



Standardization Study of Poly Herbal Formulation - Caspa Drops

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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 program for production and manufacturing of herbal drugs. WHO specifies guidelines for the assessment of the safety, efficacy and quality of herbal medicines as a prerequisite for global harmonization. Hence it is very essential to ensure that each packet sold in the market is standardized. In this research work, a polyherbal formulation (PHF), Caspa Drops has been taken to establish its standardization level using various physico-chemical parameters. For raw material various parameters such as pH, Moisture content, ash values, HPTLC fingerprinting, assays of marker compounds etc. were carried out to confirm their quality and potency. The presence of the raw materials in the finished product was carried out with the aid of sophisticated instruments. 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, Caspa Drops, Polyherbal Syrup formulation.

INTRODUCTION

Plants contain several hundred constituents and some of them are present at very low concentrations. In spite of the modern chemical, analytical procedures available, rarely any phytochemical investigations succeed in isolating and characterizing all secondary metabolites present in the plant extract. Apart from this, plant constituents vary considerably depending on several factors that impair the quality control of phytotherapeutic agents.^[1] Quality control and standardization of herbal medicines involve several steps. However, the source and quality of raw materials play a pivotal role in guaranteeing the quality and stability of herbal preparations. Thus, in the present study, Polyherbal Syrup formulation (Caspa Drops) has been selected to establish its standardization status. The key ingredients used in the formulation are Extract of *Anethum sowa* (Soa) Fruit², *Acorus calamus* (Vacha) Rhizome^[2], *Plumbago zeylanica* (Chitrak) Root^[2], *Apium graveolens* (Ajmoda) Fruit^[2], *Mentha sylvestris* (Pudina) Leaves^[2], *Aconitum heterophyllum* (Ativisha) Root Tuber^[2], *Caesalpinia crista* (Latakaranj) Seed^[2], *Embelia ribes* (Vidang) Fruit^[2],

Elettaria cardamomum (Elaichi) Fruit^[2], *Hedychium spicatum* (Shati) Rhizome^[2], *Holarrhena antidysenterica* (Indrayav) Seed^[2] and Powder of Kala namak (Black salt)^[2], Saindhav (Rock salt)^[2], *Ferula foetida* (Hingu)^[2] and *Citrus acida* Fruit Juice (Nimbuk).^[2]

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 1.

Physicochemical parameters for extracts: The physico-chemical parameters includes tests like Moisture content / Loss on drying^[3], pH^[3], Alcohol soluble extractive^[3], Water soluble extractive^[3], Determination of ash^[3], Acid insoluble ash^[3], and Water soluble ash^[3] of the relevant raw materials. The results are as tabulated in Table 2 & 3.

Estimation of Actives: Assay analysis includes estimation of Alkaloid^[4], Bitter^[4], Tannin^[4], Saponin^[4] and Citric Acid^[5] 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 physical appearance, pH, Specific gravity^[3], Viscosity.^[3] The results are as tabulated in Table 5.

Microbial Analysis^[6]: Bio-burden analysis consists of parameters like Total Bacterial Count, Total Fungal Count and Presence of pathogens such as *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and

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Table 1: Organoleptic parameters and ingredient's part used

Ingredient	Parts used	Organoleptic characters		
		Colour	Odour	Taste
SE	Fruit	Brown coloured fine powder	Aromatic	Acrid pungent
VE	Rhizome	Dark Brown coloured fine powder	Characteristic	Characteristic
CE	Root	Brown coloured fine powder	Characteristic	Acrid Bitter
AE	Fruit	Brown coloured fine powder	Strong aromatic	Acrid pungent
PE	Leaves	Translucent crystals	Strong mentholated	Acrid pungent
AVE	Root Tuber	Dark Brown coloured fine powder	Characteristic	Bitter
LE	Seed	Light Brown coloured fine powder	Characteristic	Bitter
VIE	Fruit	Dark Brown coloured fine powder	Characteristic	Astringent
EE	Fruit	Dark brown coloured viscous liquid	Pleasant Aromatic	Bitter
SHE	Rhizome	Dark Brown coloured fine powder	Characteristic	Characteristic
IE	Seed	Dark Brown coloured fine powder	Characteristic	Bitter
KN	Salt	Light Pink coloured fine powder	Characteristic sulphorus	Salty
SD	Salt	White Crystalline Powder	Characteristic	Salty
HI	Exudates	Creamish coloured fine powder	Strong aromatic	Acrid
CA	Fruit Juice	Hazy white liquid	Characteristic	Characteristic

SE: Soa Extract, VE: Vacha Extract, CE: Chitrak Extract, AE: Ajmoda Extract, PE: Pudina Extract, AVE: Ativish Extract, LE: Latakaranj Extract, VIE: Vidang Extract, EE: Elaichi Extract, SHE: Shati Extract, IE: Indrayav Extract, KN: Kala namak, SD: Saindhav HI: Hingu Powder and CA: Citrus acid Fruit Juice; NA: Not Applicable

Table 2: Physicochemical parameters

Ingredients	pH	M/S (by LOD) %
SE	5.03 ± 0.02	2.27 ± 0.06
VE	3.80 ± 0.12	2.21 ± 0.14
CE	4.44 ± 0.25	2.89 ± 0.03
AE	4.52 ± 0.15	3.82 ± 0.02
PE	4.99 ± 0.12	7.06 ± 0.25
AVE	3.73 ± 0.23	3.05 ± 0.52
LE	5.04 ± 0.35	3.19 ± 0.12
VIE	5.58 ± 0.12	4.27 ± 0.32
EE	4.04 ± 0.03	4.50 ± 0.35
SHE	4.56 ± 0.01	5.07 ± 0.35
IE	4.02 ± 0.05	2.07 ± 0.02
CA	NA	0.29 ± 0.01

SE: Soa Extract, VE: Vacha Extract, CE: Chitrak Extract, AE: Ajmoda Extract, PE: Pudina Extract, AVE: Ativish Extract, LE: Latakaranj Extract, VIE: Vidang Extract, EE: Elaichi Extract, SHE: Shati Extract, IE: Indrayav Extract, CA: Citrus acid Fruit Juice; LOD: Loss on Drying; NA: Not Applicable

Table 3: Extractive values and Ash Value of Ingredients of Caspa Drops

Ingredients	WSE (%)	ASE (%)	TA (%)	AIA
SE	88.08 ± 0.02	96.06 ± 0.01	7.66 ± 0.12	1.90 ± 0.12
VE	90.65 ± 0.01	90.27 ± 0.01	9.87 ± 0.11	31.23 ± 0.21
CE	94.32 ± 0.05	98.30 ± 0.12	4.59 ± 0.02	4.87 ± 0.15
AE	87.04 ± 0.04	86.83 ± 0.23	7.95 ± 0.12	7.94 ± 0.15
PE	76.69 ± 0.12	83.35 ± 0.24	9.57 ± 0.12	5.30 ± 0.13
AVE	96.48 ± 0.12	86.92 ± 0.21	7.85 ± 0.22	1.74 ± 0.18
LE	85.04 ± 0.05	84.49 ± 0.22	7.92 ± 0.05	2.04 ± 0.14
VIE	95.28 ± 0.02	91.77 ± 0.32	9.30 ± 0.04	2.99 ± 0.48
EE	93.12 ± 0.05	NA	NA	NA
SHE	96.00 ± 0.21	76.74 ± 0.15	5.03 ± 0.05	7.74 ± 0.16
IE	88.24 ± 0.13	76.48 ± 0.65	3.39 ± 0.13	9.56 ± 0.14
HI	9.12 ± 0.11	8.08 ± 0.01	0.65 ± 0.10	0.31 ± 0.12

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Salmonella enterica ser. Abony spp. The results are furnished in Table 6.

Heavy Metal Analysis [7]: Sample preparation for heavy metal analysis was done by MARS Express microwave digestive system. The standard solutions for detection of Lead (Pb), Cadmium (Cd), Arsenic (As) and Mercury (Hg) were prepared. Samples were then analyzed using Atomic Absorbance Spectrophotometer AA 6300, SHIMADZU and HVG-1 by preparing a calibration curve of standard. 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 Hamilton 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 60F254 (Merck) 0.2mm thickness.

Table 4: Analysis of the Active Compounds

	Alkaloid	Bitter	Tannin	Saponin	Citric Acid
SE	0.38 ± 0.12	2.79 ± 0.14	0.01 ± 0.12	41.65 ± 0.21	NA
VE	0.14 ± 0.02	2.18 ± 0.21	0.01 ± 0.14	42.21 ± 0.12	NA
CE	0.29 ± 0.03	4.61 ± 0.01	0.04 ± 0.21	44.63 ± 0.05	NA
AE	0.65 ± 0.01	5.97 ± 0.12	0.06 ± 0.32	20.14 ± 0.21	NA
PE	0.44 ± 0.05	1.99 ± 0.45	0.01 ± 0.24	14.18 ± 0.03	NA
AVE	0.04 ± 0.13	10.63 ± 0.35	0.04 ± 0.02	20.09 ± 0.45	NA
LE	0.74 ± 0.14	4.25 ± 0.12	0.05 ± 0.02	66.05 ± 0.12	NA
VIE	0.88 ± 0.11	3.31 ± 0.11	0.07 ± 0.01	34.35 ± 0.25	NA
SHE	0.11 ± 0.21	2.05 ± 0.14	0.07 ± 0.32	46.51 ± 0.21	NA
IE	0.79 ± 0.23	3.24 ± 0.32	0.08 ± 0.21	25.78 ± 0.32	NA
CA	NA	NA	NA	NA	99.98 ± 0.11

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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°C for 3 minutes

Steps involved in HPTLC analysis

Selection of plate and adsorbent: Precoated aluminum plates with Silica Gel F254 of 20 × 20 cm and 0.2 mm thickness, was used for the 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 Extract (Reference Standard / Test Drug / with 10mL of Methanol with constant shaking for 05 minutes. Heat on water bath at 90-100°C for 5 minutes, 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: 8µl/mL methanol extract of test drug under observation

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

Table 5: Analysis of the Finished Product (Caspa Drops)

Parameter	Results		
	Batch 1	Batch 2	Batch 3
Description	Dark Green coloured syrup with characteristic odour & characteristic sweet taste	Dark Green coloured syrup with characteristic odour & characteristic sweet taste	Dark Green coloured syrup with characteristic odour & characteristic sweet taste
pH	5.46	5.48	5.78
Specific gravity	1.220 g/mL	1.152 g/mL	1.256 g/mL
Viscosity	19.72 Centipoise	21.00 Centipoise	21.42 Centipoise

RESULTS

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 Rf 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.

Table 6: Results of Heavy metal content and Bio-burden in raw material of Caspa Drops

Material	Trace elements				Bio-burden					
	Pb 10ppm	Cd 0.3ppm	As 3.0ppm	Hg 1.0ppm	TBC cfu/g	TFC cfu/g	<i>E. coli</i> Ab	<i>P.a</i> Ab	<i>Sal.sp</i> Ab	<i>S.a</i> Ab
SE	3.021	0.121	1.254	Absent	14 × 10 ²	Ab	Ab	Ab	Ab	Ab
VE	2.0125	0.125	1.352	Absent	21 × 10 ²	Ab	Ab	Ab	Ab	Ab
CE	3.052	0.214	1.652	Absent	11 × 10 ²	Ab	Ab	Ab	Ab	Ab
AE	0.214	0.001	0.235	Absent	15 × 10 ²	Ab	Ab	Ab	Ab	Ab
PE	0.325	0.254	0.362	Absent	NA	NA	NA	NA	NA	NA
AVE	0.258	0.251	0.112	Absent	12 × 10 ²	Ab	Ab	Ab	Ab	Ab
LE	2.352	0.214	0.521	Absent	6 × 10 ²	Ab	Ab	Ab	Ab	Ab
VIE	1.350	0.213	1.252	Absent	68 × 10 ²	Ab	Ab	Ab	Ab	Ab
EE	3.125	0.112	2.102	Absent	NA	NA	NA	NA	NA	NA
SHE	0.125	0.152	1.258	Absent	16 × 10 ²	Ab	Ab	Ab	Ab	Ab
IE	1.025	0.214	0.357	Absent	15 × 10 ²	Ab	Ab	Ab	Ab	Ab
KN	1.582	0.254	1.685	Absent	NA	NA	NA	NA	NA	NA
SD	3.258	0.004	1.352	Absent	NA	NA	NA	NA	NA	NA
HI	3.254	0.050	1.058	Absent	12 × 10 ²	Ab	Ab	Ab	Ab	Ab
CA	0.125	0.057	0.025	Absent	NA	NA	NA	NA	NA	NA
PHP	3.121	0.251	0.352	Absent	16 × 10 ²	Ab	Ab	Ab	Ab	Ab

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DISCUSSION

Standardization of bio-active is the process of delivering a product with a specified level of one or more phyto-constituents where we can make sure about related product quality and efficacy; broadly it covers the 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 a chemical in a crude herbal extract which are, present in that particular product and establishing a standard amount of that chemical for future production. The concept of standardized extracts

definitely provides a solid platform for scientific validation of herbals.

The testing of a finished product in compliance with predetermined standard prior to release of the product for packaging and subsequent distribution is a critical factor for quality assurance.

Caspa Drops 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.

Anethum sowa (Soa) Fruit Extract (SE)

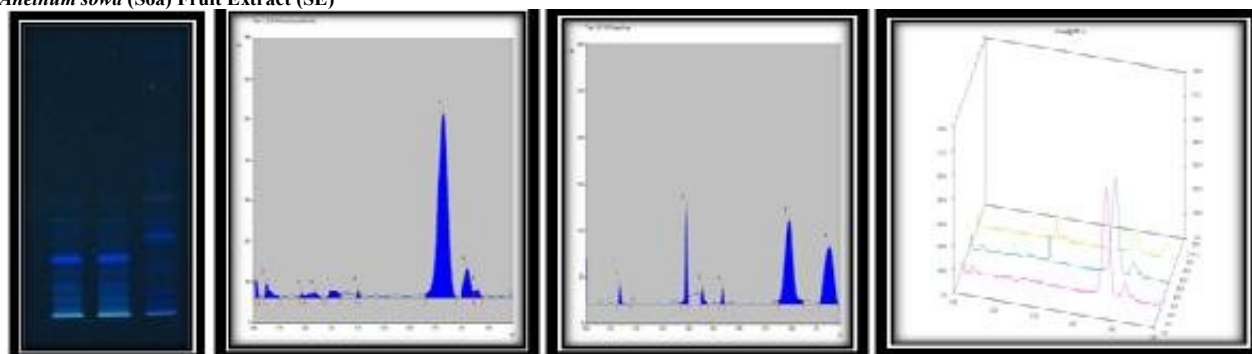


Fig. 1: It shows the HPTLC Chromatogram of SE. A: HPTLC Plate of SE at 366nm under UV. B: 2D Chromatogram of methanol extract of SE at 366nm; C: 2D Chromatogram of methanol extract of finish product at 366nm; D: 3D image of the Fingerprinting of SE and finished product (366nm). The results indicate that HPTLC Chromatogram of SE and finished product has shown the similar Rf value of 0.22 at 366nm

Acorus calamus (Vacha) Rhizome Extract (VE)

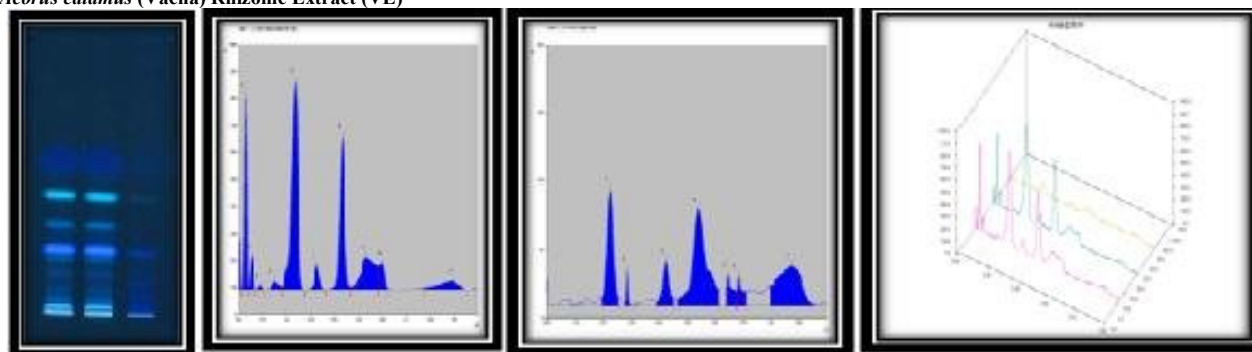


Fig. 2: It shows the HPTLC Chromatogram of VE. A: HPTLC Plate of VE at 366nm under UV. B: 2D Chromatogram of methanol extract of VE at 366nm; C: 2D Chromatogram of methanol extract of finish product at 366nm; D: 3D image of the Fingerprinting of VE and finished product (366nm). The results indicate that HPTLC Chromatogram of VE and finished product has shown the similar Rf value of 0.08, 0.41 at 366nm

Plumbago zeylanica (Chitrak) Root Extract (CE)

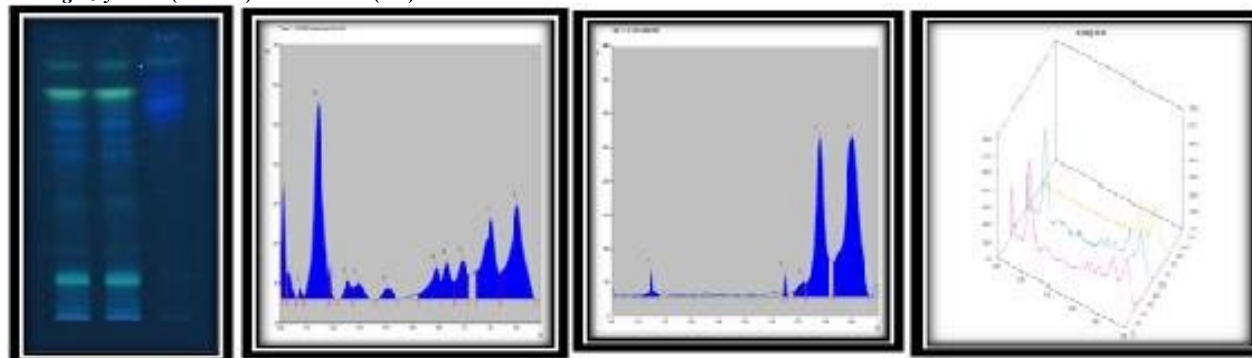


Fig. 3: It shows the HPTLC Chromatogram of CE. A: HPTLC Plate of CE at 366nm under UV. B: 2D Chromatogram of methanol extract of CE at 366nm; C: 2D Chromatogram of methanol extract of finished product at 366nm; D: 3D image of the Fingerprinting of CE and finished product (366nm). The results indicate that HPTLC Chromatogram of CE and finished product has shown the similar Rf value of 0.69, 0.77 at 366nm

Apium graveolens (Ajmoda) Fruit Extract (AE)

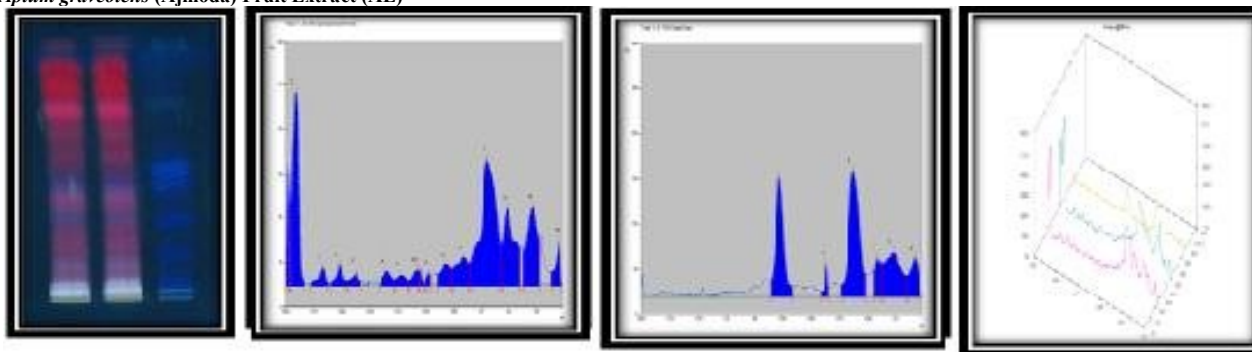


Fig. 4: It shows the HPTLC Chromatogram of AE. A: HPTLC Plate of AE at 366nm under UV. B: 2D Chromatogram of methanol extract of AE at 366nm; C: 2D Chromatogram of methanol extract of finished product at 366nm; D: 3D image of the Fingerprinting of AE and finished product (366nm). The results indicate that HPTLC Chromatogram of AE and finished product has shown the similar Rf value of 0.02, 0.13, 0.21, 0.66, at 366nm

Mentha sylvestris (Pudina) Leaves (PE)

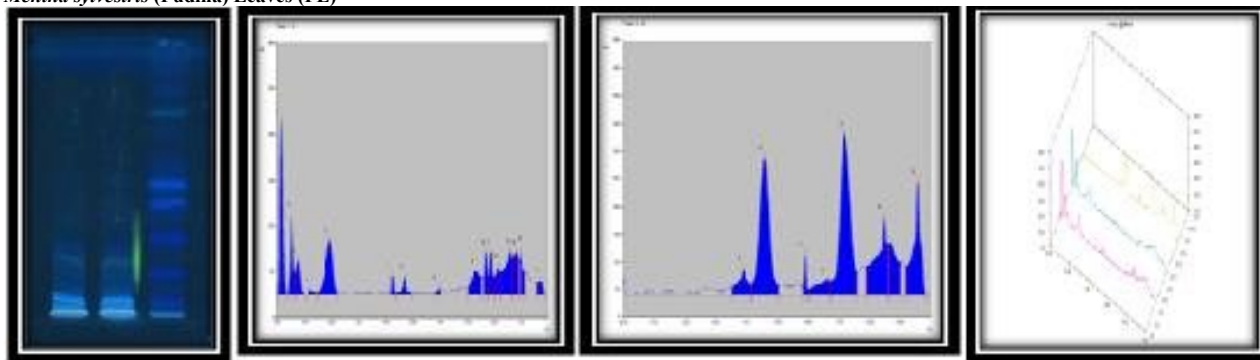


Fig. 5: It shows the HPTLC Chromatogram of PE. A: HPTLC Plate of PE at 366nm under UV. B: 2D Chromatogram of methanol extract of PE at 366nm; C: 2D Chromatogram of methanol extract of finished product at 366nm; D: 3D image of the Fingerprinting of PE and finished product (366nm). The results indicate that HPTLC Chromatogram of PE and finished product has shown the similar Rf value of 0.21, 0.30 at 366nm

Aconitum heterophyllum (Ativisha) Root Tuber Extract (AVE)

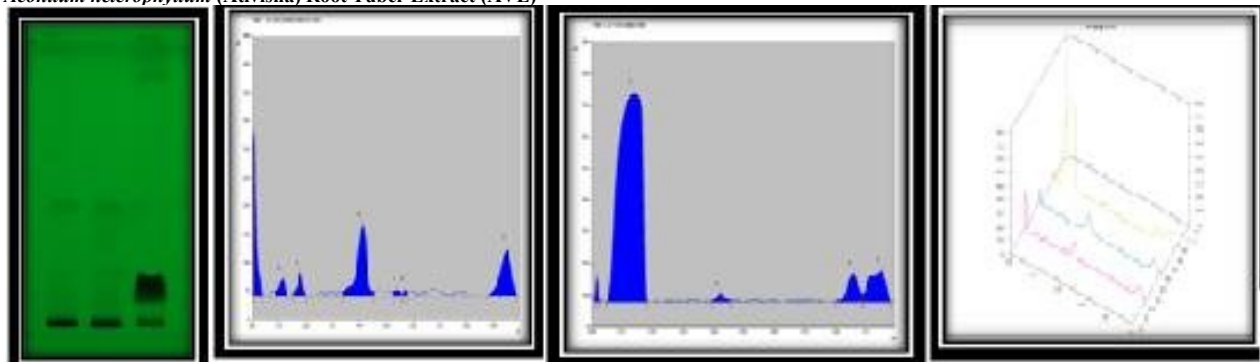


Fig. 6: It shows the HPTLC Chromatogram of AVE. A: HPTLC Plate of AVE at 254nm under UV. B: 2D Chromatogram of methanol extract of AVE at 254nm; C: 2D Chromatogram of methanol extract of finished product at 254nm; D: 3D image of the Fingerprinting of AVE and finished product (254nm). The results indicate that HPTLC Chromatogram of AVE and finished product has shown the similar Rf value of 0.57 at 254nm

Caesalpinia crista (Latakaranj) Seed Extract (LE)

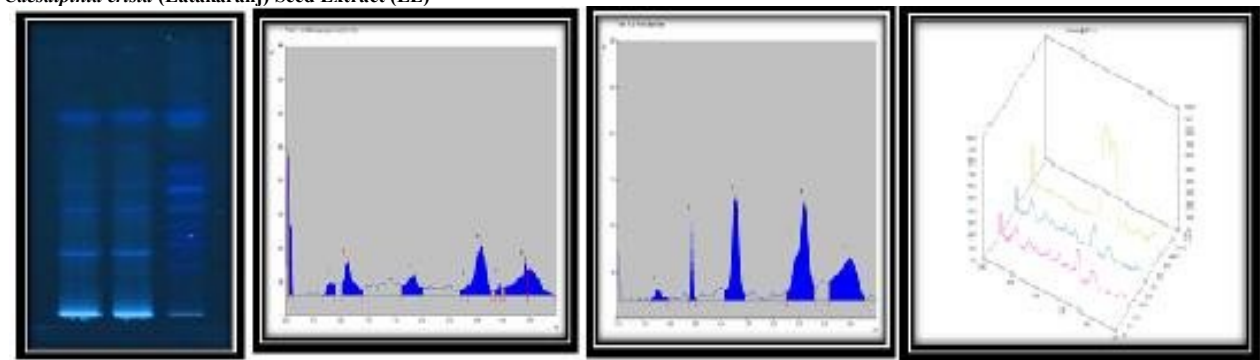


Fig. 7: It shows the HPTLC Chromatogram of LE. A: HPTLC Plate of LE at 366nm under UV. B: 2D Chromatogram of methanol extract of LE at 366nm; C: 2D Chromatogram of methanol extract of finished product at 366nm; D: 3D image of the Fingerprinting of LE and finished product (366nm). The results indicate that HPTLC Chromatogram of LE and finished product has shown the similar Rf value of 0.25, 0.36 and 0.51 at 366nm

Embelia ribes (Vidang) Fruit Extract (VIE)

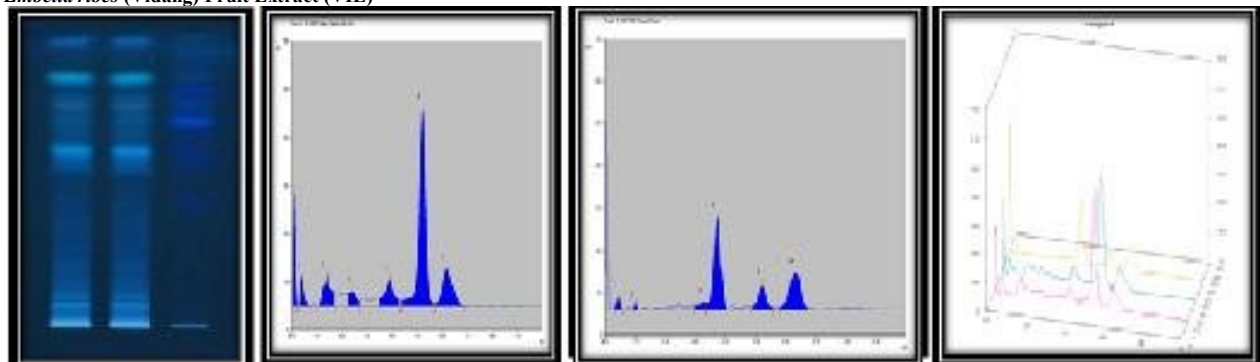


Fig. 8: It shows the HPTLC Chromatogram of VIE. A: HPTLC Plate of VIE at 366nm under UV. B: 2D Chromatogram of methanol extract of VIE at 366nm; C: 2D Chromatogram of methanol extract of finished product at 366nm; D: 3D image of the Fingerprinting of VIE and finished product (366nm). The results indicate that HPTLC Chromatogram of VIE and finished product has shown the similar Rf value of 0.58, 0.71 at 366nm

Elettaria cardamomum (Elaichi) Fruit Extract (EE)

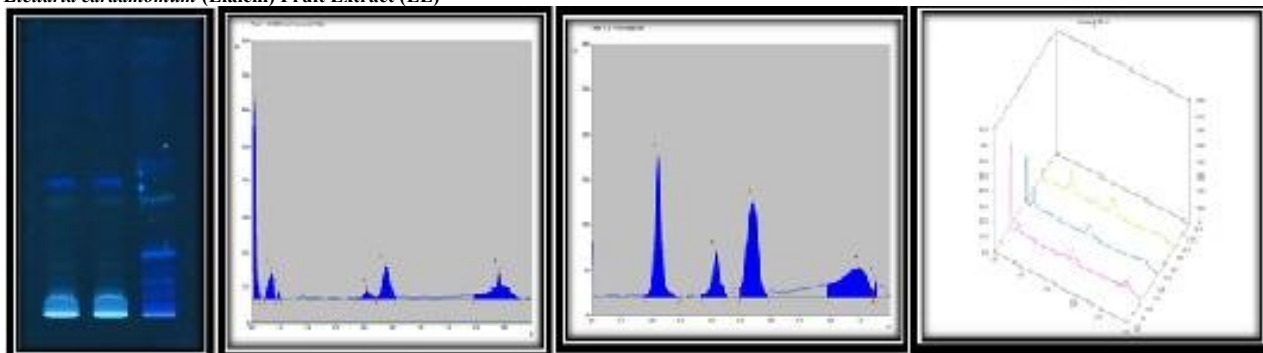


Fig. 9: It shows the HPTLC Chromatogram of EE. A: HPTLC Plate of EE at 366nm under UV. B: 2D Chromatogram of methanol extract of EE at 366nm; C: 2D Chromatogram of methanol extract of finished product at 366nm; D: 3D image of the Fingerprinting of EE and finished product (366nm). The results indicate that HPTLC Chromatogram of EE and finished product has shown the similar Rf value of 0.21, 0.38 at 366nm

Hedychium spicatum (Shati) Rhizome Extract (SHE)

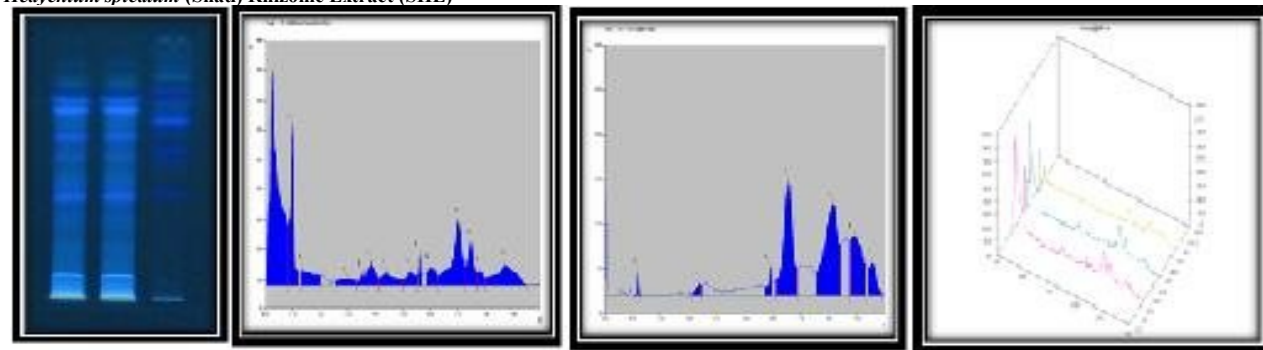


Fig. 10: It shows the HPTLC Chromatogram of SHE. A: HPTLC Plate of SHE at 366nm under UV. B: 2D Chromatogram of methanol extract of SHE at 366nm; C: 2D Chromatogram of methanol extract of finished product at 366nm; D: 3D image of the Fingerprinting of SHE and finished product (366nm). The results indicate that HPTLC Chromatogram of SHE and finished product has shown the similar Rf value of 0.39, 0.65 at 366nm

Holarrhena antidysenterica (Indrayav) Seed Extract (IE)

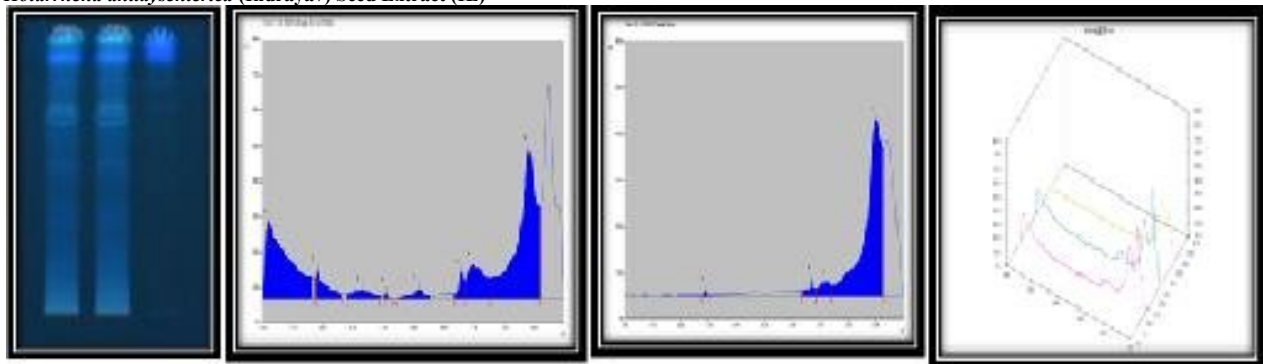


Fig. 11: It shows the HPTLC Chromatogram of IE. A: HPTLC Plate of IE at 366nm under UV. B: 2D Chromatogram of methanol extract of IE at 366nm; C: 2D Chromatogram of methanol extract of finished product at 366nm; D: 3D image of the Fingerprinting of IE and finished product (366nm). The results indicate that HPTLC Chromatogram of IE and finished product has shown the similar Rf value of 0.57, 0.73 at 366nm

Ferula foetida (Hingu) Powder (HI)

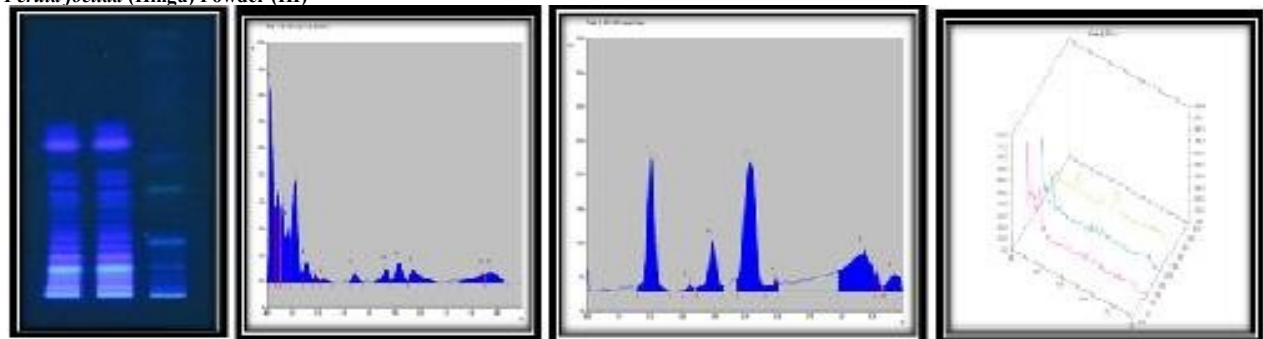


Fig. 12: It shows the HPTLC Chromatogram of HI. A: HPTLC Plate of HI at 366nm under UV. B: 2D Chromatogram of methanol extract of HI at 366nm; C: 2D Chromatogram of methanol extract of finished product at 366nm; D: 3D image of the Fingerprinting of HI and finished product (366nm). The results indicate that HPTLC Chromatogram of HI and finished product has shown the similar Rf value of 0.16, 0.33, 0.47 at 366nm

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

Sensory characters like physical appearance, colour odour and taste of the raw materials and Finish product were first evaluated for identification and purity before any further tests are undertaken.

pH and moisture content 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.

Extractive Value determines the amount of active constituents in a given amount of medicinal plant material when extracted with a solvent media such as Water and Alcohol. 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. Acid insoluble ash is the residue obtained after boiling the total ash with dilute hydrochloric acid, and igniting the washed insoluble matter.

Thus our 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 ser. Abony spp* along with yeast-moulds and four heavy metals viz; Lead, Cadmium, Arsenic & Mercury as the

consumption of which can lead to complications in one's routine life. Caspa Drops 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 reveals compliance with all the above discussed parameters and hence it can be concluded that Caspa Drops is a well standardized product at essential physico-chemical parameters.

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