



Research Article

PHYSICOCHEMICAL ANALYSIS AND PHYTOCHEMICAL EVALUATION OF *GOKSHURADI CHURNA* AND ITS HYDRO-ALCOHOLIC EXTRACT

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ABSTRACT

Drug is an essential tool of a physician included in *Chatushpada*. Many single as well as compound drugs are mentioned in classics in different contexts for internal as well as external use. It is always the cumulative impact of all the ingredients in the solution that plays a critical role in therapeutics, rather than the action of the individual medication. Synergistic action, combined action and toxicity neutralizing action and basic purpose are envisaged to serve drug combinations. One such important formulation prescribed in the Ashtanga Haridya Uttartantra is *Gokshuradi Churna* provide specifics of the drugs *Gokshuradi Churna* which was chosen for study. Acharya Vaghbhatta in Uttartantra listed numerous drugs that are useful in the treatment of *Alpa, Dushta Retas* that specifically influence the properties of *Shukrajanana* (spermatogenesis) and *Shukra Shodhana*. This formulation consists of five ingredients i.e., *Gokshura* (*Tribulus terrestris* Linn), *Ikshura* (*Asteracantha longifolia* Nees), *Mash* (*Phaseolus mungo* Linn), *Atmagupta* seeds (*Mucuna prurita* Hook) & *Shatavari* (*Asparagus racemosus* Wild). Many formulations are available in various texts under the same name *Gokshuradi Churna* with different ingredients and different *Rogadhikara*. Keeping this aim into consideration, the present study was designed to scientific evaluation of *Gokshuradi Churna* and its hydroalcoholic extract on different analytical and phytochemical parameters. The study includes macro and microscopic characters, powder microscopic characteristics, heavy metals analysis and High Performance Thin Layer Chromatography fingerprints (HPTLC). The information generated by this particulars study provides relevant pharmacognostical and physicochemical data needed for proper identification and authentication of *Gokshuradi Churna* and its hydroalcoholic extract.

INTRODUCTION

The WHO's definition of the drug/medication expresses that any substance or device utilized or interfered with to serve the patient to change or investigate the pathological status or neurotic status'. The essential tool of a physician included in *Chatushpada* is medicines. Many single as well as compound drugs are mentioned in classics in different

contexts for internal as well as external use. It is always the cumulative impact of all the ingredients in the solution that plays a critical role in therapeutics, rather than the action of the individual medication. Synergistic action, combined action and toxicity neutralizing action and basic purpose are envisaged to serve drug combinations. The ideal drug or diet element, according to Ayurveda, is that which reverses or breaks *Samprapti*.

One such important formulation prescribed in the Ashtanga Samgraha Uttartantra is *Gokshuradi Churna* provides specifics of the drugs *Gokshuradi Churna* which was chosen for study. Acharya Vaghbhat in Uttartantra listed numerous drugs that are useful in the treatment of *Alpa, Dushta Retas* that specifically

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influence the properties of *Shukrajanana* (spermatogenesis) and *Shukra Shodhana*. One of them is *Gokshuradi Churna* (chapter /40-34). *Shukrala* and *Vrishya Karma* are made up of *Gokshuradi Churna* ingredients (80%), while 60% of the ingredients have *Balya* properties. *Go-ghrut* is strongest of all the *Jangam Snehas* because it has a special capacity to adopt, i.e., *Samsakarysa Anuvartanam*, and this is *Vrushya*. *Gokshuradi Churna* is an Ayurvedic formulation mentioned in the ancient classical text *Ashtang Hridaya*.

श्वंदष्ट्रेक्षुरमाषात्मगुप्ताबीजशतावरी ।

पिबन् क्षीरेण जीर्णोऽपि गच्छति प्रमदाशतम् ॥ (अ० ह० उ० 40/34)

This formulation consists of five ingredients i.e., *Gokshura* (*Tribulus terrestris* Linn), *Ikshura* (*Asteracantha longifolia* Nees), *Mash* (*Phaseolus mungo* Linn), *Atmagupta* seeds (*Mucuna prurita* Hook) & *Shatavari* (*Asparagus racemosus* Wild). Many formulations are available in various texts under the same name *Gokshuradi Churna* with different ingredients and different *Rogadhikara*. The plant contains a diversity of biologically compounds such as alkaloids, flavonoids, terpenoids, vitamins, carbohydrates, glycosides, waxy substances, gum, phytosterols, fatty acids, minerals, polyphenols, proanthocyanins, mucilage, enzymes, amino acids, hydrocarbons, reducing sugars, essential oils, gums etc. minerals like calcium, potassium, iron, zinc,

manganese, magnesium, chromium, copper and cobalt present in various part of this plant. Keeping this aim into consideration, the present study was designed to scientific evaluation of *Gokshuradi Churna* and its hydroalcoholic extract on different analytical and phytochemical parameters. The study includes macro and microscopic characters, powder microscopic characteristics, heavy metals analysis and High Performance Thin Layer Chromatography fingerprints (HPTLC). The information generated by this particulars study provides relevant pharmacognostical and physicochemical data needed for proper identification and authentication of *Gokshuradi Churna* and its hydroalcoholic extract.

MATERIAL AND METHODS

Procurement of Raw Drugs

Seeds of *Gokshura*, *Ikshura*, *Mash*, *Atmagupta*, along with, *Shatavari* root were collected from authentic sources. All the solvent needed for extraction and excipient for *Gokshuradi Churna* extract formulation were collected from Vijay Scientific Laboratory, Haridwar.

Authentication of Raw Materials

All the herbs were authenticated (Brochure No. DG/RC/UAU-239, Date 05-10-2024) in the PG Department of Dravyaguna, Uttarakhand Ayurved University, Rishikul campus, Haridwar.

Table 1: Details of Drug Ingredients

Contents	Botanical name	Family	Parts used
<i>Gokshura</i>	<i>Tribulus terrestris</i> Linn	Zygophyllaceae	Fruit
<i>Ikshura</i>	<i>Asteracantha longifolia</i> Nees	Acanthaceae	Seeds
<i>Mash</i>	<i>Phaseolus mungo</i> Linn	Leguminosae	Seeds
<i>Atmagupta</i>	<i>Mucuna prurita</i> Hook	Leguminosae	Seeds
<i>Shatavari</i>	<i>Asparagus racemosus</i> Wild	Liliaceae	Roots

Procedure of Preparation of Churna and its Hydroalcoholic Extract

Gokshuradi Churna

- All five *Churnas* were taken in equal proportions and mixed well using mortar and pestle.
- After proper mixing, sieving was done through the sieve (85#).
- The prepared *Churna* was filled in an airtight stainless-steel container.

Hydroalcoholic Extract

Quantity of sample- 150 gm

Ratio of solvent - 50% water + 50% ethanol.

Solvent used - 500ml ethanol +500ml water (total 1000ml)

- 1150gm of sample of *Gokshuradi Churna* ingredients was transferred into a clean filter paper (thimble) and inserted into a Soxhlet

(500ml) assembly fitted with 1000ml round bottom flask (R.B flask).

- The whole assembly was heated for 8hrs in a 1000ml heating mantle for a day.
- This process was repeated till the solvent in soxhlet became colourless.
- After the extraction, the sample went through the process of filtration.
- The solvent was then evaporated by using the distillation and rotatory evaporation process.
- Extract of *Gokshuradi Churna* ingredients is ready for analysis.

Macroscopic Study

The collected sample of *Gokshura* (*Tribulus terrestris* Linn) seed, *Ikshura* (*Asteracantha longifolia* Nees) seeds, *Mash* (*Phaseolus mungo* Linn) seed, *Atmagupta* (*Mucuna prurita* Hook) seed, *Shatavari* (*Asparagus racemosus* Wild) root, were studied organoleptically with naked eye and magnifying lens with the help of pharmacognostical procedure i.e., shape, size, surface, taste, odour, color, texture and findings were recorded

Powder Microscopy (Anonymous API, 2008)

For microscopic study, 5gm of the powder of above mentioned drugs were taken separately. The powdered material was taken on 85 mesh sieve and allowed in slow running water for washing away the minerals. Then the microscopic slides were prepared either by soaking a pinch of fine powder in distilled water for 1 hour and staining in saffranin for 2-4 minutes, followed by addition of 1-2 drops of conc. HCl, then observed characters under microscope and figures were drawn with the help of mirror type camera lucida.

Physico-chemical parameters

Physico-chemical parameters such as moisture content (loss on drying at 105°C), water soluble extractive value, alcohol soluble extractive value, hexane soluble extractive value, total ash value, acid insoluble ash value and water soluble ash and pH values were calculated (Tripathi & Sikarwar, 2015).

Heavy metals Analysis

OBSERVATION AND RESULTS

Macroscopic and Microscopic Characters

Organoleptic Characters

Table 3: Results of Organoleptic characters of Churna and its Extract

S. no.	Parameters	<i>Gokshuradi Churna</i>	<i>Gokshuradi Extract</i>
1.	Appearance	Cremamish yellow colour	Dark brown colour
3.	Odour	Characteristics	Characteristics
4.	Taste	Astringent	Bitter
5	Texture	Granular powder	Paste

Physicochemical Characters

Table 4: Physio-chemical Parameters of Churna & its Extract

S.no.	Parameters	<i>Gokshuradi Churna</i>	<i>Gokshuradi Extract</i>
1.	Total ash content	7.55%	3.16%
2.	Acid-insoluble ash content	0.88%	0.00%
3.	Water-soluble extractive	25.87%	90.75%
4.	Alcohol-soluble extractive	18.15%	26.78%
5.	Loss on drying	9.54	11.68%
6.	pH (1%)	6.57	7.08
7.	Particle size by sieve method		
	20 mesh (Pass%)	74.15%	

Heavy metals are toxic and generally occur through earth in plants. Mainly four types of heavy metals harmful for us they are Pb, Cd, As and Hg. As. These heavy metals detected through Atomic Absorption Spectrophotometer as per standard method (Ansari S H, 2006).

High Performance Thin Layer Chromatography (HPTLC)

HPTLC is an invaluable quality assessment tool for the evaluation of botanical material. It allows for the analysis of a vast number of compounds both efficiently and cost-effectively. Additionally, in this in single analysis, numerous samples can be run by decreasing analytical time. With HPTLC, a similar analysis can be viewed by using different wavelengths of light, thus providing a complete profile of the plant than typically observed with more specific analyses. Performing thin-layer chromatographic separation on HPTLC layers has several advantages over those on conventional layers.

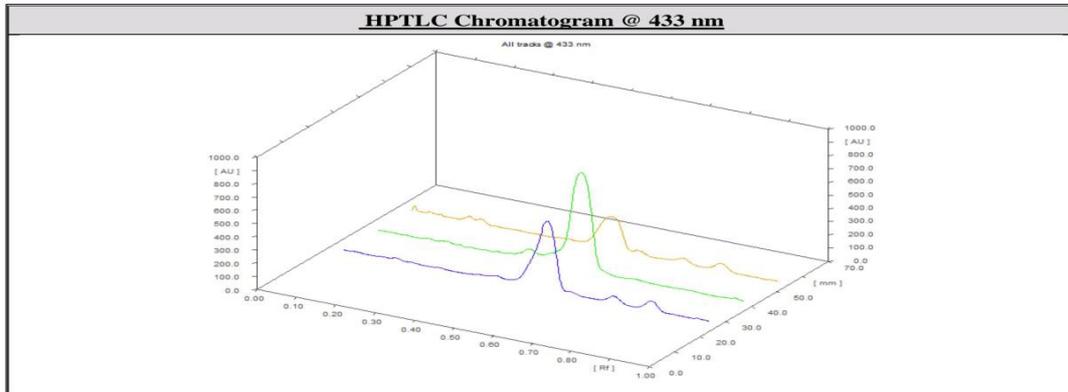
Preparation of Test Solution

- 1 gm of the sample was weighed accurately in an iodine flask.
- 2 Then 20ml methanol was added to the flask and refluxed for 15 min in the water bath.
- 3 It was then filtered with the help of Whatman filter paper No. 1.
- 4 Then obtained filtrate was used for HPTLC fingerprinting.

40 mesh (Pass%)	42.42%	NA
60 mesh (Pass%)	18.08%	
80 mesh (Pass%)	13.80%	
100 mesh (Pass%)	9.82 %	

Table 5: Instrumental Analysis

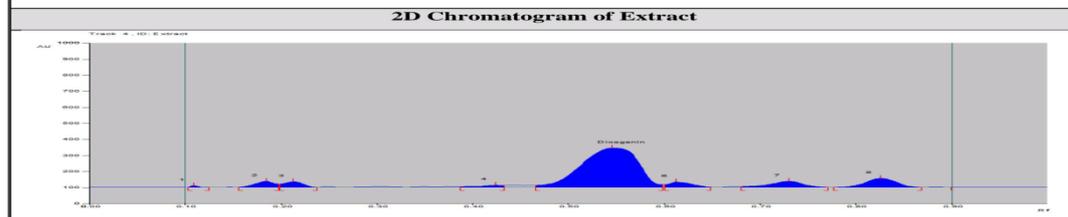
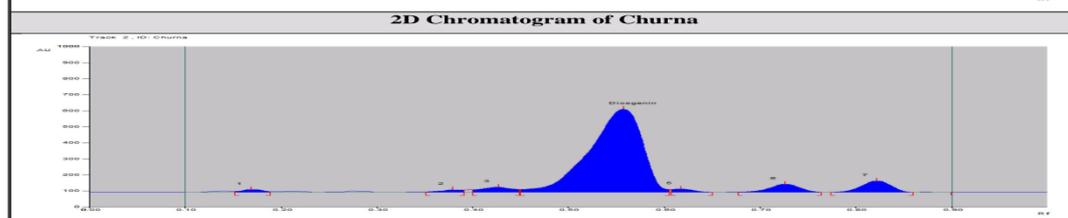
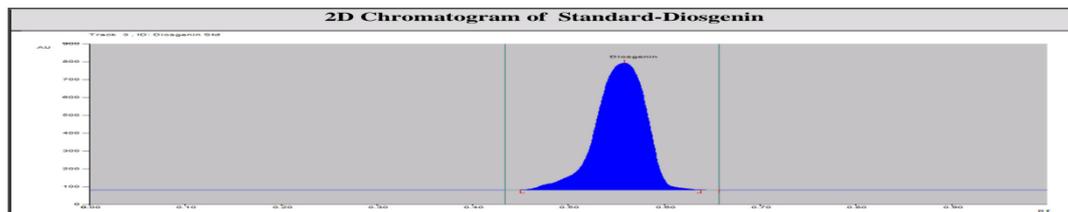
S.no		Gokshuradi Churna	Gokshuradi Churna Extract
1.	Assay of Diosgenin by HPTLC	0.662%	0.332%
2.	Assay of Shatavarin by HPTLC	0.364%	0.182%
3.	Assay of L-dopa by HPTLC	0.186%	0.022%

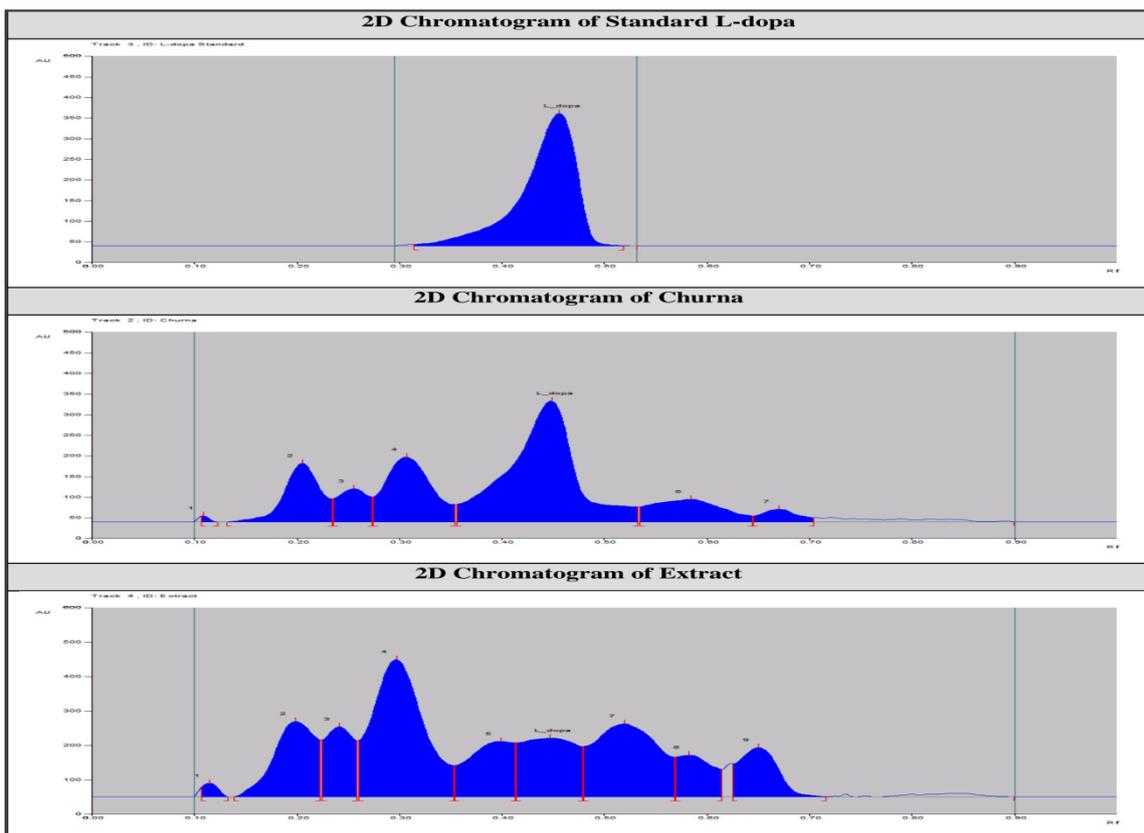
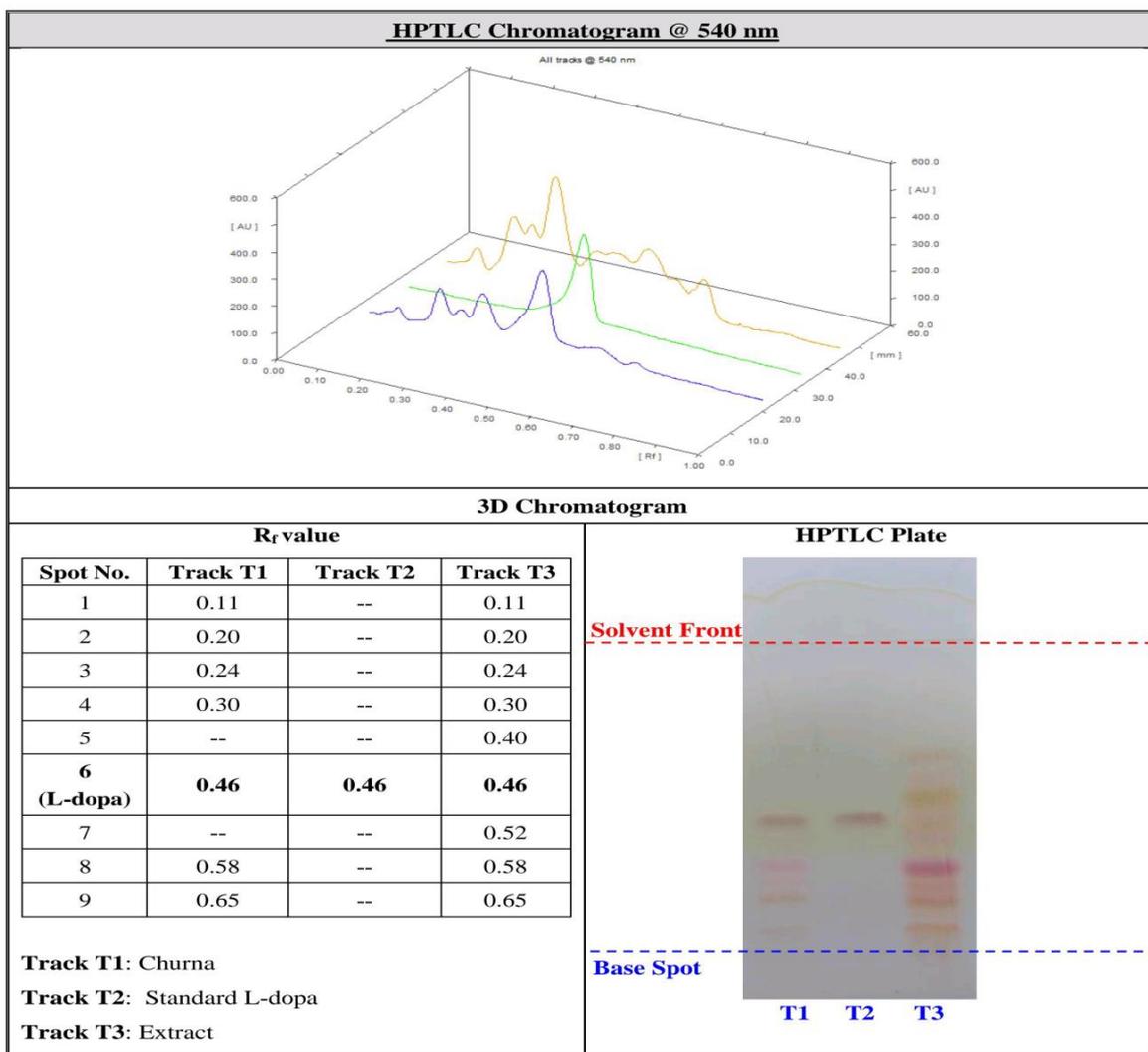


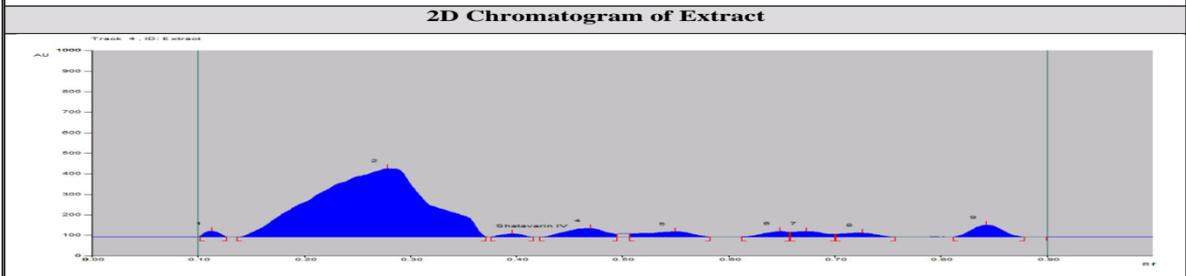
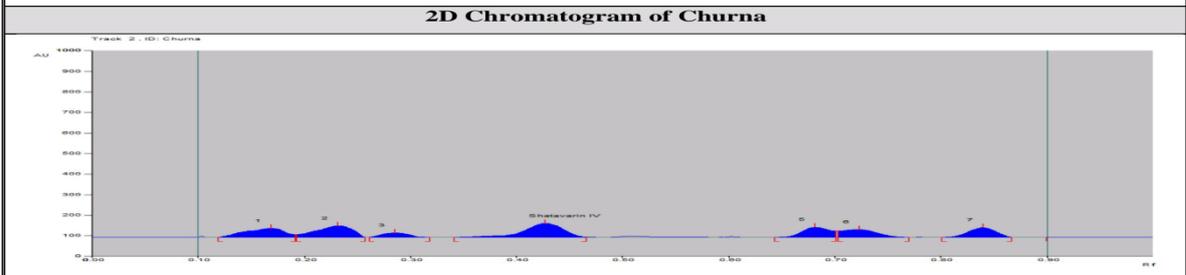
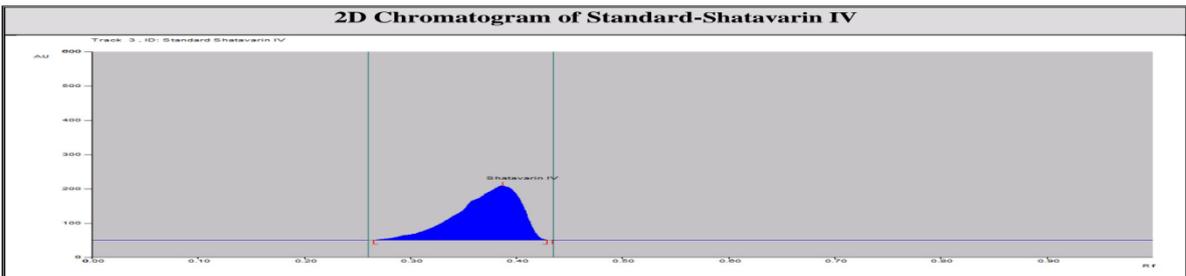
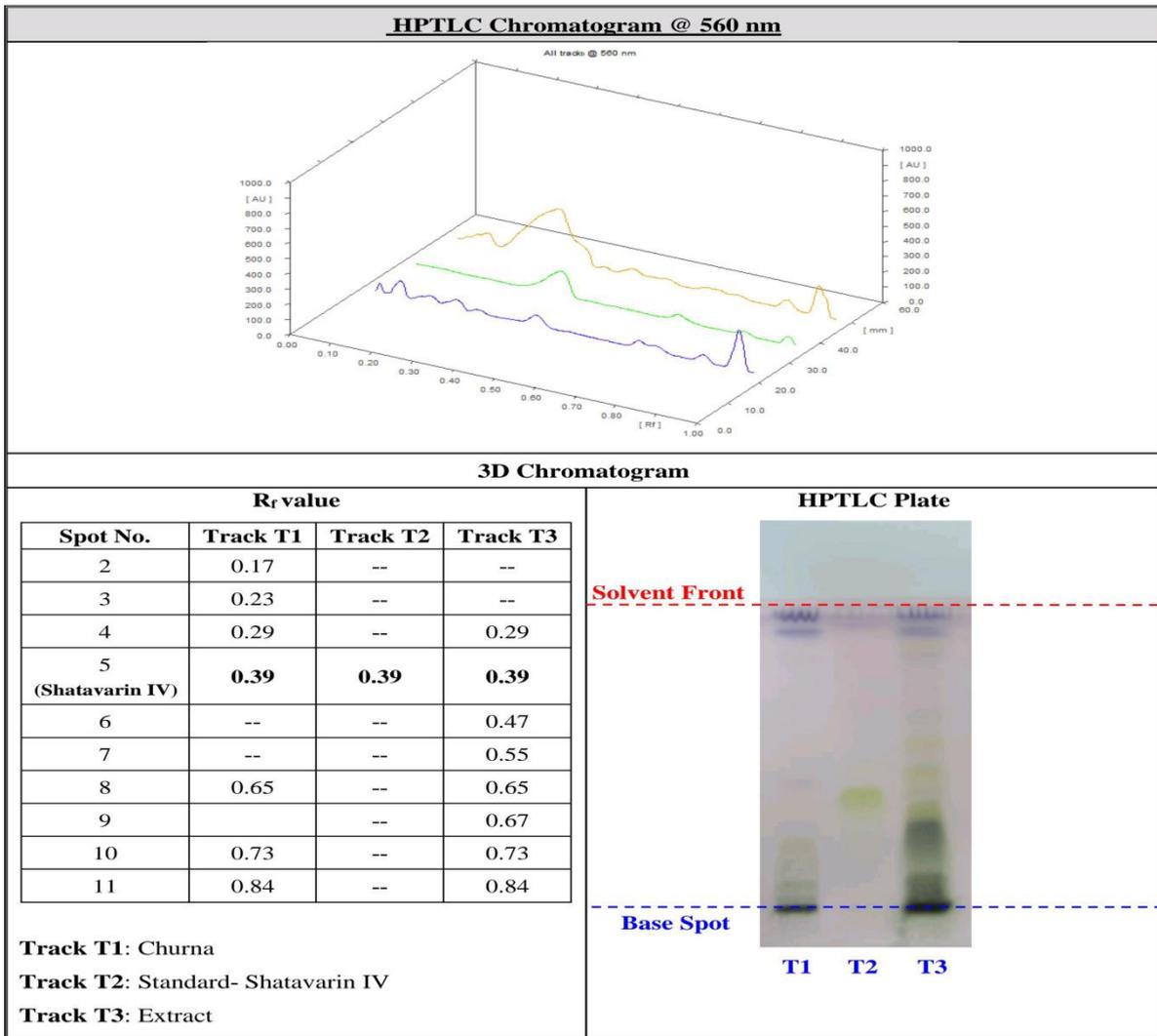
R _f value			
Spot No.	Track T1	Track T2	Track T3
1	--	--	0.11
2	0.18	--	0.18
3	--	--	0.21
4	0.38	--	--
5	0.42	--	0.42
6 (Diosgenin)	0.56	0.56	0.56
7	0.61	--	0.61
8	0.73	--	0.73
9	0.83	--	0.83

Track T1: Churna
Track T2: Standard-Diosgenin
Track T3: Extract

HPTLC Plate
Solvent Front
Base Spot
T1 T2 T3







Heavy Metal Analysis**Table 6: Heavy metals analysis of Gokshuradi Churna & its Extract**

S.no.	Parameters	Permissible Limits	Gokshuradi Churna	Gokshuradi extract
1.	Mercury	NMT 1 ppm	0.147 ppm	0.243 ppm
2.	Cadmium	NMT 0.3 ppm	0.089 ppm	0.023 ppm
3.	Arsenic	NMT 3 ppm	0.650 ppm	0.815 ppm
4.	Lead	NMT 10 ppm	1.341 ppm	0.470 ppm

Microbial Contamination**Table 7: Test for specific pathogens**

S.no.	Pathogens	Gokshuradi Churna	Gokshuradi Extract	Permissible Limits
1.	<i>E. coli</i>	Absent	Absent	Absent/g
2.	<i>Salmonella spp.</i>	Absent	Absent	Absent/g
3.	<i>S. aureus</i>	Absent	Absent	Absent/g
4.	<i>Pseudomonas aureus</i>	Absent	Absent	Absent/g

DISCUSSION

Acharyas have mentioned a variety of formulations, including *Panchavidha Kashaya Kalpana* in which *Churna Kalpana* is the *Upkalpana* of *Kalka Kalpana*. *Churna* is a powder of either a single drug or a combination of two or more drugs. The drugs are finely powdered and passed through a filter cloth to make the powder extremely fine so it can be used both internally and externally. The fineness of the powder used for oral administration should be at least 85 number mesh. *Churna* is the most basic Ayurvedic formulation that may be manufactured easily and quickly. It has excellent therapeutic value and cost-effective than any other pharmaceutical preparation.

Extraction, as the term is used in pharmacy, involves the separation of medicinally active portion from plant or animal tissues using selective solvents through standard extraction procedures. (API, part 1 vol IX) The required components are dissolved by the use of selective solvents known as a menstruum, and the undissolved part is called marc. The final product is called galenical. The rate of extraction depends upon the rate of diffusion of the solute through the liquid boundary layer in case of the solid-liquid type of extraction and through the layer at the interface in case of the liquid-liquid type of extraction.

Organoleptic Characteristics

The characteristics of a material that are felt through the sense organs- eyes, nose, tongue and skin are called organoleptic characteristics. These characteristics are helpful for assessing the quality of the substance to ensure that there is no contamination in the finished product. In order to make sure that a product complies with the standards, organoleptic testing is crucial. *Gokshuradi Churna* had a creamish yellow colour appearance of granular powder

consistency with characteristic odour while the extract had a typical dark brown coloured paste appearance with characteristic odour and taste.

Total Ash Value

The total ash value is an estimation of inorganic content to determine the authenticity and purity of the formulation. The total ash was found to be 7.55% in *Churna* and 3.16% in extract which exhibited less inorganic content in both samples. It means that both the formulations possessed a very lesser amount of contamination, substitution, adulteration in the product.

Acid Insoluble Ash

This ash value particularly indicates the contamination with silicious material, e.g. earthy matter and sand. (Garg NK, *et. al*, Ayurveda and Herbal Formulations, b, 2021). The obtained value of acid insoluble ash in *Churna* and Extract are 0.88% and 0.00% successively which showed less silicious material in both the samples.

Water-soluble extractive

Water-soluble extractive value is applied for the drugs which contain water-soluble constituents. Water soluble extractive value for *Churna* was 25.87% and for extract was 90.75% which indicates *Extract* had more water-soluble constituents than *Churna*.

Alcohol-soluble Extractive

It is applied to drugs that contain alcohol-soluble constituents. The alcohol-soluble extractive value for *Churna* was 18.51% and 26.58% for extract which indicates that *Churna* have lesser alcohol-soluble constituents than extract.

Loss on drying/Moisture content

Loss on drying at 105°C signifies the amount of moisture content present in the formulation. Higher moisture content may trigger enzymatic or hydrolytic reactions and microbial contamination, altering the chemical composition and medicinal properties of the formulation. It should be minimized to prevent the decomposition of the formulation. In prepared *Gokshuradi Churna* and extract, moisture content was 9.54% and 11.6% which indicates that *Churna* has better stability than Extract.

pH

pH value helps to determine the nature of the sample that is whether it is acidic or alkaline in nature. The pH value is one of the main factors influencing the quality of the medicines. It always controls many chemical and microbiological reactions. These pH values may be taken for quality control measures at various levels of processing and may be an important marker as well as an early indicator in case of any gross variation or spoiling of the formulation. Low pH restricts the growth of microbes, while high pH facilitates their growth. The pH of *Gokshuradi Churna* and its extract was 6.57 and 7.08, respectively.

Particle Size

It refers to the dimensions of particles in a substance, material or sample usually expressed in the average diameter, radius or volume of the particles. It can impact the drug's efficacy and safety. Smaller particles may facilitate quick absorption and assimilation of formulation in the gastrointestinal system. The number of particles for *Churna* which passed through #20 was 74.15%, through #40 was 42.42%, through #60 was 18.08%, through #80 was 13.80%, through #100 was 9.82% respectively.

Heavy metal analysis

Heavy metals (mercury, cadmium, lead and arsenic) in *Churna* and extract were

- Lead- [*Churna*-1.341 and extract-0.470]
- Cadmium- [*Churna*-0.089 and extract-0.023]
- Arsenic- [*Churna*-0.650 and extract-0.815]
- Mercury- [*Churna*-0.147 and extract-0.243]

All were found within permissible limits which exhibited the safety and non-toxicity of the formulations.

HPTLC

- The Rf values for *Gokshuradi Churna* at 433nm wavelength were 0.18, 0.42, 0.38, 0.61, 0.73, 0.83 (6 spots) and *Gokshuradi* extract in gm were 0.18, 0.42, 0.38, 0.61, 0.73, 0.83 (7 spots).
- At a wavelength of 540nm, Rf values for *Gokshuradi Churna* were 0.11, 0.20, 0.24, 0.30, 0.58, 0.65 (6 spots) and extract were 0.11, 0.20, 0.24, 0.30, 0.58, 0.65 (8 spots).

- At a wavelength of 560nm, Rf values for *Gokshuradi Churna* were 0.17, 0.23, 0.29, 0.65, 0.65, 0.73, 0.84 (6 spots) and extract were 0.29, 0.47, 0.55, 0.65, 0.67, 0.73, 0.84 (7 spots).

Rf Value

Rf value refers to the ratio of the distance moved by the solute to the distance moved by the solvent on a thin layer of an absorbent. Rf value of a compound under a given condition is characteristic and can be used to identify the compound by comparison with the reference standard. The intensity of the color of the compound under test can be utilized for quantitative estimation of the active principle in the drug. This Rf value suggests that the active biological ingredients were present in both the samples. Many Rf values of *Gokshuradi Churna* and extract were the same at 433nm such as 0.11, 0.20, 0.42, at 540nm 0.11, 0.20, 0.24, 0.30, 0.58, 0.65 and at 560nm 0.29, 0.39, 0.65, 0.73, 0.84. This showed that most of the compounds of *Gokshuradi Churna* were present in extract.

CONCLUSION

1. *Gokshuradi Churnais* a formulation quoted by Acharya Vagbhata in Uttartantra Adhyaya 40/34 comprising of three ingredients i.e., *Gokshura*, *Ikshura*, *Masha*, *Atmagupta* and *Shatavari*.
2. A common problem with Ayurvedic preparations is that they are difficult to administer and are generally unpalatable. This urged the need for the conversion of *Gokshuradi Churna* into other dosage forms i.e., extract for quick absorption and easily palatability.
3. *Gokshuradi Churna* was prepared as per the procedure mentioned in Sharangdharaa Samhita and extract was prepared by contemporary method of extraction with the help of Soxhlet apparatus.
4. The yield of *Gokshuradi Churna* and extract was 91.6% and 30% respectively.
5. Physico-chemical analysis ensures the efficacy and safety of all formulations and all parameters required for the quality control of final formulations (*Gokshuradi Churna* and extract) were within permissible limits.
6. *Gokshuradi Churna* exhibited better phytochemical constituents as compared to *Gokshuradi Churna* extract.
7. Hence, there would be no exaggeration in concluding that this plant is accepted as most sacred for its high medicinal values.

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