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Research Article

A COMPREHENSIVE STANDARDIZATION STUDY OF A POLY HERBAL FORMULATION: URAL TABLET

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ABSTRACT
More than half the world's population is dependent on herbal drugs and in order to enter into the global market it is vital to maintain the quality. Initially the crude drugs were identified by comparison only with the standard description available. At present due to advancement in the chemical knowledge of crude drugs various methods like botanical, chemical and biological methods are being used for estimating active constituents present in the crude drugs in addition to its physical constants. According to pharmaceutical manufacturers association of U.S. "quality is the sum of all the factors which contribute directly or indirectly to the safety, effectiveness and acceptability of the product. In this research work, a polyherbal formulation (PHF), Ural Tablet has been taken to establish its standardization level using various physic chemical parameters such as pH, Moisture content, ash values, HPTLC fingerprinting and assays of marker compounds with the aid of sophisticated instruments and was carried out to check the presence of the raw materials in comparison with the finished product. Study results revealed that product was well standardized at selected analytical parameters. All the tests were carried out in triplicates after which their SD was calculated. All the raw materials as well as finished product were found free from heavy metal and microbial contaminants.
Keywords: Standardization, HPTLC, Ural Tablet, Polyherbal formulation.

INTRODUCTION

The adulteration and substitution of herbal drugs is the burning problem in herbal industry and it has caused a major effect in the commercial use of natural products. It is a practice of substituting the original crude drug partially or fully with other substances which is either free from or inferior in therapeutic and chemical properties or addition of low grade or spoiled drugs or entirely different drug similar to that of original drug which do not confirm with the official standards[1,2]. Hence it is very essential to ensure that every product that reaches the market is free of any kind of adulteration and even the WHO has enforced the standardization of a product which is essential to know the quality of the product. Thus in the present study, Polyherbal Tablet (Ural Tablet) has been selected to establish its standardization status. The key ingredients used in the formulation are extract of *Boerhaavia diffusa* (Punarnava) Root[3], *Tribulus terrestris* (Gokshur) Fruit[3], *Crataeva nurvala* (Varun) Bark[3], *Berginia ligulata* (Pashanbhed) Root[3], Powder of *Sodii carbonas* (Swarjika kshar)[3], *Potasii carbonas* (Yava kshar) [3], Chandraprabha vati, *Raphanus sativus* (Mulika)[3] and Kala namak (Black salt)[3].

MATERIAL AND METHODS

All the Protocols were followed from standard Pharmacopoeia's.

Organoleptic parameters

Organoleptic parameters 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 1.

Physicochemical parameters for extracts

The physiochemical parameters include tests like pH[4], Moisture content/ Loss on drying[4], Water soluble extractive[4] and determination of ash[4] of the relevant raw materials. The results are as tabulated in Table 2 & 3.

Estimation of Actives

Assay analysis includes estimation of Alkaloid[5], Withanolide[5], Glycyrrhizin[5] and Saponin[5], Tannin[5], Assay of Swarjikakshar[6] value in respective extracts. The results are as tabulated in Table 4.

Evaluation of Standardization Parameters selected for Finished Product

The finished product was analyzed for its description, diameter, thickness, hardness, disintegration time (DT), Moisture and dissolution. The results are as tabulated in Table 5.

Microbial Analysis

Bio burden analysis consists of parameters like Total Bacterial Count, Total Fungal Count and Presence of *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus and Salmonella spp*[7]. The results are as given in Table 6.

Heavy Metal Analysis

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[8]. The results are as given in Table 6.

HPTLC Fingerprinting of Raw Materials and Finished Product

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, aluminium sheet precoated with Silica Gel 0F254 (Merck) 0.2mm thickness.

Chromatographic Conditions

Application Mode	CAMAG Linomat 5 – Applicator
Filtering System	Whatman filter paper No.41
Stationary Phase	MERCK - TLC / HPTLC 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	8 mm
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± 5^0C for 3 minutes

Steps involved in HPTLC analysis

Selection of plate and adsorbent

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

Sample solution

Extract

Extract 1.0g of the sample Extract (Reference Standard/Test Drug/with 10mL of Methanol with constant shaking for 5minutes. Heat on a water bath at 90-100^{0}C for 5minutes, Filter it through Whatman filter paper No.41. Use the filtrate 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 Extract.

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

Track 3: 8 µl/mL methanol extract of Poly Herbal formulation.

RESULTS

Table 1: Organoleptic parameters and ingredient's part used

Ingredient	Parts used	Organoleptic characters		
		Colour	Odour	Taste
PE	Root	Brown	Characteristic	Pungent
GE	Fruit	Brown	Characteristic	Bitter
VE	Bark	Brown	Characteristic	Characteristic
PAE	Root	Brown	Characteristic	Astringent
SJ	Mineral	White Crystalline	Odourless	Alkaline Taste
YP	Mineral	Off White	Characteristic	Salty
CV	Formulation	Brown	Characteristic	Bitter & Acrid
MP	Kshar	Greyish	Characteristic	Salty
KN	Salt	Light Pink	Characteristic sulphorus	Salty

PE: Punarnava Ext, **GE**: Gokshur Ext, **VE**:Varun Ext, **PAE**: Pashanbhed Ext, **SJ**: Swarjikakshar Powder; **YP**: Yavakshar Powder; **CV**: Chandraprabha vati, **MP**: Mulika Powder; **KN**: Kala namak

Table 2: Physicochemical parameters

Ingredients	pH	M/S (by LOD) %
PE	5.64 ± 0.01	2.03 ± 0.05
GE	5.31 ± 0.05	1.28 ± 0.07
VE	4.52 ± 0.05	3.02 ± 0.12
PAE	5.14 ± 0.06	2.97 ± 0.01

PE: Punarnava Ext, **GE**: Gokshur Ext, **VE**:Varun Ext, **PAE**: Pashanbhed Ext, **LOD**: Loss on Drying

Table 3: Extractive values and Ash Value of Ingredients of Ural Tablet

Ingredients	WSE (%)	TA (%)
PE	99.92 ± 0.02	9.82 ± 0.05
GE	87.84 ± 0.05	4.33 ± 0.02
VE	95.28 ± 0.04	5.32 ± 0.18
PAE	81.36 ± 0.01	4.58 ± 0.12

PE: Punarnava Ext, **GE**: Gokshur Ext, **VE**: Varun Ext, **PAE**: Pashanbhed Ext, **WSE**: Water Soluble Extractive; **TA**: Total Ash

Table 4: Estimation of the Active Constituent

Assay	PE	GE	VE	PAE	SJ
Alkaloid	0.26 ± 0.01	NA	NA	NA	NA
Saponin	NA	13.31 ± 0.05	20.77 ± 0.02	NA	NA
Tannin	NA	NA	NA	16.62 ± 0.13	NA
Assay of Swarjikakshar	NA	NA	NA	NA	99.91 ± 0.15

PE: Punarnava Ext, **GE**: Gokshur Ext, **VE**:Varun Ext, **PAE**: Pashanbhed Ext, **SJ**: Swarjikakshar Powder; **NA**: Not Applicable

Table 5: Standardization parameters for the finished product (Ural Tablet)

Parameter	Results		
	Batch 1	Batch 2	Batch 3
Description	Yellow coloured biconvex round film coated tablet	Yellow coloured biconvex round film coated tablet	Yellow coloured biconvex round film coated tablet
Average Weight	540.00mg	538.00mg	538.00mg
Diameter	10.40mm	10.38mm	10.36mm
Thickness	5.73mm	5.71mm	5.72mm
Hardness	4.1Kg/cm^2	4.1Kg/cm^2	4.2Kg/cm^2
DT	20mins 01secs	24mins 18secs	23mins 28secs
Moisture	3.80%	3.50%	4.00%
Dissolution	88.48%	86.68%	84.96%

Table 6: Results of Heavy metal content and Bio-burden in raw material of Ural Tablet

Material	Trace elements				Bio-burden					
	Pb 10ppm	Cd 0.3ppm	As 3.0ppm	Hg 1.0ppm	TBC Cfu/g	TFC Cfu/g	*E. coli* Ab	*P.a* Ab	*Sal.sp* Ab	*S.a* Ab
PE	0.152	0.115	1.025	Absent	14 x 10^2	Absent	Absent	Absent	Absent	Absent
GE	1.526	0.154	0.350	Absent	21 x 10^2	Absent	Absent	Absent	Absent	Absent
VE	0.157	0.165	0.517	Absent	11 x 10^2	Absent	Absent	Absent	Absent	Absent
PAE	0.574	0.201	2.351	Absent	15 x 10^2	Absent	Absent	Absent	Absent	Absent
SJ	2.545	0.106	2.148	Absent	NA	NA	NA	NA	NA	NA
YP	2.147	0.206	1.256	Absent	NA	NA	NA	NA	NA	NA
CV	2.198	0.274	1.254	Absent	NA	NA	NA	NA	NA	NA
MP	1.125	0.198	0.584	Absent	68 x 10^2	Absent	Absent	Absent	Absent	Absent
KN	0.578	0.548	0.367	Absent	NA	NA	NA	NA	NA	NA
UT	0.598	1.246	2.157	Absent	16 x 10^2	Absent	Absent	Absent	Absent	Absent

PE: Punarnava Ext, **GE**: Gokshur Ext, **VE**:Varun Ext, **PAE**: Pashanbhed Ext, **SJ**: Swarjikakshar Powder; **YP**: Yavakshar Powder; **CV**: Chandraprabha vati, **MP**: Mulika Powder; **KN**: Kala namak; **UT**: Ural Tablet; **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,* ***Sal.sp***: *Salmonella spp.,* **S.a**: *Staphylococcus aureus,* **NA**: Not Applicable

HPTLC

In HPTLC analysis, the sample shows comparison of individual extract & Powder with finished product. The visualization of TLC plates was carried out in all 3 different wavelengths i.e. 254nm, 366nm and 540 nm. From this only the best visualization result was selected and included in our study along with its 3D image. The R_f value thus found during this study indicates the prominent presences of raw material in the finished product which is used to establish its quantitative presence. The Results are as depicted in Figure 1-6**.**

***Boerhaavia diffusa* (Punarnava) Root Ext**

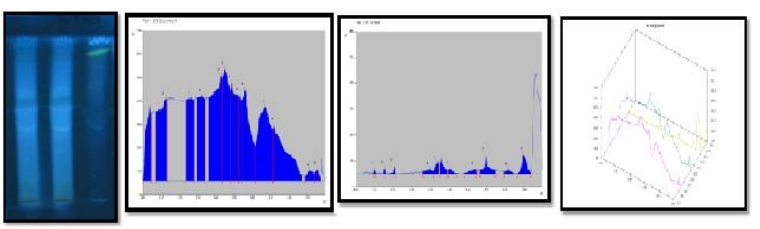

Figure 1: It shows the HPTLC Chromatogram of Punarnava Ext. **A**: HPTLC Plate of PE at 366nm under UV. **B**: 2D Chromatogram of methanol extract of Punarnava Ext. at 366nm; **C**: 2D Chromatogram of methanol extract of finish product at 366nm; **D**: 3D image of the Fingerprinting of Punarnava Ext. and finish product (366nm). The results indicate that HPTLC Chromatogram of PE and finished product has shown the similar R_f value of 0.89 at 366nm.

***Tribulus terrestris* (Gokshur) Fruit Ext**

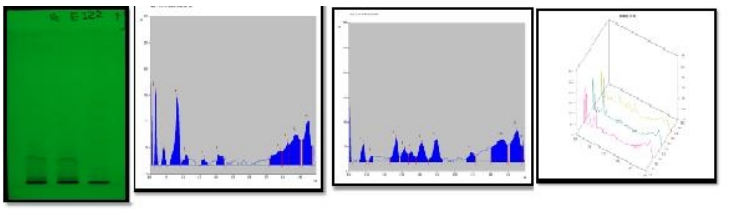

Figure 2: It shows the HPTLC Chromatogram of Gokshur Ext. **A**: HPTLC Plate of GE at 254nm under UV. **B**: Chromatogram of methanol extract of GE. at 254nm; **C**: 2D Chromatogram of methanol extract of finish product at 254nm; **D**: 3D image of the Fingerprinting of GE and finish product (254nm). The results indicate that HPTLC Chromatogram of GE and finished product has shown the similar R_f value of 0.42 at 254nm

***Crataeva nurvala* (Varun) Bark Ext**

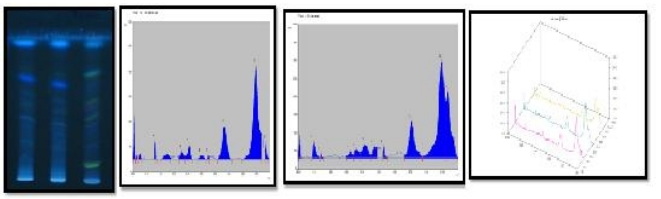

Figure 3: It shows the HPTLC Chromatogram of Varun Ext. **A**: HPTLC Plate of VE at 366nm under UV. **B**: 2D Chromatogram of methanol extract of VE at 366nm; **C**: 2D Chromatogram of methanol extract of finish product at 366nm; **D**: 3D image of the Fingerprinting of VE and finish product (366nm). The results indicate that HPTLC Chromatogram of VE and finished product has shown the similar R_f value of 0.52 and 0.68 at 366nm.

***Berginia ligulata* (Pashanbhed) Root Ext**

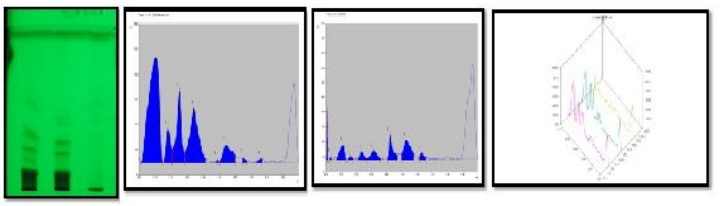

Figure 4: It shows the HPTLC Chromatogram of Pashanbhed Ext. **A**: HPTLC Plate of PAE at 254nm under UV. **B**: 2D Chromatogram of methanol extract of PAE at 254nm; **C**: 2D Chromatogram of methanol extract of finish product at 254nm; **D**: 3D image of the Fingerprinting of PAE and finish product (254nm). The results indicate that HPTLC Chromatogram of PAE and finished product has shown the similar R_f value of 0.41 at 254nm.

Chandraprabha Vati

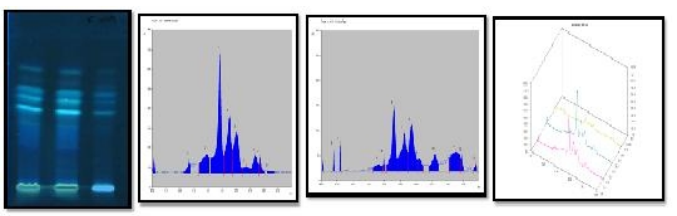

Figure 5: It shows the HPTLC Chromatogram of Chandraprabha Powder. **A**: HPTLC Plate of CV at 366nm under UV. **B**: 2D Chromatogram of methanol extract of CV at 366nm; **C**: 2D Chromatogram of methanol extract of finish product at 366nm; **D**: 3D image of the Fingerprinting of CV and finish product (366nm). The results indicate that HPTLC Chromatogram of CV and finished product has shown the similar R_f value of 0.45, 0.50, 0.55 and 0.70 at 366nm.

***Raphanus sativus* (Mulika) Powder**

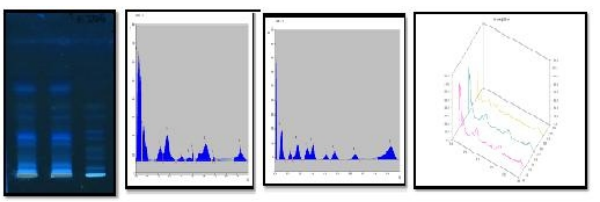

Figure 6: It shows the HPTLC Chromatogram of Mulika Kshar Powder. **A**: HPTLC Plate of MP at 366nm under UV. **B**: 2D Chromatogram of methanol extract of MP at 366nm; **C**: 2D Chromatogram of methanol extract of finish product at 366nm; **D**: 3D image of the Fingerprinting of MP and finish product (366nm). The results indicate that HPTLC Chromatogram of MP and finished product has shown the similar R_f value of 0.21, 0.41, 0.52 and 0.64 at 366nm.

DISCUSSION AND CONCLUSION

Herbal medicines are completely prepared from plant origin materials which are a direct source and prone to contamination, deterioration and variation in composition which directly can result in batch to batch variation. Thus it is essential to standardize the product that assures that each unit contains the amount of ingredients claimed on the label.

Ural Tablet is a Polyherbal 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 physical and chemical parameters.

The testing method for each parameter has been standardized and validated and the protocols have been adopted from standard reference books.

Sensory characters like Taste, appearance, odour of the drug were first evaluated for identification and purity before any further tests are undertaken.

The pH and moisture were found in the stipulated limit as they play important role in reflecting quality of product. Variation in them can encourage microbial growth and can cause deterioration followed by hydrolysis.

Water and Alcohol Soluble Extractive tests determine the amount of active constituents extracted with water and alcohol respectively from a given amount of medicinal plant material.

Total ash was performed to measure the total amount of material remaining after ignition. This test is important to control adulteration.

WHO also specifies limits for the presence of contaminants like Pathogenic micro-organisms along with yeast-moulds and the presence of heavy metals as the consumption of which can lead to complications in one's routine life.

HPTLC method helps in confirming the qualitative as well as quantitative presence of the raw material in the finished product. Thus present standardization study reveals compliance with all the above selected parameters and hence it can be concluded that Ural tablet is a well standardized product at essential physico-chemical parameters.

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