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QUALITY CHARACTERIZATION AND HPTLC FINGERPRINTING OF *HINGWADI CHOORNA*: A POLYHERBAL FORMULATION

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ABSTRACT

Ayurveda is known as the oldest healing science. Rising use of herbal drugs by the human is forcing the driving force to evaluate the health claim of these agents and to develop standards for a quality, purity, safety and efficacy of the drug. The present study was conducted with the aim to evaluate purity and strength of *Hingwadi choorna*. The prepared polyherbal formulation was subjected to determine the physical constants (pH determination, refractive index, specific gravity, total solids, reducing and non-reducing sugar) and HPTLC fingerprinting. The analysis revealed the physico-chemical constant such as loss on drying 10.98%, total ash 15.32%, acid insoluble ash 1.4%, water soluble ash 12.8%, alcohol soluble extractive value 16.64% and water soluble extractive value 43.90%. HPTLC finger printing profile showed different band patterns at different wavelength under short UV, long UV and at 254nm, 366nm, and 620nm after derivatisation with vanillin sulphuric acid spraying reagent. Unique R_f patterns were recorded. *Hingwadi choorna* was authenticated according to pharmacopeial standards as its analysis was important to ensure the purity and strength of drug.

Keywords: Purity and Strength, *Hingwadi choorna*, HPTLC fingerprinting, physical constants

INTRODUCTION

Respiratory tract infections accounts about more than 50% of patients attending pediatric OPD in developing and even developed countries worldwide[1, 2]. Use of Ayurvedic drugs in the form of herbomineral formulation has been increased all over the world due to their huge therapeutic effect and less adverse effect as compared to other medicines. A polyherbal

Ayurvedic formulation *Hingwadi choorna* which is known *Swasahara yoga* was prepared using different drugs including *Ferula foetidaLinn, Black salt, Rubia cordifoliaLinn, Piper longumLinn, Sida cordifoliaLinn, Zizyphus jujubeLam* and *Citrus medica*. Quality assurance of Ayurvedic formulations is an important step to study physico-chemical profile and safety of drug, as the standardization of drug is emerging aspect in Ayurvedic drug preparation. Leading population of world now relies on phytotherapy for health care as per World Health Organization (WHO) [3, 4]. WHO consider phytotherapy is safe, cost effective and more significant without any side effect[5]. Ayurvedic Pharmacopoeia of India and AYUSH also provides monograph on the preparation of Ayurvedic formulation that aided in standardization of drug. To fulfill the requirement of the emerging trend, the prepared polyherbal formulation *Hingwadi choorna* was subjected to determine the physical constants (pH determination, refractive index, specific gravity, total solids, reducing and non-reducingsugar) and HPTLC fingerprinting as per pharmacopeial procedures.

MATERIAL AND METHODS

Collection of raw drugs

Raw drugs were purchased from SDM Ayurveda Pharmacy Kuthpady, Udupi, Karnataka for preparation of *Hnigwadi choorna*. The syrup was standardized and authenticated at Pharmacognosy department of SDM Center for Research in Ayurveda and Allied Sciences, Udupi, Kuthpady, Karnataka and a specimen (845/16122101) is being maintained for future reference. The prepared *choorna* was subjected for characterization and HPTLC finger printing.

Preparation of *choorna*

Hingwadi choorna was prepared with different drugs which are mentioned in table number 1. The drugs were identified morphologically with the description given in Quality standard of Indian medical plants. Seven *bhavans* with *matulunga swarasa* was given to this powdered herb and was subjected for authentication.

Physical evaluation

Determination of physical evaluation includes loss on drying at 105^{o}C, total ash, acid insoluble ash, water soluble ash, alcohol soluble extractive, water soluble extractive, microbial load evaluation and successive extractive values by Soxhlet extraction method, were carried out as procedures mentioned in Ayurveda Pharmacopoeia of India[6].

HPTLC finger printing

Sample preparation

1g of *Hingwadi choorna* powder was extracted with 10 ml of ethanol (90%) and filtered. The filtrate was then made up to 10.0ml in separate standard flask.

Mobile phase

The solvent system containing Toluene: Ethyl acetate (9.0:1.0) gave optimum separation in alcohol extract and hence was used for HPTLC study.

Method

3,6 and 9µl were applied on aluminum plates precoated with gel 60 F_{254} of 0.2mm thickness (Merck, Darmstadt, Germany) using a CAMAG LINOMANT 5 applicator[7].The plates were developed in the CAMAG glass twin trough chamber previously saturated with the mobile phase. The plate was derivatized using vanillin sulfuric acid (VS) and heated at 105C till the spots appeared[8, 9].The developed plates were visualized in the CAMAG visualizing

chamber and scanned using CAMAG SCANNER 4 at 254 nm, 366 nm and 610 nm (post derivatization with VS) with the help of CAMAG WinCATS software. Rf values and densitograms were recorded.

RESULTS AND DISCUSSION

Physical invariant

Physical evaluation of *Hingwadi choorna* for the standardization was determined according to AYUSH protocols which are mentioned in the Table number 2.

HPTLC fingerprinting

Rf values and color of the spots in chromatogram developed in Toluene: Ethyl acetate (9.0:1.0 v/v) for *Hingwadi choorna* were recorded in a Table number 3. TLC photo-documentation unveiled the presence of many phyto constituents with different Rf values. HPTLC densitometric scan of the plates showed numerous bands under short UV, long UV and 620 nm (after derivatisation).On photo documentation 9 spots under short UV, 13 spots under long UV and 16 spots under white light following derivatisation with vanillin-sulphuric acid spray reagent (Table 3,Fig: 1).Rf values by densitometric scan at 254nm showed a total of 11peaks of which Rf of 0.16(29.69%) and 0.37(27.19%) were prominent, at 366nm it showed a total of 10peaks of which Rf of 0.16(66.24%) was prominent, at 620nm it showed 13 peaks of which 0.03(14.40%) was the major peak (Fig 2a-2c).

DISCUSSION

Standardization is the process of developing and agreeing upon technical standards. Physico- chemical parameters such as loss on drying which reveals the moisture content 10.98%, total ash indicating total inorganic content was found to be 15.32%, acid insoluble ash refers to that part of acid insoluble portion of total ash mainly silica 1.49%, water soluble ash refers to inorganic content without water insoluble inorganic salts like silica 12.8%, alcohol soluble extractive value shows percentage of active constituents soluble in alcohol 16.64%, water soluble extractive value shows percentage of active constituents soluble in water 43.90%. These physico-chemical standardization parameters are conducted to ascertain the standards for processed formulations. HPTLC fingerprinting also revealed active constituents at different R_f values. Standardization of *Hingwadi choorna* reveals compliance with all the above discussed parameters and hence it can be concluded that *Hingwadi choorna* is a well standardized product at essential pharmacopeial parameters. The results obtained from the study could be utilized as a reference for setting limits.

CONCLUSION

For the quality assured Ayurvedic products, the physico- chemical standardization is of utmost importance including standardization by HPTLC for qualitative identification of active compounds in the formulation. HPTLC fingerprinting profile of *Hingwadi choorna* has disclosed many active constituents at different R_f values. The set of data obtained in the present investigation can serve as standard for the identification of *choorna*. The results will be helpful in sustaining and reproducibility of drug as well as to ensure the quality and safety of drug.

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Table 1: Different parts of drugs used in formulation

Name of drugs	Used part	Quantity
Ferula foetida Linn.	Latex	4 *ratti*(500mg)
Black salt	Crystal	1 *masha*(1gm)
Rubia cardifolia Linn.	Root	1 *masha*(1gm)
Piper longum Linn.	Fruit	1 *masha*(1gm)
Sida cardifolia Linn.	Root	1 *masha*(1gm)
Zizyphus jujube Lam.	Fruit	1 *masha*(1gm)

Table 2: Results of standardization parameters of *Hingwadi choorna*

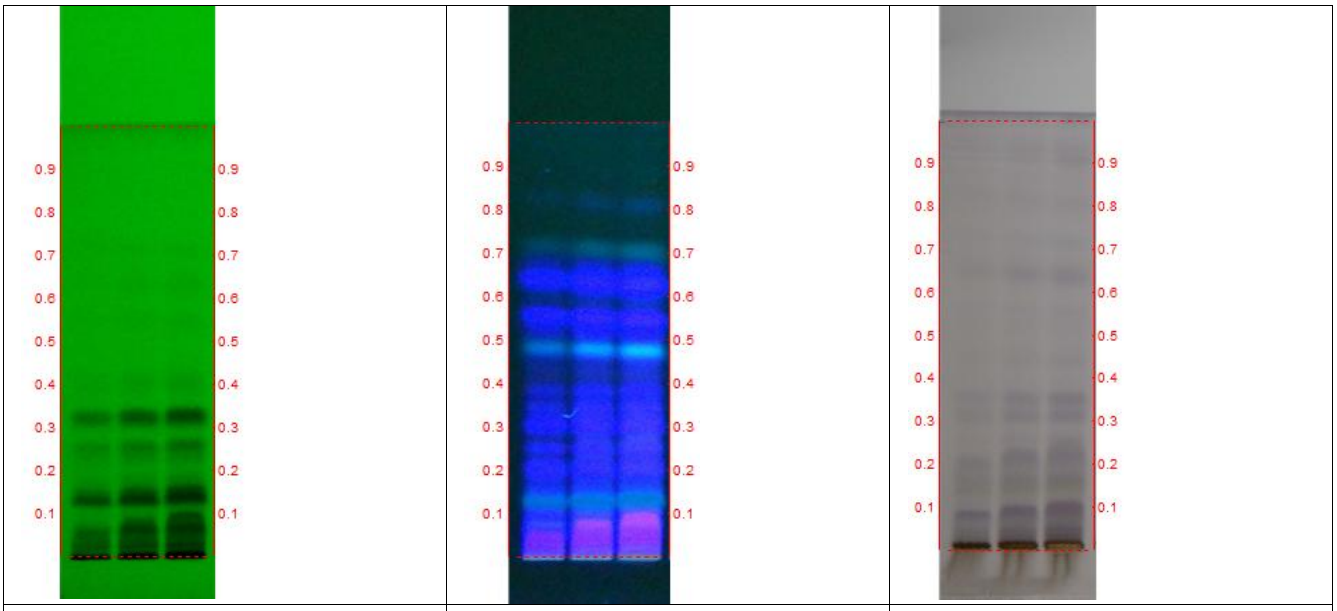
Parameter	Results n = 3 %w/w
Loss on drying	10.98
Total Ash	15.32
Acid Insoluble Ash	1.49
Water soluble Ash	12.8
Alcohol soluble extractive value	16.64
Water soluble extractive value	43.90

Table 3: R_f values of samples

Short UV	Long UV	Post derivatisation
0.04 (D. green)	0.04 (F. blue)	0.04 (Purple)
0.08 (D. green)	0.08 (F. blue)	0.08 (Purple)
0.10 (D. green)	-	-
0.14 (D. green)	0.14 (F. blue)	0.14 (Purple)
-	-	0.17 (Purple)
-	0.21 (F. blue)	0.21 (Purple)
-	0.25 (F. blue)	0.25 (Purple)
0.27 (D. green)	-	-
-	0.31 (F. blue)	0.31 (Purple)
0.33 (D. green)	-	-
-	0.35 (F. blue)	0.35 (Purple)
-	0.39 (F. blue)	-
0.41 (L. green)	-	-
-	-	0.45 (Purple)
-	0.48 (F. blue)	-
-	-	0.52 (Purple)
0.56 (L. green)	0.56 (F. blue)	0.56 (Purple)
0.63 (L. green)	-	-
-	0.65 (F. blue)	0.65 (Purple)
0.71 (L. green)	0.71 (F. blue)	0.71 (Purple)
-	0.81 (F. blue)	0.81 (Purple)
-	-	0.91 (Purple)
-	-	0.94 (Purple)

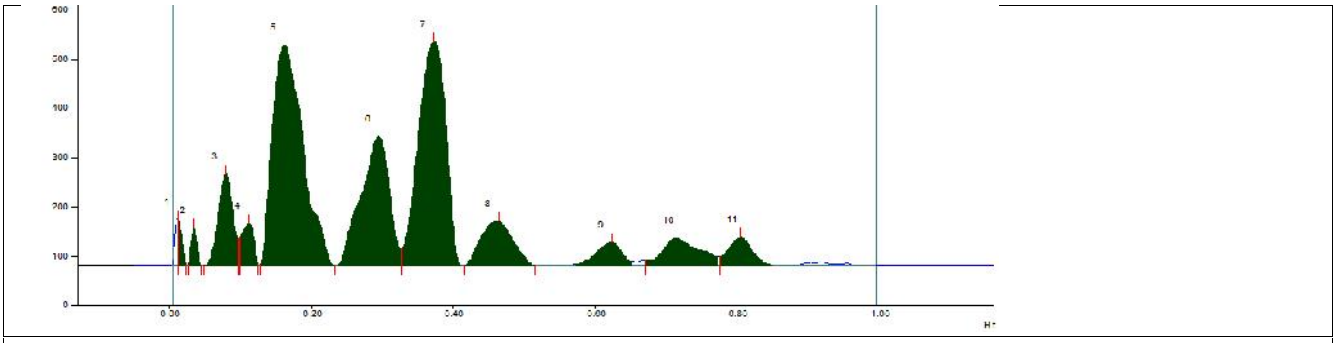
*F – Fluorescent; L –Light; D – Dark

Figure 1. HPTLC photo documentation of ethanol extract of *Hingwadi choorna* at short UV, long UV and post derivatisation under white light. Solvent system – Toluene: Ethyl Acetate (9.0: 1.0), Track 1: 3µl; Track 2: 6µl, Track 3: 9µl



Short UV	Long UV	Post derivatisation

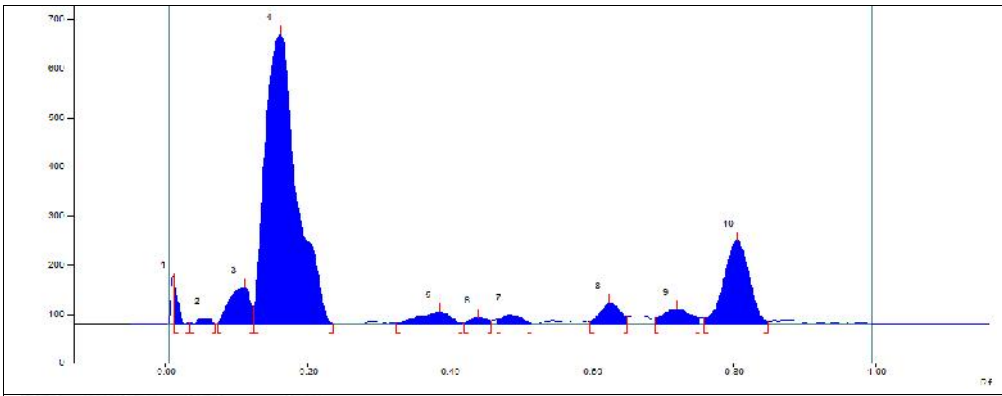
Figure 2. Densitometric scan of *Hingwadi choorna*



Track 3, ID: Hingwadi choorna

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.01 Rf	95.3 AU	0.01 Rf	95.3 AU	5.09 %	0.02 Rf	1.4 AU	441.7 AU	0.95 %
2	0.03 Rf	0.1 AU	0.04 Rf	78.6 AU	4.19 %	0.05 Rf	1.1 AU	457.5 AU	0.98 %
3	0.05 Rf	0.2 AU	0.08 Rf	188.1 AU	10.04 %	0.10 Rf	58.2 AU	2776.9 AU	5.95 %
4	0.10 Rf	58.7 AU	0.11 Rf	86.5 AU	4.62 %	0.13 Rf	4.1 AU	1068.0 AU	2.29 %
5	0.13 Rf	1.2 AU	0.16 Rf	449.3 AU	23.98 %	0.23 Rf	0.1 AU	13849.4 AU	29.69 %
6	0.24 Rf	1.1 AU	0.30 Rf	264.1 AU	14.09 %	0.33 Rf	34.4 AU	7553.6 AU	16.19 %
7	0.33 Rf	35.4 AU	0.37 Rf	456.2 AU	24.34 %	0.41 Rf	0.7 AU	12687.0 AU	27.19 %
8	0.42 Rf	0.1 AU	0.46 Rf	90.9 AU	4.85 %	0.52 Rf	0.8 AU	2983.7 AU	6.40 %
9	0.57 Rf	3.5 AU	0.62 Rf	49.2 AU	2.63 %	0.65 Rf	8.2 AU	1268.9 AU	2.72 %
10	0.67 Rf	10.6 AU	0.72 Rf	57.1 AU	3.05 %	0.77 Rf	18.5 AU	2137.6 AU	4.58 %
11	0.78 Rf	19.0 AU	0.81 Rf	58.6 AU	3.13 %	0.85 Rf	2.2 AU	1428.9 AU	3.06 %

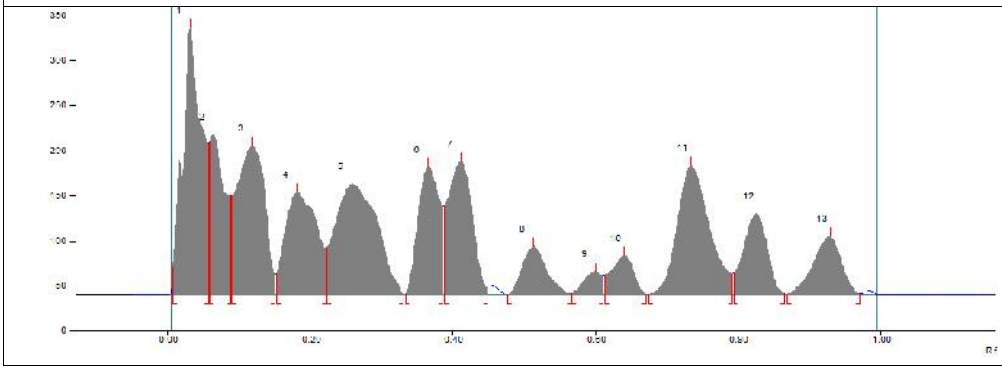
Fig 2a. At 254nm



Track 3, ID: Hingwadi choorna

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.01 Rf	85.4 AU	0.01 Rf	85.4 AU	8.06 %	0.03 Rf	3.0 AU	349.1 AU	1.24 %
2	0.04 Rf	2.5 AU	0.06 Rf	11.4 AU	1.08 %	0.07 Rf	0.0 AU	147.5 AU	0.52 %
3	0.07 Rf	1.2 AU	0.11 Rf	75.2 AU	7.10 %	0.12 Rf	27.6 AU	1559.4 AU	5.54 %
4	0.13 Rf	33.6 AU	0.16 Rf	589.7 AU	55.65 %	0.24 Rf	0.9 AU	18631.5 AU	66.24 %
5	0.33 Rf	1.4 AU	0.39 Rf	24.2 AU	2.29 %	0.42 Rf	1.0 AU	759.4 AU	2.70 %
6	0.42 Rf	1.7 AU	0.44 Rf	13.1 AU	1.23 %	0.46 Rf	6.5 AU	231.8 AU	0.82 %
7	0.47 Rf	9.1 AU	0.49 Rf	18.3 AU	1.73 %	0.52 Rf	0.2 AU	369.9 AU	1.32 %
8	0.60 Rf	5.3 AU	0.63 Rf	42.1 AU	3.97 %	0.65 Rf	15.2 AU	867.0 AU	3.08 %
9	0.69 Rf	12.3 AU	0.72 Rf	30.5 AU	2.87 %	0.75 Rf	12.3 AU	868.2 AU	3.09 %
10	0.76 Rf	12.2 AU	0.81 Rf	169.7 AU	16.01 %	0.85 Rf	5.7 AU	4344.2 AU	15.44 %

Fig 2b. At 366nm



Track 3, ID: Hingwadi choorna

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.01 Rf	29.5 AU	0.03 Rf	296.7 AU	18.71 %	0.06 Rf	67.6 AU	5721.3 AU	14.40 %
2	0.06 Rf	169.4 AU	0.06 Rf	177.7 AU	11.21 %	0.09 Rf	09.1 AU	2677.7 AU	6.74 %
3	0.09 Rf	109.1 AU	0.12 Rf	165.1 AU	10.42 %	0.15 Rf	21.3 AU	4673.7 AU	11.76 %
4	0.15 Rf	22.3 AU	0.18 Rf	114.0 AU	7.19 %	0.22 Rf	50.9 AU	3580.0 AU	9.01 %
5	0.22 Rf	51.8 AU	0.26 Rf	122.5 AU	7.73 %	0.33 Rf	0.8 AU	5145.6 AU	12.95 %
6	0.34 Rf	1.3 AU	0.37 Rf	141.7 AU	8.93 %	0.39 Rf	98.5 AU	3038.5 AU	7.65 %
7	0.39 Rf	98.6 AU	0.41 Rf	147.5 AU	9.31 %	0.45 Rf	8.8 AU	3605.8 AU	9.07 %
8	0.48 Rf	0.0 AU	0.51 Rf	53.6 AU	3.38 %	0.57 Rf	1.0 AU	1301.7 AU	3.28 %
9	0.57 Rf	1.2 AU	0.60 Rf	25.6 AU	1.61 %	0.61 Rf	21.7 AU	454.6 AU	1.14 %
10	0.61 Rf	21.3 AU	0.64 Rf	43.5 AU	2.75 %	0.67 Rf	0.2 AU	950.3 AU	2.39 %
11	0.68 Rf	0.1 AU	0.74 Rf	143.1 AU	9.03 %	0.79 Rf	22.8 AU	4642.6 AU	11.68 %
12	0.80 Rf	23.3 AU	0.83 Rf	89.5 AU	5.64 %	0.87 Rf	0.5 AU	2125.2 AU	5.35 %
13	0.87 Rf	0.3 AU	0.93 Rf	65.0 AU	4.10 %	0.97 Rf	0.9 AU	1825.8 AU	4.59 %

Fig 2c. After derivatisation (At 620nm)

Source of Support: Nil
Conflict Of Interest: None Declared

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