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Research Article

ANALYTICAL PROFILE OF *PUNARNAVADI GHRITA*- AN AYURVEDIC FORMULATION

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ABSTRACT

Punarnavadi Ghrita, a traditional Ayurvedic formulation, is used in the management of *Madatyaya* (alcoholism). It comprises *Punarnava* (*Boerhaavia diffusa*), *Yashtimadhu* (*Glycyrrhiza glabra*), *Godugdha* (cow's milk), and *Goghrita* (cow's ghee). The present study focuses on the analysis of this *Ghrita* as per the general guidelines for drug development of Ayurvedic formulations. This formulation was prepared following the general procedure of *Bhaishajya Kalpana*, ensuring the authenticity and quality of raw ingredients through rigorous organoleptic assessment. Physicochemical analyses were conducted to determine parameters such as optical rotation, iodine value, saponification value, specific gravity, and free fatty acid content. Phytochemical screening revealed the presence of alkaloids, phenols, and terpenoids. HPTLC analysis at 254nm and 366nm wavelengths identified multiple peaks, indicating a complex profile of active compounds. Heavy metal, pesticide residue, and microbial contamination tests confirmed the safety of the formulation, with no harmful contaminants detected. The findings offer a comprehensive profile of *Punarnavadi Ghrita,* establishing quality benchmarks for its therapeutic use and providing a reference standard for future research. This study underscores the formulation's potential in replenish *Ojadhatu* and mitigate the adverse effects of alcoholism, aligning with ancient Ayurvedic wisdom.

INTRODUCTION

The term '*Madya'*, derived from Sanskrit, signifies a state of intoxication and joy. Ancient texts, such as those by *Acharyas Charaka* and *Sharangadhara*, describe *Madya* (alcohol) as a *Drava dravya* with significant cultural and medicinal relevance. It is noted for its *Laghu* (light), *Ushna* (hot), *Teekshna* (sharp), *Aasu* (quickly absorbed nature), and so on *Gunas*, exhibiting both beneficial and detrimental effects depending on usage[1]. Benefits include exhilaration, increased appetite, and improved digestion, while overuse leads to mental disturbances and physical ailments[2].

Madatyaya, or alcoholism, presents in stages with symptoms ranging from mild exhilaration to severe delirium and unconsciousness.

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The condition's progression reflects the exacerbation of symptoms, which may start with excitement and joy and escalate to severe mental and physical disturbances. Ancient texts provide detailed descriptions of these stages and recommend specific treatments based on the dominant *Dosha* affected, aiming to restore balance and mitigate the adverse effects of excessive alcohol consumption[3,4].

In the ancient text *Chakradutta*, Chapter 18 focuses on the treatment of alcoholism, where a polyherbal formulation known as *Punarnavadi Ghrita* is mentioned. This formulation consists of four ingredients, including *Punarnava* (*Boerhaavia diffusa*), *Yashtimadhu* (*Glycyrrhiza glabra*), *Godugdha* (cow's milk), and *Goghrita* (cow's ghee). *Punarnavadi Ghrita* is revered for its ability to replenish the '*Ojadhatu*' in the human body, which is often depleted due to excessive consumption of *Madya* (alcohol) [5].

AIM AND OBJECTIVE

1. To evaluate the quality of the drug by using different analytical techniques.
2. To prepare a profile of the drug.

MATERIALS AND METHODS

Name and detail of drugs: *Punarnavadi ghrita (1 Prastha ghrita*-768 ml)

Table 1: Contents of *Punarnavadi ghrita*

S.No	Name of drug	Botanical name (only for herbal drugs)	Family (only for herbal drugs)	Part used (only for herbal drugs)	Quantity
1	*Yashtimadhu*	*Glycyrrhiza glabra*	*Fabaceae*	Root	128g
2	*Punarnava*	*Boerhavia diffusa*	*Nyctaginaceae*	Whole plant	576g
3	*Godugdha*	-	-	-	768ml
4	*Goghrita*	-	-	-	768ml

Collection of Drugs

Punarnava and *Yastimadhu* were collected from authorized dealers situated in Thiruvananthapuram. Agmark *Goghritha* (cow's ghee) was purchased from authorized dealer. *Godugdha* (cow's milk) was purchased from a FSSAI licensed dairy unit.

Method of Preparation

Punarnavadi ghrita was a preparation made from *Yashtimadhu, Punarnava, Goghrita*, and *Godugdha*, as mentioned in Chakradatta. The *Ghrita* was prepared according to the general procedure of *Bhaishajya Kalpana*. All the raw drugs were thoroughly washed and dried in the shade. *Punarnava (Kwatha dravya)* was pulverized into a coarse powder, and 16 times water was added. This mixture was heated over a medium flame and reduced to one-fourth of its original volume. The mixture was then filtered through a muslin cloth to obtain *Punarnava kwatha*. Since one of the *Dravadravya* was *Kwatha*, one-sixth of the *Kalka dravya yashtimadhu* was taken, powdered, and passed through sieve number 85. A sufficient quantity of water was added to the *Kalka dravya* to prepare a homogeneous blend.

Ghrita was taken in a stainless-steel vessel and mildly heated. *Kalka* was added and stirred thoroughly while adding *Punarnava kwatha* in a specific ratio. This mixture was heated for 3 hours with constant stirring, maintaining the temperature between 50° and 90° during the first hour of heating. Milk was taken in the same quantity of *Ghrita*, It was added at the beginning of *Mridupaka* and boiled. The heating was stopped, and the mixture was allowed to stand overnight. The next day, heating was resumed, and the boiling mixture was observed for the subsidence of froth (*Phena santi*). The *Kalka* was constantly checked for the formation of *Varti (Madhyama paka lakshana)*. The *Varti* was exposed to flame to confirm the absence of a cracking sound, indicating the absence of moisture. The heating was stopped when the *Kalka* formed a *Varti* and the froth subsided. The mixture was then filtered while hot (about 80°) through a muslin cloth and allowed to cool. It was packed in a tightly closed glass container to protect it from light and moisture.

Analytical study

At CareKeralam, the physico-chemical analysis of *Punarnavadi Ghrita* followed a rigorous methodology, adhering to standard procedures outlined in the Ayurveda Pharmacopeia of India (API). The analysis encompassed a range of parameters to ensure a comprehensive evaluation. For physicochemical assessment, key parameters such as optical rotation (1% solution), iodine value, saponification value, specific gravity, rancidity test, refractive index, free fatty acid, peroxide value, acid value, and mineral oil were meticulously examined. Additionally, the phytochemical composition was scrutinized, including the presence of alkaloids, flavonoids, glycosides, saponins, phenol, carbohydrate, terpenoids, and tannins. To detect the phytoconstituents within the formulation, High-Performance Thin Layer Chromatography (HPTLC), test for specific pathogens, heavy metal, pesticide residue, microbial contamination, and aflatoxin analysis, was employed, providing insights into its chemical profile and therapeutic potential.

OBSERVATION AND RESULTS

Physico-chemical Properties

Table 1: Physico-chemical properties

S.no	Parameters	Result
1	Optical Rotation	+0.02
2	Iodine value	34.04
3	Saponification value	219.15
4	Rancidity test	Absent

5	Refractive index at 25°C	1.4590
6	Free fatty acid	1.66 %w/w
7	Peroxide value	17.22
8	Acid value	3.26
9	Specific gravity	0.9146
10	Mineral oil	Absent

Table 2: Organoleptic Evaluation

Colour	Odour	Taste	Texture
Yellow colour	Characteristic and pleasant odour	Sweet and bitter taste	Smooth, soft, and greasy texture

Phyto-chemical screening

Table 3: Phyto-chemical Properties

S.no	Parameters	Result
1	Alkaloids	Present
2	Flavonoids	Absent
3	Glycosides	Absent
4	Phenol	Present
5	Saponins	Absent
6	Tannins	Absent
7	Carbohydrate	Absent
8	Terpenoids	Present

HPTLC analysis

The observation and data obtained in the HPTLC analysis of a methanolic extract of *Punarnavadi ghrita* revealed 11 peaks in UV short of 254nm with max Rf value -0.01, 0.08, 0.14, 0.17, 0.24, 0.29, 0.36, 0.42, 0.49, 0.54, 0.91 in Track 1. In Track 2, 11 peaks were identified with max Rf value -0.02, 0.08, 0.14, 0.17, 0.24, 0.28, 0.36, 0.42, 0.49, 0.54, 0.91. In Track 3, 10 peaks are identified with max Rf value -0.02, 0.07, 0.13, 0.17, 0.29, 0.36, 0.42, 0.50, 0.54, 0.90.

Graph 1: Track 1 values in UV short of 254nm

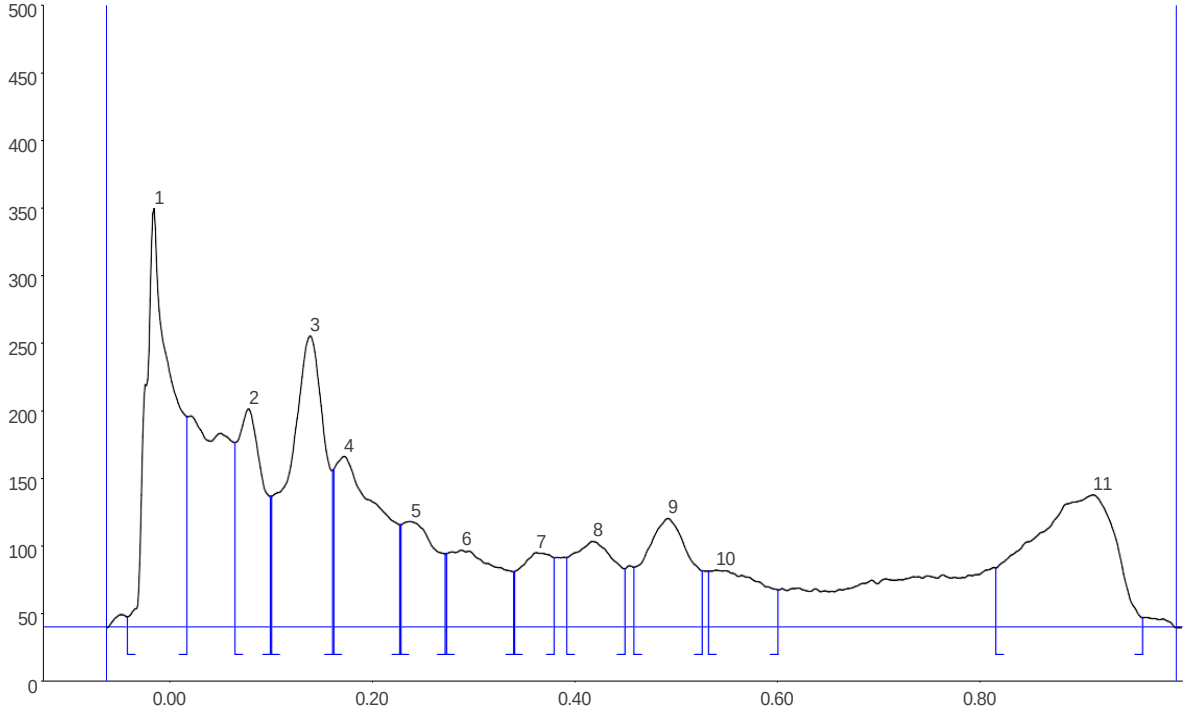


Table 4: Track 1 values in UV short of 254nm

Peak	Start Rf	Max Rf	End Rf	Area (AU)	Area %
1	-0.04	-0.01	0.02	7518.8	16.17
2	0.06	0.08	0.10	3862.5	8.31
3	0.10	0.14	0.16	7201.0	15.49
4	0.16	0.17	0.23	5286.8	11.37
5	0.23	0.24	0.27	2470.8	5.31
6	0.27	0.29	0.34	2706.6	5.82
7	0.34	0.36	0.38	1627.3	3.50
8	0.39	0.42	0.45	2602.5	5.60
9	0.46	0.49	0.53	3348.7	7.20
10	0.53	0.54	0.60	2063.8	4.44
11	0.82	0.91	0.96	7808.4	16.79

Graph 2: Track 2 values in UV short of 254nm

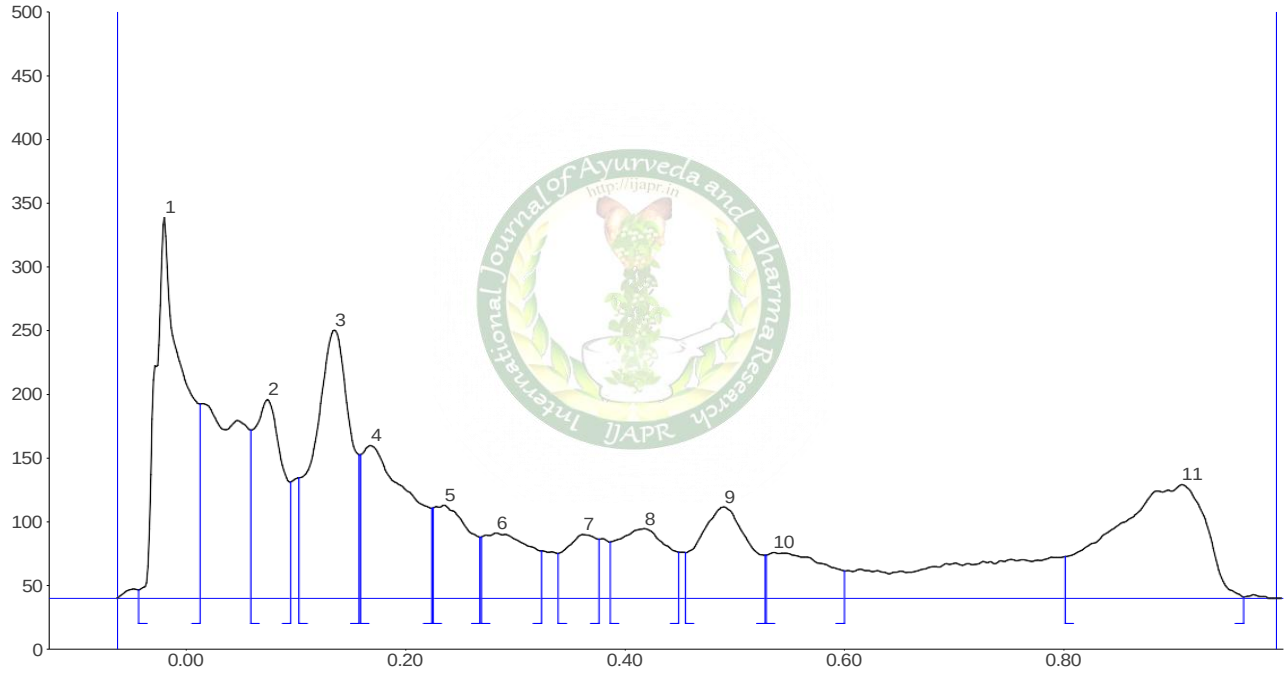


Table 5: Track 2 values in UV short of 254nm

Peak	Start Rf	Max Rf	End Rf	Area (AU)	Area%
1	-0.04	-0.02	0.01	7213.4	16.93
2	0.06	0.08	0.10	3863.0	9.07
3	0.10	0.14	0.16	6594.5	15.48
4	0.16	0.17	0.22	4976.6	11.68
5	0.23	0.24	0.27	2196.1	5.15
6	0.27	0.28	0.32	2063.1	4.84
7	0.34	0.36	0.38	1385.0	3.25
8	0.39	0.42	0.45	2404.4	5.64
9	0.46	0.49	0.53	3056.6	7.17
10	0.53	0.54	0.60	1766.5	4.15
11	0.80	0.91	0.96	7094.0	16.65

Graph 3: Track 3 values in UV short of 254nm

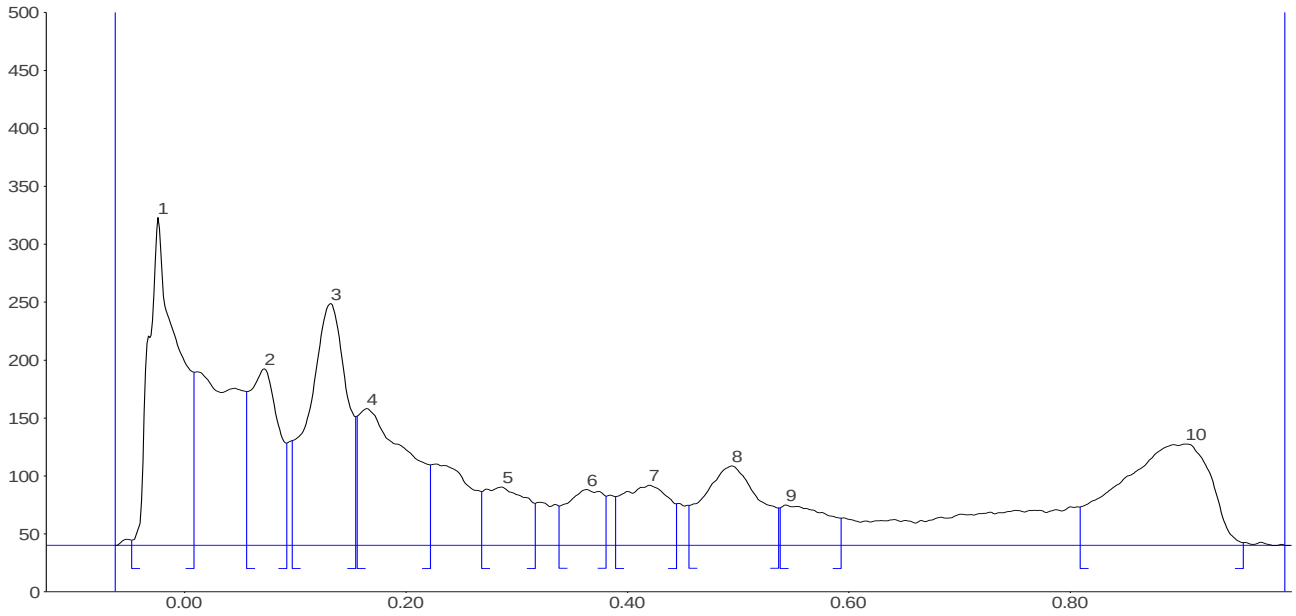


Table 6: Track 3 values in UV short of 254nm

Peak	Start Rf	Max Rf	End Rf	Area (AU)	Area%
1	-0.05	-0.02	0.01	7133.0	18.09
2	0.06	0.07	0.09	3828.9	9.71
3	0.10	0.13	0.16	6787.2	17.21
4	0.16	0.17	0.22	4994.3	12.66
5	0.27	0.29	0.32	1817.0	4.61
6	0.34	0.36	0.38	1503.9	3.81
7	0.39	0.42	0.44	2070.5	5.25
8	0.46	0.50	0.54	3215.7	8.15
9	0.54	0.54	0.59	1359.7	3.45
10	0.81	0.90	0.96	6727.7	17.06

In UV long of 366nm 12 peaks were obtained in Track 1 with max Rf value -0.02, 0.02, 0.06, 0.12, 0.16, 0.25, 0.29, 0.35, 0.38, 0.63, 0.85, 0.93. In track 2, 12 peaks were obtained with max Rf value -0.02, 0.02, 0.06, 0.12, 0.16, 0.25, 0.29, 0.35, 0.63, 0.84, 0.92, 0.95. In track 3, 11 peaks were obtained with max Rf value - 0.02, 0.01, 0.05, 0.11, 0.16, 0.25, 0.29, 0.35, 0.64, 0.84, 0.90.

Graph 4: Track 1 values in UV short of 366nm

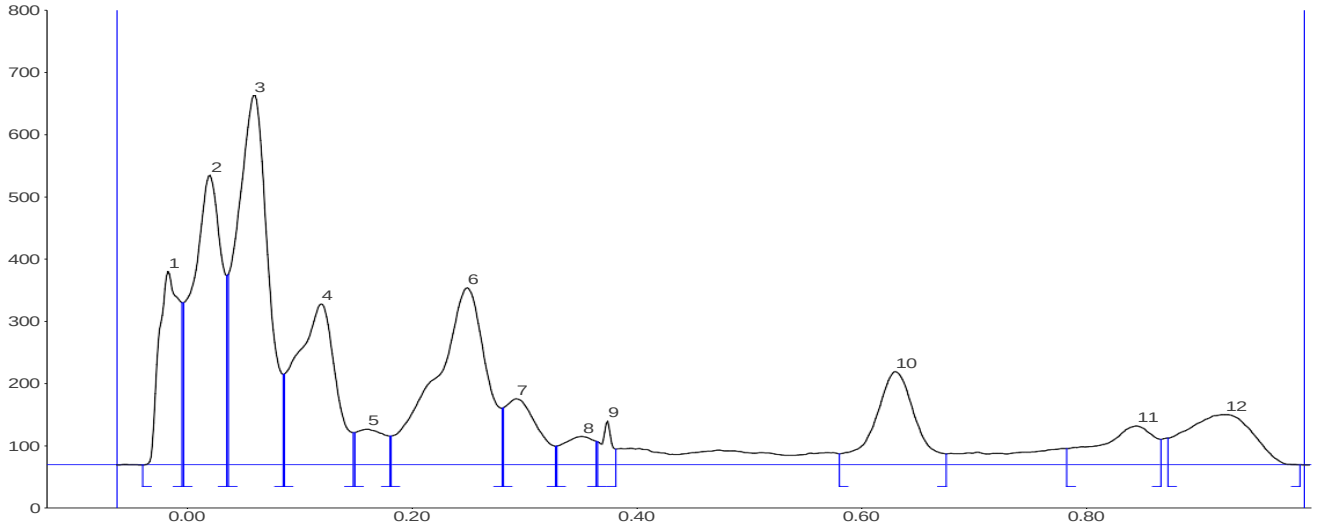


Table 7: Track 1 values in UV long of 366nm

Peak	Start Rf	Max Rf	End Rf	Area (AU)	Area%
1	-0.04	-0.02	-0.00	5193.9	7.33
2	-0.00	0.02	0.04	11351.9	16.02
3	0.04	0.06	0.09	15499.6	21.88
4	0.09	0.12	0.15	8385.8	11.84
5	0.15	0.16	0.18	1384.8	1.95
6	0.18	0.25	0.28	11973.1	16.90
7	0.28	0.29	0.33	2840.1	4.01
8	0.33	0.35	0.37	1166.2	1.65
9	0.37	0.38	0.38	596.9	0.84
10	0.58	0.63	0.68	5014.3	7.08
11	0.78	0.85	0.87	2852.7	4.03
12	0.87	0.93	0.99	4579.2	6.46

Graph 5: Track 2 values in UV short of 366 nm

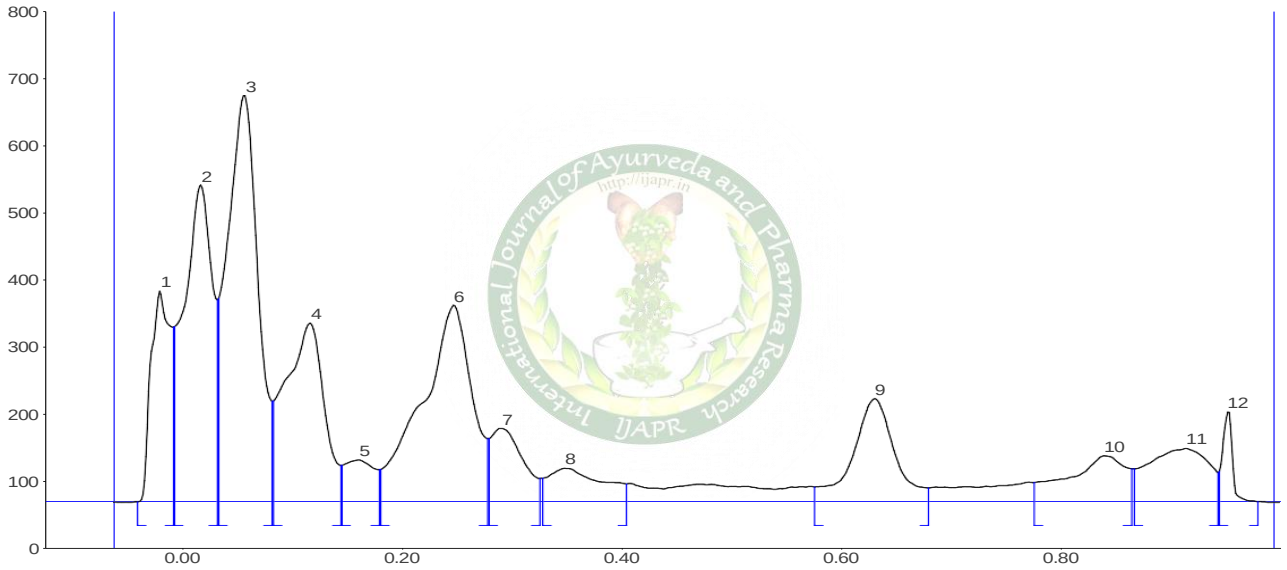


Table 8: Track 2 values in UV long of 366 nm

Peak	Start Rf	Max Rf	End Rf	Area (AU)	Area%
1	-0.04	-0.02	-0.01	5215.6	6.99
2	-0.01	0.02	0.03	11404.8	15.29
3	0.03	0.06	0.08	15811.8	21.20
4	0.08	0.12	0.14	8742.3	11.72
5	0.15	0.16	0.18	1604.2	2.15
6	0.18	0.25	0.28	12393.5	16.62
7	0.28	0.29	0.33	3005.1	4.03
8	0.33	0.35	0.41	2343.7	3.14
9	0.58	0.63	0.68	5463.9	7.33
10	0.78	0.84	0.87	3362.8	4.51
11	0.87	0.92	0.94	4094.4	5.49
12	0.94	0.95	0.98	1132.8	1.52

Graph 6: Track 3 values in UV short of 366 nm

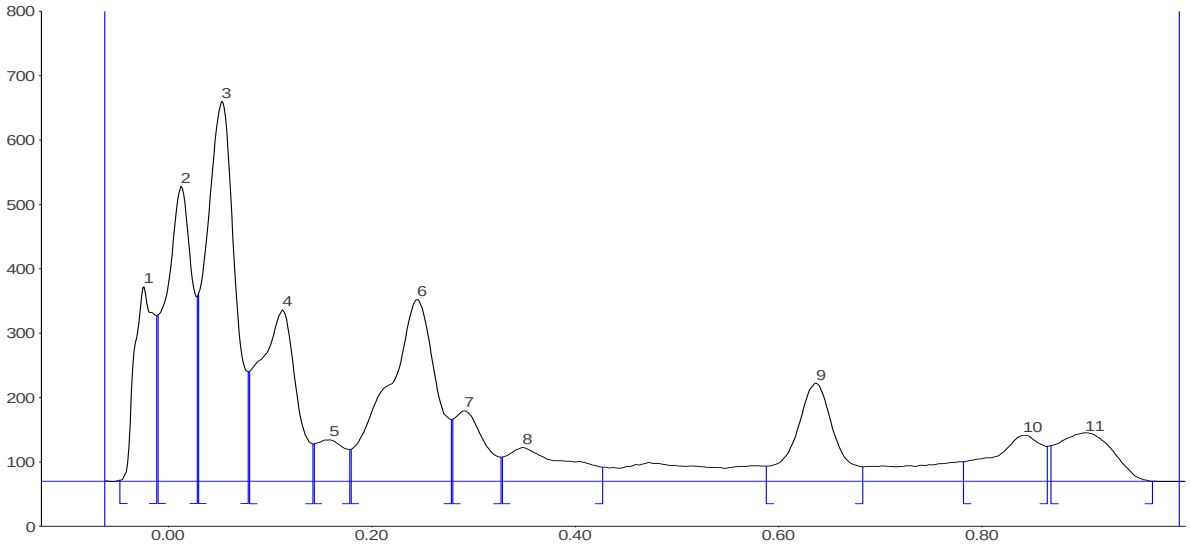
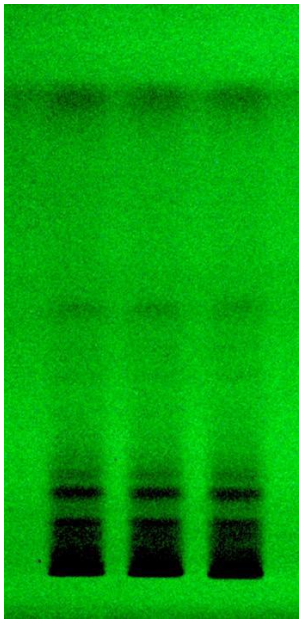


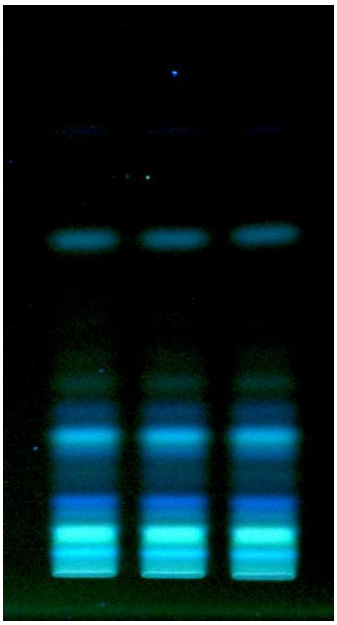
Table 9: Track 3 values in UV long of 366nm

Peak	Start Rf	Max Rf	End Rf	Area (AU)	Area%
1	-0.05	-0.02	-0.01	5467.2	7.39
2	-0.01	0.01	0.03	11143.5	15.07
3	0.03	0.05	0.08	15609.0	21.10
4	0.08	0.11	0.14	8998.8	12.17
5	0.14	0.16	0.18	1711.8	2.31
6	0.18	0.25	0.28	12569.4	16.99
7	0.28	0.29	0.33	3037.9	4.11
8	0.33	0.35	0.43	2916.5	3.94
9	0.59	0.64	0.68	5396.2	7.30
10	0.78	0.84	0.87	3333.4	4.51
11	0.87	0.90	0.97	3783.7	5.12

Figure 1: HPTLC photo documentation of the sample of *Punarnavadi ghrita*



Short UV



Long UV

Test for heavy metals

Table 10: Test for heavy metals

S.no	Parameters	Result
1	Arsenic	BDL
2	Cadmium	BDL
3	Lead	0.41 ppm
4	Mercury	Not detected

Test for pesticide residue

Table 11: Test for pesticide residue

S.no	Parameters	Result
1	Alanchlor	Not Detected
2	Aldrin and Dieldrin	Not Detected
3	Azinphos-methyl	Not Detected
4	Bromopropylate	Not Detected
5	Chlordane	Not Detected
6	Chlorfenvinphos	Not Detected
7	Chlorpyrifos	Not Detected
8	Chlorpyrifos Methyl	Not Detected
9	Cypermethrin	Not Detected
10	DDT	Not Detected
11	Deltamethrin	Not Detected
12	Diazinon	Not Detected
13	Dichlorvos	Not Detected
14	Dithiocarbamates	Not Detected
15	Endosulfan	Not Detected
16	Endrin	Not Detected
17	Ethion	Not Detected
18	Fenitrothion	Not Detected
19	Fenvalerate	Not Detected
20	Fonofos	Not Detected
21	Heptachlor	Not Detected
22	Hexachloro Benzene	Not Detected
23	Hexachlorocyclohexane isomers	Not Detected
24	Lindane	Not Detected
25	Malathion	Not Detected
26	Methidathion	Not Detected
27	Parathion	Not Detected
28	Parathion methyl	Not Detected
29	Permethrin	Not Detected
30	Phosalone	Not Detected
31	Pineronyl butoxide	Not Detected
32	Pirimiphos-methyl	Not Detected

33	Pyrethrins	Not Detected
34	Quintozene	Not Detected

Test for microbial contamination

Table 12: Test for microbial contamination

S.no	Parameter	Result
1	Total plate count for bacteria	25 CFU/ml
2	Total Yeast and Mold Count	<10 CFU/ml
3	Enterobacteriaceae	<10 CFU/ml

Test for specific pathogens

Table 13: Test for specific pathogens

S.no	Parameters	Result
1	E.coli	Absent
2	Pseudomonas aeruginosa	Absent
3	Salmonella sp.	Absent
4	Staphylococcus aureus	Absent

Test for Aflatoxins

Table 14: Test for Aflatoxins

S.no	Parameters	Result
1	B1	Not detected
2	B2	Not detected
3	G1	Not detected
4	G2	Not detected

DISCUSSION

The present formulation was created using two plant ingredients, whose authenticity was confirmed through organoleptic assessment, which involves evaluating the ingredients based on their sensory properties such as appearance, colour, odour, and taste. To ensure the quality and consistency of the formulation, various physiochemical parameters and phytochemical parameters (such as the presence of specific active compounds) were analysed. High-Performance Thin-Layer Chromatography (HPTLC) was employed to further investigate the formulation. The densitometric analysis at wavelengths of 254nm and 366nm revealed 11 distinct peaks, indicating the presence and concentration of several active compounds. These findings establish a reliable reference standard for future research, providing a baseline for comparison and validation of similar formulations.

Further quality control testing showed the formulation to be free from harmful heavy metals. Arsenic, cadmium, and mercury were not detected, and lead was found at 0.41 ppm, well within permissible limits. Pesticide residue analysis also confirmed the absence of harmful agricultural chemicals.

Microbial contamination was minimal, with a total bacterial count of 25 CFU/ml and yeast and mold count under 10CFU/ml. No Enterobacteriaceae were detected. Importantly, the formulation was free from pathogenic microorganisms such as *E. coli*, *Salmonella*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*. The aflatoxin analysis further validated the safety of the formulation, with no detectable levels of aflatoxin B1, B2, G1, or G2.

CONCLUSION

This comprehensive analysis confirms the high quality, safety, and consistency of the formulation. The combination of organoleptic, physicochemical, and phytochemical evaluations, along with advanced chromatographic techniques, establishes this formulation as a standard for future research. The absence of toxic elements, harmful pesticides, and pathogens, along with the confirmation of active compounds, ensures the formulation's suitability for therapeutic use. These findings provide a solid foundation for the validation of similar Ayurvedic formulations, ensuring their authenticity and safety for clinical application.

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