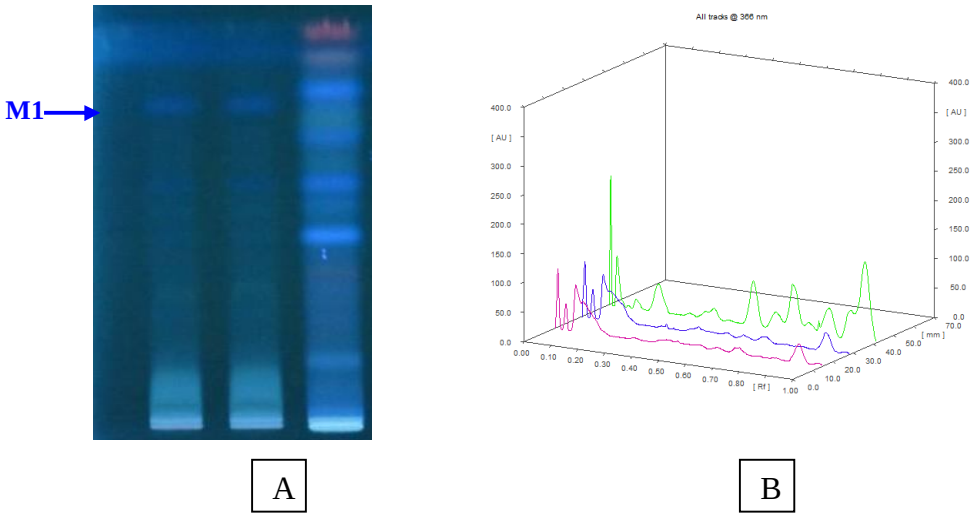


Figure 10: It shows the HPTLC Chromatogram of JP**.**

Track 1: reference standard **Track 2:** test drug **Track 3:** Trichup Tablet
A: HPTLC Plate of JP at 366nm under UV.
B: 3D image of the Fingerprinting of JP and finished product (366nm). The results indicate that HPTLC Chromatogram of JP and finished product has shown the similar R_f value of 0.78 at 366nm

***Tinospora cordifolia* (Guduchi) Stem Extract (GU)**

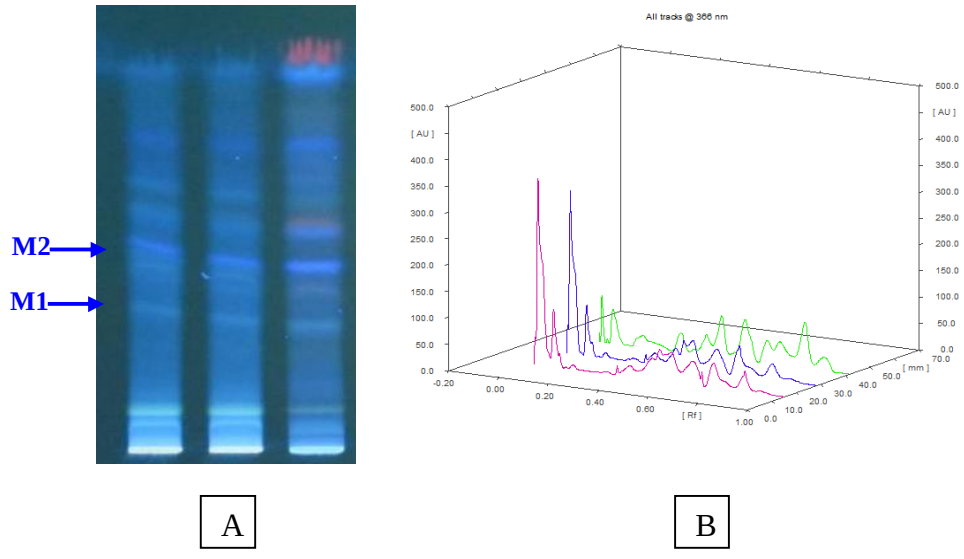


Figure 11: It shows the HPTLC Chromatogram of GU.

Track 1: reference standard **Track 2:** test drug **Track 3:** Trichup Tablet
A: HPTLC Plate of GU at 366nm under UV.
B: 3D image of the Fingerprinting of GU and finished product (366nm). The results indicate that HPTLC Chromatogram of GU and finished product has shown the similar R_f value of 0.30 and 0.40 at 366nm

***Tribulus terrestris* (Gokshur) Fruit Extract (GO)**

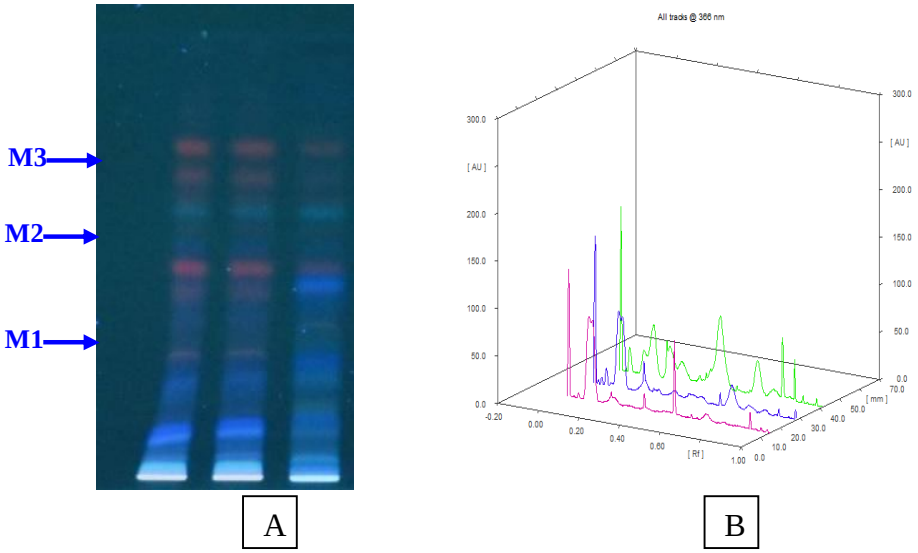


Figure 12: It shows the HPTLC Chromatogram of GO.

Track 1: reference standard **Track 2:** test drug **Track 3:** Trichup Tablet

A: HPTLC Plate of GO at 366nm under UV.

B: 3D image of the Fingerprinting of GO and finished product (366nm). The results indicate that HPTLC Chromatogram of GO and finished product has shown the similar R_f value of 0.50, 0.61 and 0.75 at 366nm

Triphala Churna (TC)

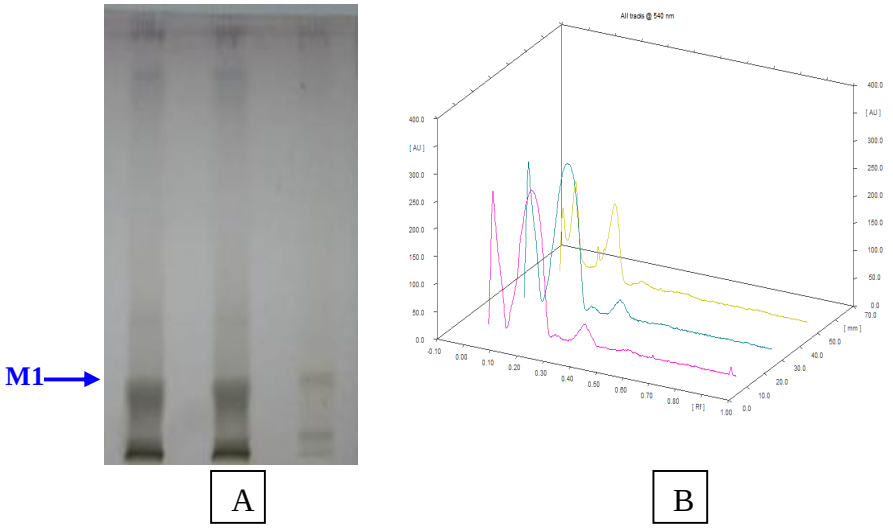


Figure 13: It shows the HPTLC Chromatogram of TC.

Track 1: reference standard **Track 2:** test drug **Track 3:** Trichup Tablet

A: HPTLC Plate of TC at 540nm under UV.

B: 3D image of the Fingerprinting of TC and finished product (540nm). The results indicate that HPTLC Chromatogram of TC and finished product has shown the similar R_f value of 0.17 at 540nm

DISCUSSION AND CONCLUSION

Herbal medicines are prepared using plants and minerals collected directly from the natural sources. Therefore, they are considered very prone to contamination, deterioration and variation in composition. This can be controlled by monitoring the growing conditions, harvesting time, harvested plant part, absence of toxic materials, analytical controls and by establishing pharmacognostical standards. Hence, standardization is a very important process where one can be ensured about the physical quality and therapeutic efficacy of product. By enlarge, Standardization covers overall qualitative and quantitative part of analysis.

Qualitative analysis mainly covers the identification of the constituent(s) present in a particular product, whereas the quantitative analysis is accomplished by measuring the level of marker chemical constituents in herbal extracts as well as in finished product. The concept of standardized extracts definitely provides a solid platform for scientific validation of Ayurvedic medicines.

Trichup Tablet is a herbo-mineral Ayurvedic propriety product manufactured and marketed by Vasu Healthcare Pvt. Ltd. As a part of standardization procedure, the finished product and the raw materials of three different batches were analyzed for various physicochemical parameters.

The testing method for each parameter was standardized and validated. The protocols for the same were adopted from standard reference books.

Organoleptic characters like physical appearance, colour, odour and taste of the raw materials and finish product were first evaluated for identification and batch to batch uniformity before any further tests are undertaken.

pH and moisture content play important role in reflecting quality of product. These parameters were found well within the limits during the analysis and thereby confirmed the consistency in quality of product.

Extractive Value determines the amount of active constituents in medicinal plant material when extracted with a solvent media such as Water. These values provide an indication of the extent of polar, medium polar and non-polar compounds present in the plant material. Thus from the above study we can conclude that all our extracts have good solubility in water which is a polar solvent.

The total ash usually consists of carbonates, phosphates silicates and silica that include the physiological ash which is derived from the plant tissue itself and non physiological ash which is the residue of the adhering material to the plant material e.g. sand and soil. Total ash was performed to measure the total amount of material remaining after ignition. This test is important to control adulteration and the results show that the ash values were much within the prescribed limits.

WHO has specified the limits for the presence of contaminants like four pathogenic micro-organisms viz *E.coli, Staphylococcus aureus, P.aeruginosa* & *Salmonella enterica* along with yeast-moulds and four heavy metals viz; Lead, Cadmium, Arsenic and Mercury as the consumption of which can lead to complications in one's routine life. Trichup Tablet was found in full compliance of the permissible microbial and heavy metal limits.

HPTLC study confirmed the qualitative as well as quantitative presence of the raw material in the finished product.

Present standardization study revealed Trichup Tablet in full compliance with all the above discussed parameters hence it can be concluded that Trichup Tablet is a well standardized product at essential physicochemical parameters.

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