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Original article

# Quality standards for Hutabhugādi cūrṇa (Ayurvedic Formulary of India)

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ABSTRACT

In India, herbal medicines are mainly based on the Ayurvedic system. The main drawback of traditional medicines is a lack of standardized products. Standardization of any herbal formulation is essential in order to assess the quality, purity, safety, and efficacy of drugs based on the analysis of their active properties. Testing of Ayurvedic preparations using scientific methodologies will add to quality and authenticity of the product. This article reports standardization parameters for Hutabhugādi cūrṇa (HC) used traditionally in the treatment of Agnimāndya (digestive impairment), Pāndu (anemia), Sopha (edema), and Ārsa (piles). The formulation was prepared as per Ayurvedic Formulary of India, and it was standardized by organoleptic characterization, macro–microscopic evaluation, physicochemical testing, and thin-layer chromatography/high-performance thin-layer chromatography profiling employing a standard methodology. Results of the experiments conducted provided diagnostic characteristics to identify and standardize the formulation prepared using official ingredients of HC. Based on the data obtained, a monograph on quality standards for HC is proposed. The monograph based on the present investigation results would serve as a document to control the quality of HC.
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## 1. Introduction

Many modern medicines are directly or indirectly derived from higher plants.[1] All medicines, whether synthetic or of plant origin, should fulfill the basic requirements of being safe and effective.[2,5] Standardization of herbal medicines is the process of prescribing a set of standards or inherent characteristics, constant parameters, and definitive qualitative and quantitative values that carry an assurance of quality, efficacy, safety, and reproducibility. Quality of raw materials, good agricultural practices, and good manufacturing practices play fundamental roles in guaranteeing the quality and stability of herbal preparations.[3] Specific standards are worked out by experimentation and observations, which would lead to the process of prescribing a set of characteristics exhibited by the particular herbal medicine. Hence, standardization is a tool used in the quality control process.[4]

Several problems, which are not applicable to synthetic drugs, often influence the quality of herbal drugs. Regulatory authorities must ensure that consumers get pure, safe, potent, and efficacious medicines, which are prepared by rigidly following various quality standards prescribed for raw materials and finished products. These procedures would logically apply to all types of modern and traditional medications.

It is common to have many plant ingredients in a single herbal formulation. Due to the complex nature and variability of the constituents, herbal preparations are likely to have variations right from the stage of collection of raw materials. In the past, due to the absence of a standard reference for identification, it was difficult to establish the quality control measures for polyherbal formulations. However, nowadays, efforts have been made so that herbal preparations comply with the consistent standards through modern analytical techniques.

Among Ayurvedic preparations, Hutabhugādi cūrṇa (HC) is prescribed for diseases such as Agnimāndya (digestive impairment), Pāndu (anemia), Sopha (edema), and Ārsa (piles).[6] In the present study, HC was subjected to organoleptic, macro–microscopic, physicochemical, and high-performance thin-layer chromatography (HPTLC) characterizations. HC was prepared using the following ingredients: Hutabhuga (*Plumbago zeylanica*),

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Ajamoda (*Apium leptophyllum*), Saindhava lavaṇa (rock salt), Magadha (*Piper longum*), Marica (*Piper nigrum*), and Pathya (*Terminalia chebula*), as per the standard method of preparation of cūrṇa; this work has been taken up with the objective of contributing to herbal pharmacopeias by deriving consistent standards, proposing rapid authentication fingerprints for the selected phytomedicine, and preparing a concise monograph on the quality.

## 2. Materials and methods

### 2.1. Collection and identification of plant samples

Dry raw samples required for the study were collected from the raw drug section of SDM Ayurveda pharmacy, Udupi. The samples were authenticated using macro–microscopic examination; voucher specimens (No. SDM/UGC-MRP/HC/01-06) have been deposited in the crude drug museum of Pharmacognosy Department of SDMCRAAS, Udupi.

### 2.2. Preparation of HC

HC was prepared following the procedure detailed in Ayurvedic Pharmacopoeia of India (API).[7] All the ingredients except Saindhava lavaṇa were washed properly so that there was no microbial load.[8] The washed and dried raw drugs of pharmacopeial quality were finely powdered. Saindhava lavaṇa was roasted in a stainless steel pan on low flame till free from moisture and was then powdered. The individual raw drug powders were passed separately through a

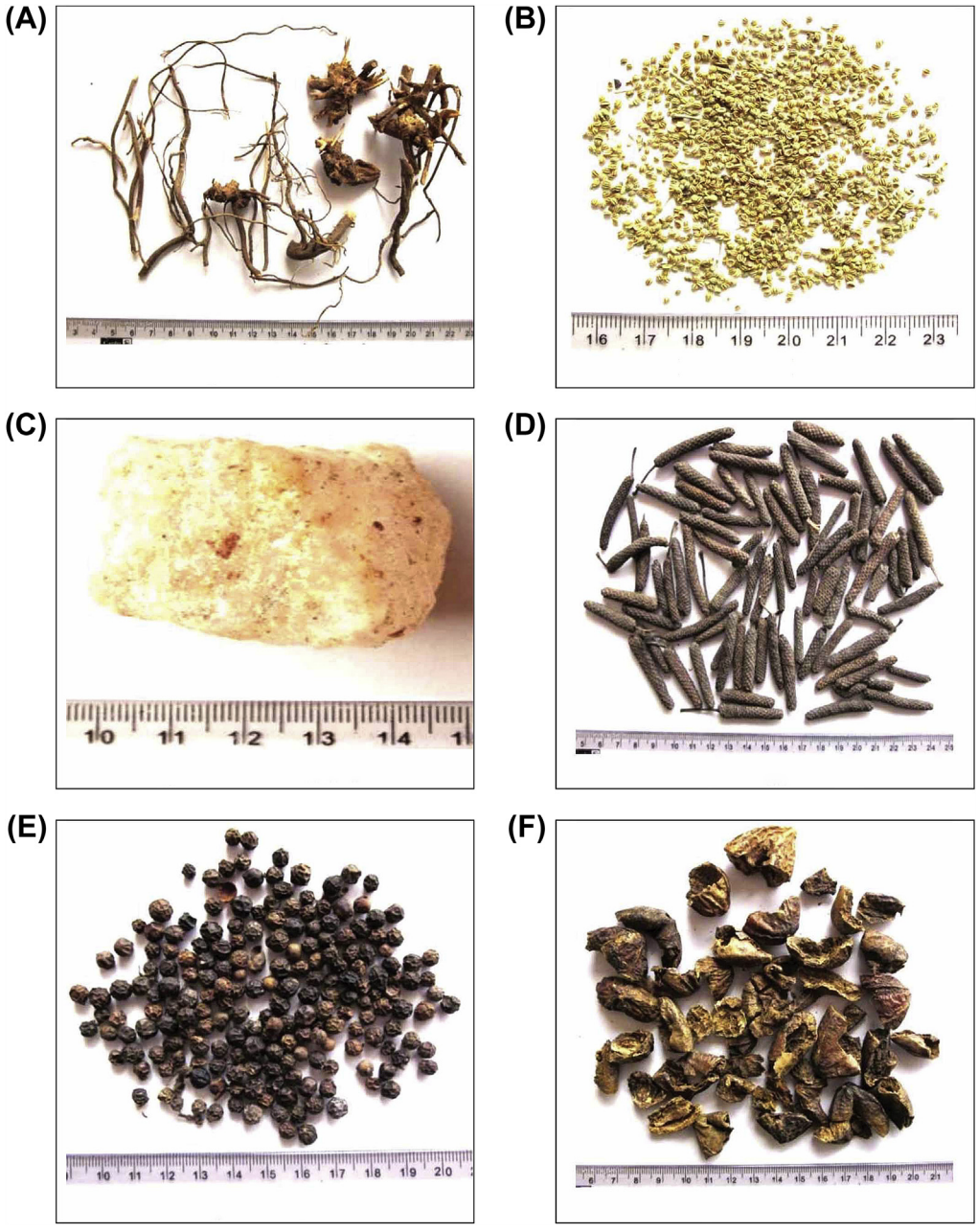



**Fig. 1.** Raw drugs used in Hutabhugādi cūrṇa: (A) Citraka, (B) Ajamoda, (C) Saindhava lavaṇa, (D) Pippali, (E) Marica, and (F) Haritaki.

sieve (number 44), followed by another (number 85). Each ingredient was weighed separately and mixed together in the proportion specified; the mixture was passed through sieve number 44 to obtain a homogenous blend and packed in an air-tight container.[7] One kilogram of the formulation was prepared at the laboratory using standardized ingredients. For detection of possible substitution, another set of the formulation was similarly prepared with *Trachyspermum ammi* (Linn.) Sprague ex Turril (Yavāni API)—a common substituent of Ajamoda [*A. leptophyllum* (Pers.) F. V. M. ex Benth.].

Organoleptic examination, macro–microscopy, and physicochemical studies, viz., total ash, water-soluble ash, acid-insoluble ash, water- and alcohol-soluble extractive, loss on drying at 105°C, pH, microbial load evaluation, and successive extractive values by Soxhlet extraction method, were carried out as per the standard procedures mentioned in Ayurveda Pharmacopoeia of India.[9]

### 2.3. Thin-layer chromatography/HPTLC

#### 2.3.1. Sample preparation

*2.3.1.1. Ingredients.* Ingredients (each 1 g) were extracted with 10 mL of ethanol (90%) and filtered. The filtrates were made up to 10 mL in separate standard flasks.

*2.3.1.2. Formulation.* HC (5 g) was successively extracted with 150 mL of chloroform and ethanol using a Soxhlet apparatus. The filtrates were made up to 10 mL of solvent in a standard flask.

#### 2.3.2. Mobile phase

The solvent system containing toluene:ethyl acetate:formic acid (10:5:1) gave optimum separation for chloroform extract and, hence, was used for the HPTLC study. The comparative fingerprint

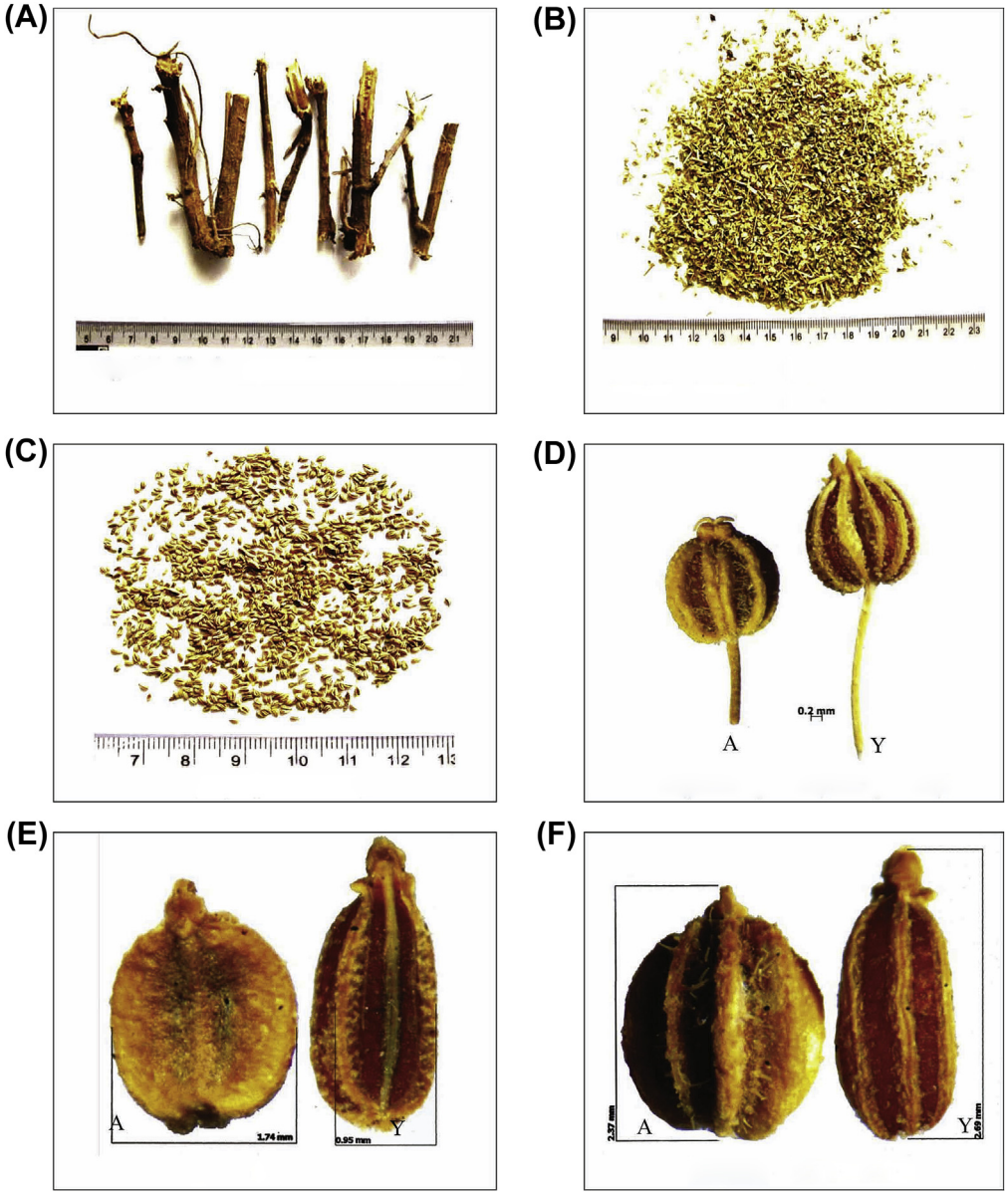



**Fig. 2.** Adulterants in raw drugs used in Hutabhugādi cūrṇa: (A) Citraka—stem pieces as foreign matter, (B) Ajamoda—bracts, leaves, and stalks of inflorescence as foreign matter, (C) Yavāni, (D) Ajamoda and Yavāni, (E) inner surface, and (F) outer surface.

of HC, prepared using substituent *T. ammi*, was developed using toluene:ethyl acetate (10:1).

### 2.3.3. Method

Chloroform extract of HC (4 μL) was applied on aluminum plates precoated with silica gel 60 $F_{254}$ of 0.2 mm thicknes (Merck, Darmstadt, Germany) using a CAMAG LINOMAT 5 applicator.[10] 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 105°C till the spots appeared.[11,12] The developed plates were visualized in the CAMAG visualizing chamber and scanned using CAMAG SCANNER 4 at 254 nm, 366 nm, 540 nm (prederivatization) and 610 nm (postderivatization with VS). With the help of CAMAG WinCATS software, $R_f$ values and densitograms were recorded.

## 3. Results and discussion

There is no monograph on the standardization of HC in Ayurvedic Pharmacopoeia of India (Part II—Formulations). There is a report on the standardization of HC, although the analysis is based on *T. ammi* as Ajamoda instead of the official source *A. leptophyllum*.[13] Apart from using *A. leptophyllum* as the true ingredient for HPTLC fingerprint profile, the present study also includes macroscopic features of the adulterants and a detailed atlas of microscopic features of the individual and compounded powders. Data derived from the present study may be used to prepare a monograph on standardization of HC for academia and industry.

Macroscopic features of ingredients of HC were recorded (Fig. 1). Possible adulterants and substitutes of the ingredients were also analyzed; Citraka was found to be adulterated with parts of stem along with the official part—the roots, and Ajamoda with bracts,

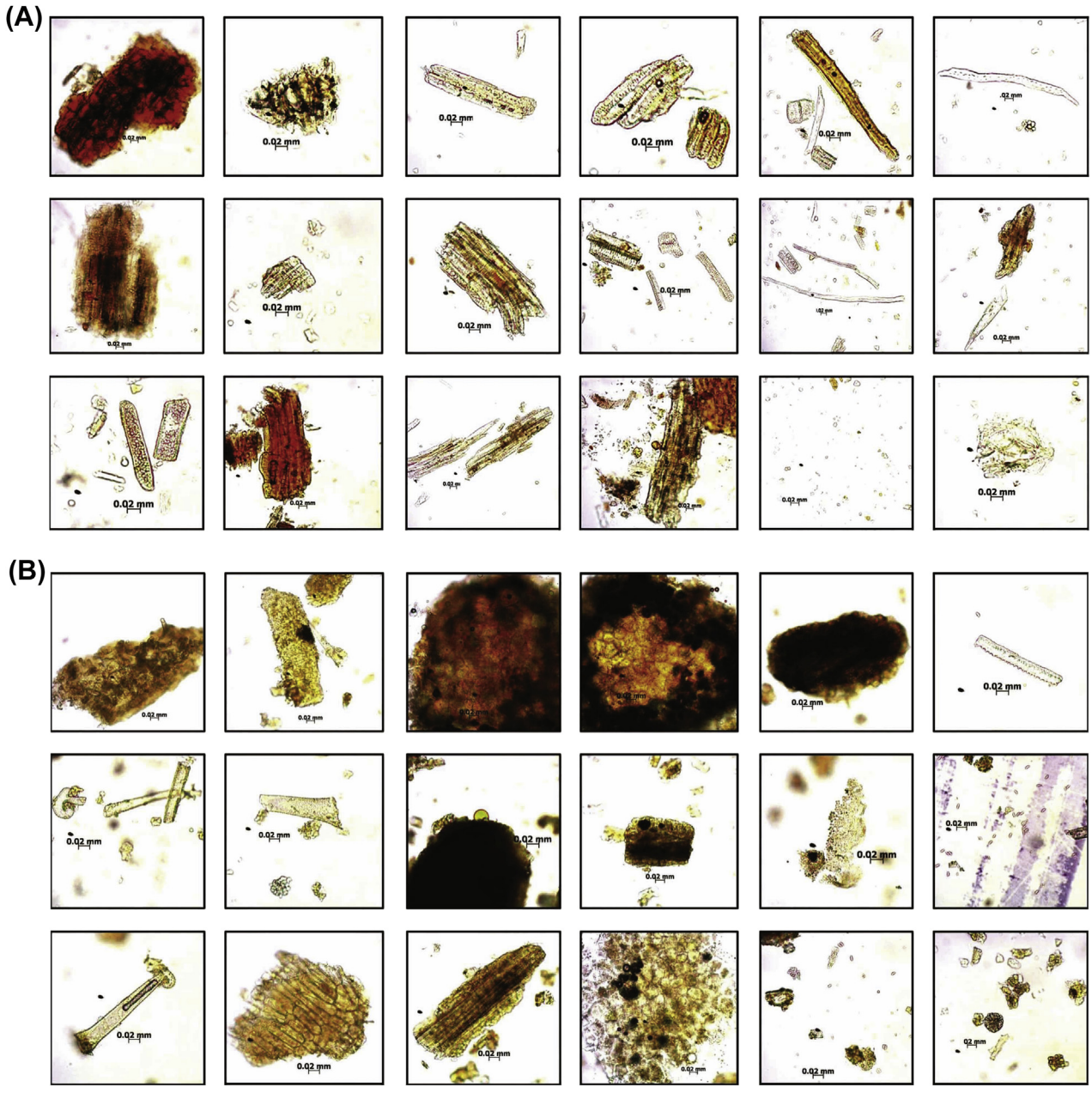



**Fig. 3.** Microscopic features of powder of (A) Citraka, (B) Ajamoda, (C) Pippali, (D) Marica, and (E) Haritaki, in Hutabhugādi cūrṇa, and (F) microscopic features of Hutabhugādi cūrṇa.

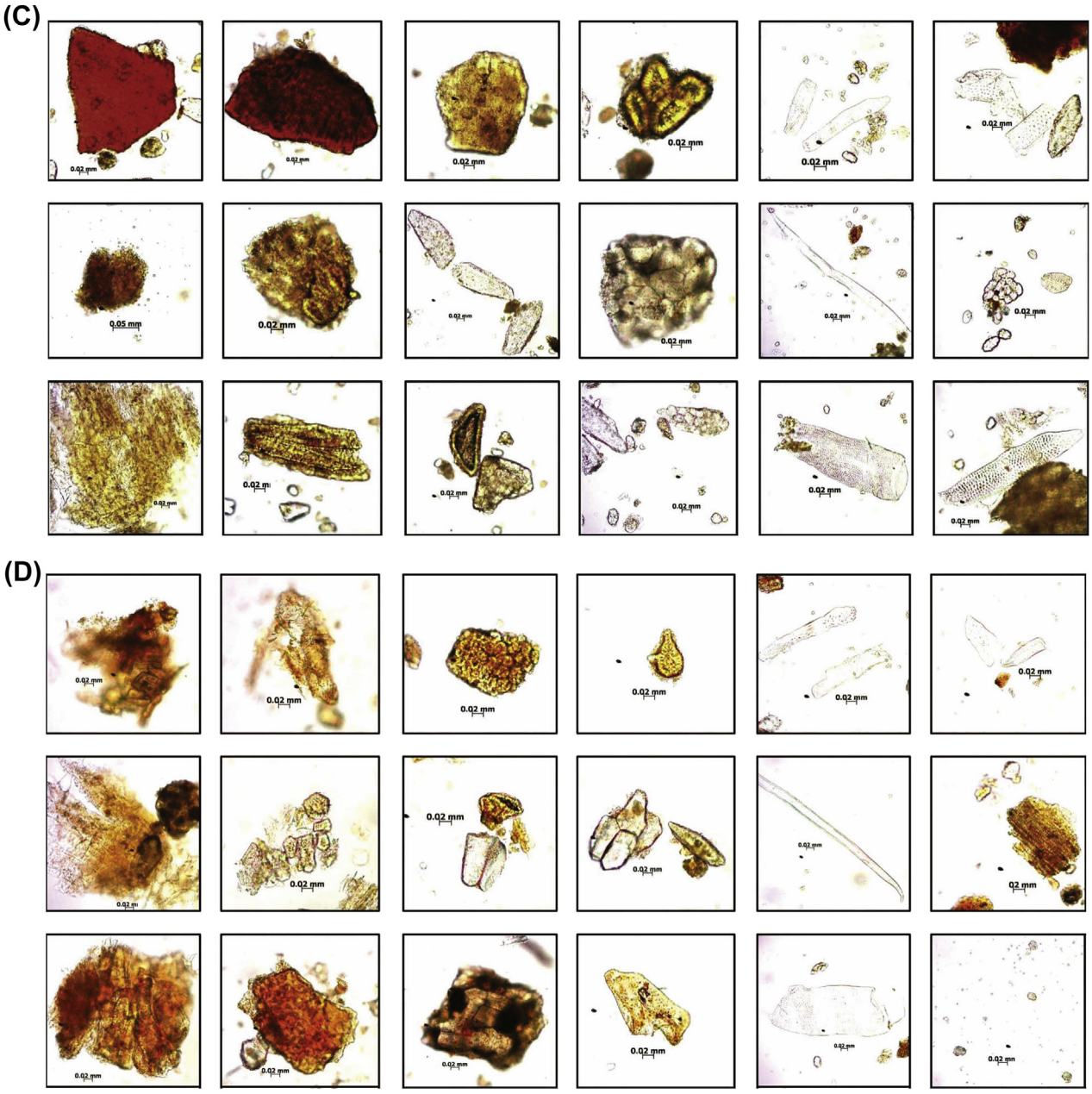



**Fig. 3.** (*continued*).

leaves, and stalks of the inflorescence bearing fruits. Distinguishing macroscopic features of Yavāni (*T. ammi*), a common substitute for Ajamoda (*A. leptophyllum*), have been documented. Ajamoda was found to have the following characteristics: occurring as entire cremocarps, occasionally as separate mericarps, usually with attached pedicel and bifid stylopod, cremocarps glabrous, ovoid to conical, yellow to yellowish green; separated mericarps broadly ovoid, about 1.5–2.5 mm long and 1.2–2 mm wide, more or less curved, outer surface convex with five equally distinct, longitudinal primary ridges; at the summit curved stylopodium, inner surface flat, showing darker and light-colored longitudinal bands, odor; aromatic; taste, slightly bitter giving a sensation of warmth to tongue. Yavāni has the following characteristics: mostly occurring as separated mericarps, grayish brown, ovoid, compressed, 2.5–2.8 mm long and 0.8–1 mm wide with pale colored protuberances; five ridges and six vittae in each mericarp, usually separate; five primary ridges pale in color; odor, characteristic; thymolic; taste pungent (Fig. 2).

HC is a yellowish-light brown fine powder with a characteristic odor, and has a salty, astringent, and pungent taste. Diagnostic characteristics of Citraka in HC are the following: parenchyma with reddish-brown content and starch grains; entire or fragments of pitted sclereids, fibers, and vessels; and thin and few thick-walled fibers often with pits (Fig. 3A). Diagnostic characteristics of Ajamoda in HC are entire or fragments of glandular trichome with unicellular stalk and unicellular head, both with pits on the surface; parquetry cells of the pericarp; fragments of epicarp in surface view showing cicatrix and stomata; and cells of cotyledon with greenish-yellow oil drops, rosette crystals, and few elongated simple starch grains (Fig. 3B).

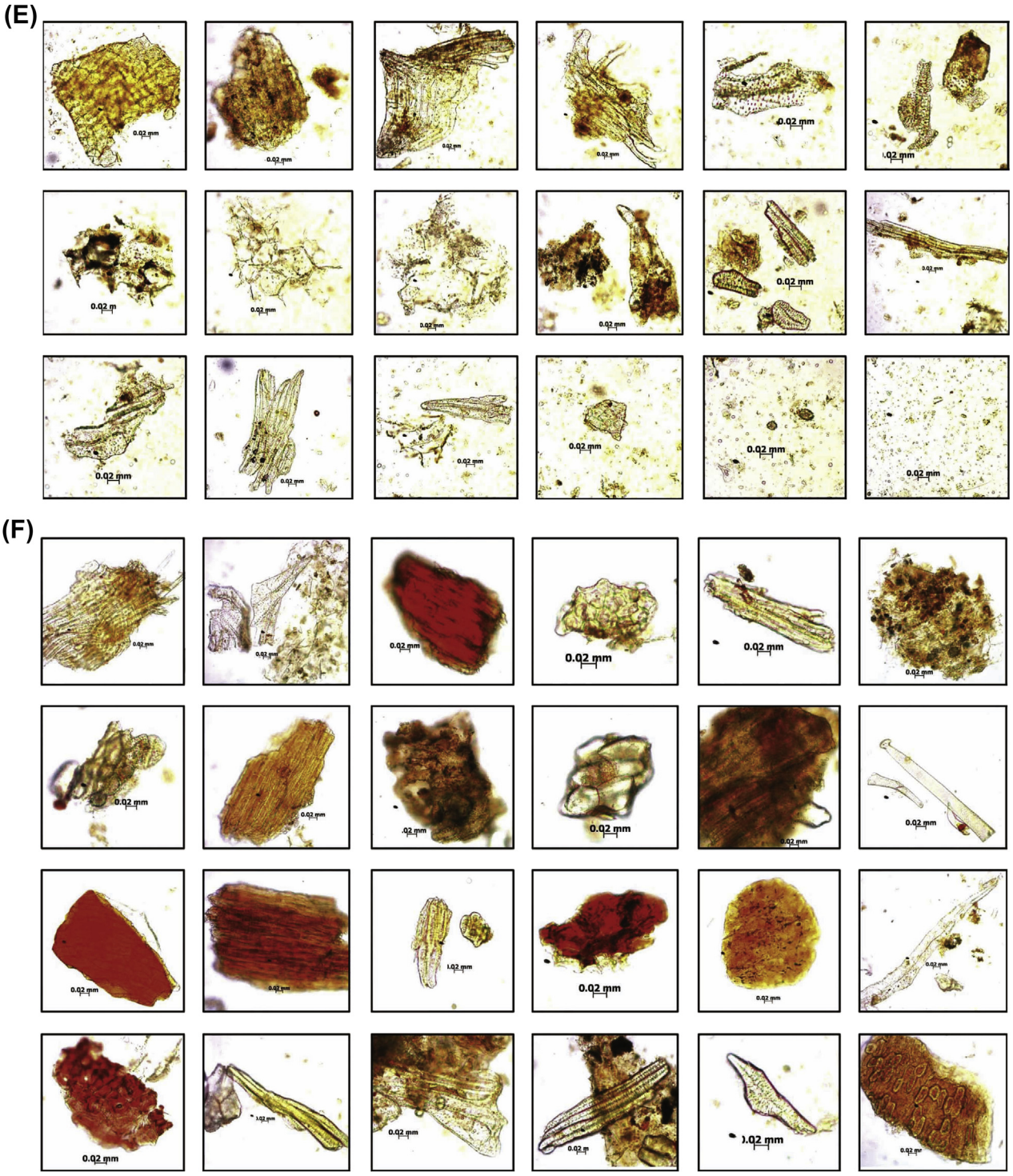



**Fig. 3.** (*continued*).

Diagnostic characteristics of Pippali in HC are as follows: round, oval to elongated stone cells in groups; cells of pericarp with pink content; fragments or entire pitted vessels; polygonal shiny cells of perisperm; and few thin-walled fibers and starch grains (Fig. 3C). Diagnostic characteristics of Marica in HC are as follows: small stone cells in groups, sometimes elongated; entire or fragments of pitted vessels; polygonal shiny cells of perisperm; and few thin-walled fibers and starch grains (Fig. 3D). The following are the diagnostic characteristics of Haritaki in HC: polygonal cells of epicarp with underlying mesocarp cells; group of fibers forming irregular parquetry arrangement; plenty of elongated pitted sclereids and few stone cells; parenchyma of mesocarp with tannin content; thin-walled fibers that are often pitted; few rosette crystals; and plenty of small and simple starch grains (Fig. 3E). Occurrence of the above characteristics in HC is a clear indication of incorporation of official ingredients in the

**Table 1**
Physicochemical constants of raw materials used for the preparation of Hutabhugādi cūrṇa.

| Name of the ingredients | Results expressed as % w/w ($n = 3$) | | | | | | |
|---|---|---|---|---|---|---|---|
| | FM | LOD | TA | AIA | WSA | WSE | ASE |
| Hutabhuga (Citraka) | Nil | 9.54 ± 0.14 | 2.44 ± 0.21 | 0.40 ± 0.28 | 0.55 ± 0.07 | 9.54 ± 0.53 | 6.63 ± 0.22 |
| | 44.26 | 12.66 ± 0.08 | 1.998 ± 0 | 0.45 ± 0.07 | 1.20 ± 0.14 | 11.33 ± 0.33 | 7.89 ± 1.19 |
| Deviation[a] | +44.26 | +3.12 | −0.44 | +0.05 | −0.65 | −1.79 | −1.26 |
| Ajamoda | Nil | 14.30 ± 0.04 | 9.49 ± 0.14 | 0.60 ± 0.14 | 5.54 ± 0.50 | 23.45 ± 0.21 | 21.56 ± 0.73 |
| | 39.16 | 12.03 ± 0.02 | 11.59 ± 0.01 | 1.10 ± 0.14 | 6.14 ± 0.50 | 29.50 ± 1.18 | 21.04 ± 0.06 |
| Deviation[b] | +39.16 | −2.27 | −2.1 | −0.5 | −0.6 | −6.05 | +0.52 |
| Saindhava lavana | Nil | 0.35 ± 0 | 93.76 ± 0.36 | 0.15 ± 0.07 | 92.01 ± 0.15 | 99.69 ± 0.30 | 2.48 ± 1.27 |
| Magadha (Pippali) | Nil | 13.07 ± 0.66 | 5.90 ± 0.14 | 0.30 ± 0 | 3.45 ± 0.07 | 11.09 ± 0.07 | 7.75 ± 0.32 |
| Marica | Nil | 13.55 ± 0.96 | 4.15 ± 0.35 | 0.25 ± 0.07 | 2.60 ± 0.14 | 8.16 ± 0.40 | 9.21 ± 0.11 |
| Pathya (Haritaki) | Nil | 10.60 ± 0.70 | 3.55 ± 0.07 | 0.40 ± 0 | 2.40 ± 0.14 | 59.47 ± 1.73 | 37.72 ± 0.40 |

AIA = acid-insoluble ash; ASE = alcohol-soluble extractive; FM = foreign matter; LOD = loss on drying at 105°C; TA = total ash; WSA = water-soluble ash; WSE = water-soluble extractive.
[a] Sample with 44.26% stems as foreign matter.
[b] Sample with 39.16% bracts, leaves, stalks, etc. as foreign matter.

formulation (Fig. 3F). Substitution or omission of any of these ingredients will be revealed by the microscopic examination of a pinch of HC.

Loss on drying, which reveals the moisture content; foreign matter, which is the percentage of materials other than the part to be used; total ash, which is the indication of total inorganic content; acid-insoluble ash, which is the acid-insoluble part of total ash, mainly silica; water-soluble ash, which is the water-soluble part of total ash indicating inorganic content without water-insoluble inorganic salts such as silica; and alcohol- and water-soluble extractives indicating the percentage of active constituents soluble in ethanol and water were analyzed for all the raw drugs used in the preparation. Ingredients with these physicochemical constants would render specific chemical nature when compounded to the formula—HC. A sample of Citraka with 44.26% of foreign matter (stem pieces) was analyzed to track the difference in physicochemical constants; a slight difference was observed in all the physicochemical constants described above in comparison to the sample with no foreign matter (entirely roots). Similarly, Ajamoda with bracts, leaves, stalks, etc. as foreign matters was analyzed and compared with the sample with no foreign matter (entirely fruits), to observe differences in the constants (Table 1).

HPTLC finger printing profiles of HC using toluene:ethyl acetate:formic acid (10:5:1) as the solvent system are given in Fig. 4 and $R_f$ values in Table 2. In Table 2, the fingerprint patterns of chloroform extract of ingredients and HC at 254 nm are given. Citraka, Ajamoda, Pippali, Marica, Haritaki, and HC showed four, 11, 10, 10, five, and eight spots (all green), respectively. Eight spots occurring in HC were due to five of the herbal ingredients (Saindhava lavana was not analyzed by TLC/HPTLC, as it is non-herbal) used for the formulation. Out of the eight spots in HC, spots with $R_f$ values of 0.25, 0.77, and 0.82 occurred in Ajamoda, Pippali, and Marica, respectively. Spots in HC corresponding to $R_f$ values of more than one ingredient would be merging of more than one compound with same $R_f$ values. A spot with an $R_f$ value of 0.34 was from Ajamoda and those with 0.42 and 0.69 were from Marica; hence, these spots are specific to these ingredients and thus help in their detection in HC. A spot with an $R_f$ value of 0.57 was observed in both Ajamoda and Pippali; hence, the spot in HC corresponds to a mixture of a minimum of two compounds with the same $R_f$ values. Spots with an $R_f$ value of 0.98 were observed in all the tracks. Spots with $R_f$ values very near to 1 may correspond to a mixture of many very-low-polarity compounds from all the ingredients added to HC.

Table 2 also represents the fingerprint pattern of chloroform extract of ingredients and HC at 366 nm. Citraka, Ajamoda, Pippali, Marica, Haritaki, and HC showed five, 16, seven, 12, three, and 11 spots (of different fluorescent colors), respectively. Out of 11 spots occurring in HC, 10 were due to five of the herbal ingredients used for the formulation, a spot with an $R_f$ value of 0.85 may be an artifact formed after compounding of the ingredients to HC. Excluding spots with an $R_f$ value of 0.85 in HC, spots with $R_f$ values 0.15, 0.35, and 0.63 occurred in Ajamoda and 0.76 in Marica; hence, these spots are specific to these ingredients and

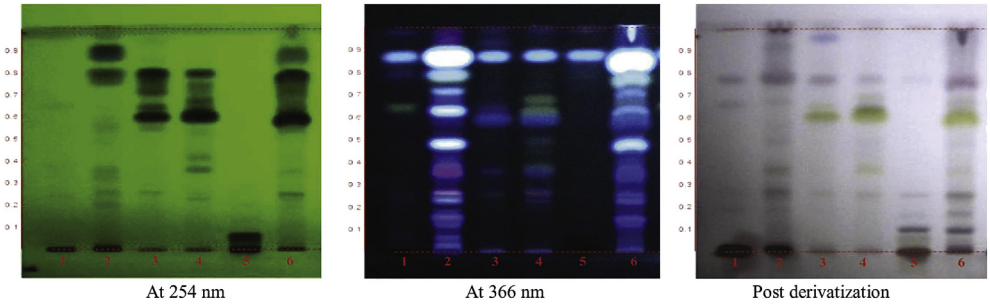



**Fig. 4.** TLC photo documentation of chloroform extract of ingredients with Hutabhugādi cūrṇa (5 μL). TLC = thin-layer chromatography.

**Table 2**
$R_f$ values of chloroform extract of ingredients and HC at 254 nm and 366 nm, and under white light post derivatization with VS.

| Citraka | Ajamoda | Pippali | Marica | Haritaki | HC |
|---|---|---|---|---|---|
| *$R_f$ values at 2546 nm* | | | | | |
| — | — | — | — | 0.07 D green | — |
| — | — | — | — | 0.13 green | — |
| — | 0.20 green | — | — | 0.20 green | — |
| 0.22 green | — | — | 0.22 green | — | — |
| — | **0.25 green** | **0.25 green** | **0.25 green** | — | **0.25 green** |
| — | — | — | — | 0.27 green | — |
| — | **0.34 green** | — | — | — | **0.34 green** |
| 0.37 green | 0.37 green | 0.37 green | 0.37 green | — | — |
| — | — | — | **0.42 green** | — | **0.42 green** |
| — | 0.49 green | — | — | — | — |
| — | **0.57 green** | **0.57 green** | — | — | **0.57 D green** |
| — | — | 0.63D green | 0.63 D green | — | — |
| 0.66 green | — | 0.66 D green | — | — | — |
| — | — | — | **0.69 D green** | — | **0.69 D green** |
| — | — | 0.73 D green | 0.73 D green | — | — |
| — | **0.77 D green** | **0.77 D green** | **0.77 D green** | — | **0.77 D green** |
| — | **0.82 D green** | **0.82 D green** | **0.82 D green** | — | **0.82 D blue** |
| — | 0.87 D blue | 0.87 green | — | — | — |
| — | 0.93 D green | — | — | — | — |
| **0.98 green** | **0.98 green** | **0.98 green** | **0.98 green** | **0.98 green** | **0.98 green** |
| **4** | **11** | **10** | **10** | **5** | **8** |
| *$R_f$ values at 366 nm* | | | | | |
| — | 0.02 F L blue | — | — | — | — |
| 0.06 F L blue | 0.06 F L blue | — | — | — | — |
| — | 0.11 F L blue | — | — | — | — |
| — | **0.15 F L pink** | — | — | — | **0.15 F D blue** |
| — | 0.17 F L blue | — | — | — | — |
| **0.21 F L blue** | **0.21 F L blue** | — | **0.21 F blue** | — | **0.21 F D blue** |
| — | **0.25 F pink** | **0.25 F brown** | **0.25 F red** | **0.25 F brown** | **0.25 F blue** |
| — | — | — | 0.28 F blue | — | — |
| — | **0.35 F pink** | — | — | — | **0.35 F D blue** |
| — | — | 0.37 F L blue | 0.37 F blue | — | — |
| — | **0.49 F blue** | — | **0.49 F green** | — | **0.49 F blue** |
| — | **0.59 F L blue** | **0.59 F blue** | **0.59 F blue** | — | **0.59 F blue** |
| — | **0.63 F blue** | — | — | — | **0.63 F blue** |
| 0.65 F green | | 0.65 F blue | 0.65 F green | — | — |
| — | **0.70 F blue** | — | **0.70 F green** | — | **0.70 F L blue** |
| — | 0.73 F green | — | — | — | — |
| — | — | — | **0.76 F brown** | — | **0.76 F green** |
| — | 0.79 F green | — | 0.79 F red | — | — |
| — | — | — | — | — | 0.85 F blue |
| 0.88 F blue | 0.88 F M blue | 0.88 F L blue | 0.88 F L blue | 0.88 F L blue | — |
| — | — | 0.95 F blue | — | — | — |
| **0.98 F blue** | **0.98 F pink** | **0.98 F blue** | **0.98 F blue** | **0.98 F red** | **0.98 F L blue** |
| **5** | **16** | **7** | **12** | **3** | **11** |
| *$R_f$ values under white light post derivatization with VS* | | | | | |
| — | — | — | — | **0.09 blue** | **0.09 blue** |
| **0.16 L brown** | **0.16 L brown** | — | — | **0.16 L blue** | **0.16 violet** |
| **0.24 L brown** | **0.24 L brown** | **0.24 L brown** | **0.24 L brown** | **0.24 L blue** | **0.24 blue** |
| — | **0.36 green** | — | **0.36 L green** | — | **0.36 L green** |
| — | — | — | | — | 0.46 L green |
| — | 0.50 violet | — | 0.50 L green | — | — |
| — | **0.57 L brown** | — | — | — | **0.57 green** |
| — | — | 0.62 green | — | — | — |
| **0.67 L brown** | **0.67 violet** | **0.67 L green** | — | — | **0.67 L brown** |
| **0.78 violet** | **0.78 violet** | **0.78 violet** | — | **0.78 violet** | **0.78 violet** |
| — | 0.92 green | — | — | — | — |
| — | — | 0.96 blue | — | — | — |
| **0.98 violet** | **0.98 violet** | **0.98 violet** | **violet** | **0.98 violet** | **0.98 violet** |
| **5** | **7** | **4** | **3** | **5** | **9** |

D = dark; F = fluorescent; HC = Hutabhugādi cūrṇa; L = light; VS = vanillin–sulfuric acid.
Texts in bold are spots with corresponding $R_f$ values in HC and ingredients.

help in their detection in HC. Spots with an $R_f$ value of 0.21 were observed in Citraka, Ajamoda, and Marica; 0.25 in Ajamoda, Pippali, Marica, and Haritaki; 0.49 in Ajamoda and Marica; and 0.59 in Ajamoda, Pippali, and Marica. Spots with an $R_f$ value of 0.98 were observed in all the tracks.

Furthermore, Table 2 represents the fingerprint pattern of chloroform extract of ingredients and HC under white light post derivatization with VS. Citraka, Ajamoda, Pippali, Marica, Haritaki, and HC showed five, seven, four, three, five, and nine spots (of different colors), respectively. Out of nine spots occurring in HC, eight were due to five of the herbal ingredients used for the formulation; a spot with an $R_f$ value of 0.46 was formed from compounding of the ingredients to HC. Excluding the spots with an $R_f$ value of 0.46 in HC, spots with an $R_f$ value of 0.09

**Table 3**
Physicochemical parameters of HC prepared using AL and TA.

| Parameters (% w/w) | HC prepared using AL | HC prepared using TA | Deviation |
|---|---|---|---|
| Loss on drying at 105°C | 9.22 ± 1.36 | 9.85 ± 0.01 | +0.631 |
| Total ash | 13.18 ± 0.42 | 12.70 ± 0.06 | −0.48 |
| Acid-insoluble ash | 0.38 ± 0.18 | 0.10 ± 0.00 | −0.28 |
| Water-soluble ash | 11.32 ± 0.11 | 11.68 ± 0.11 | +0.29 |
| Alcohol-soluble extractive | 24.67 ± 7.46 | 31.78 ± 0.53 | +7.11 |
| Water-soluble extractive | 42.50 ± 0.33 | 42.33 ± 0.24 | −0.16 |
| pH | 3.52 ± 0.37 | 4.19 ± 0.01 | +0.67 |

AL = *A. leptophyllum*; HC = Hutabhugādi cūrṇa; TA = *T. ammi*.

occurred in Haritaki and 0.57 in Ajamoda only; hence, these spots help in the detection of these ingredients in HC. Spots with an $R_f$ value of 0.16 were observed in Citraka, Ajamoda, and Haritaki; 0.36 in Ajamoda and Marica; 0.67 in Citraka, Ajamoda, and Pippali; and 0.78 in Citraka, Ajamoda, Pippali, and Haritaki. Spots with $R_f$ values of 0.24 and 0.98 were observed in all the tracks.

Color of the spots may vary slightly, depending on the concentration of the compound; hence, compounds were not considered different when the color of the spots was different. All the three wavelengths used for fingerprinting were evaluated to be diagnostic in identification of different ingredients.

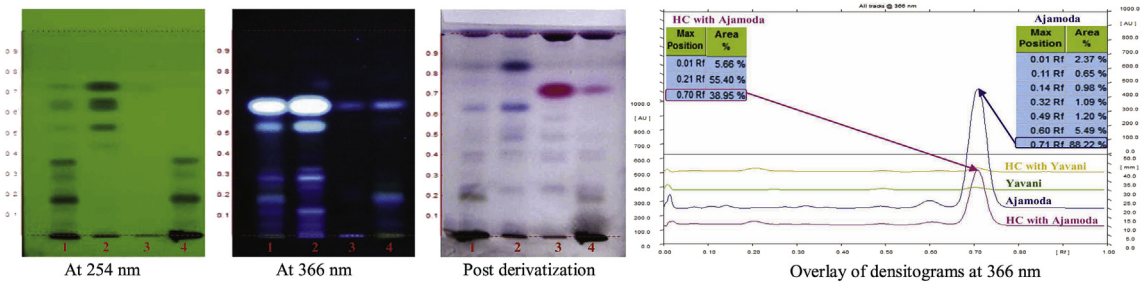
A comparative study of HC prepared using *T. ammi* (Yavāni API), a common substitute for Ajamoda, was undertaken for the purpose of detection of adulteration/substitution of Ajamoda in HC. When Yavāni was used as a substitute for Ajamoda, appreciable variation was observed in a few physicochemical parameters such as alcohol-soluble extractive (+7.11%), acid-insoluble ash (−0.28%), and pH (+0.67) (Table 3).

The HPTLC method used for the detection of possible substitution of Ajamoda in HC with Yavāni is presented in Table 4 and Fig. 5. The $R_f$ values of spots would aid in determining the substitution. A three-dimensional overlay of a densitogram at 366 nm (Fig. 5) was found to differentiate between Ajamoda, Yavāni, and HC prepared using them. Ajamoda showed a unique peak with an $R_f$ value of 0.71, which is not found in Yavāni. After derivatization with VS, Yavāni showed a spot with an $R_f$ value of

**Table 4**
Comparison of $R_f$ values of HC prepared using Ajamoda (official) and Yavani (substitute).

| At 254 nm | | | | At 366 nm | | | | Post derivatization | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| HC with Ajamoda | Ajamoda (AL) | Yavāni (TA) | HC with Yavāni | HC with Ajamoda | Ajamoda (AL) | Yavāni (TA) | HC with Yavāni | HC with Ajamoda | Ajamoda (AL) | Yavāni (TA) | HC with Yavāni |
| 0.06 L green | — | — | 0.06 L green | **0.06 F L blue** | **0.06 F blue** | — | 0.06 F L Blue | — | — | — | — |
| — | — | — | | **0.12 F blue** | **0.12 F blue** | — | | — | — | — | — |
| 0.18 green | — | — | 0.18 green | 0.18 F G blue | — | **0.18 F L blue** | **0.18 F G blue** | 0.18 L brown | — | — | 0.18 L brown |
| — | — | — | | **0.24 F L blue** | **0.24 FL blue** | — | 0.24 F L green | **0.24 L blue** | **0.24 L blue** | **0.24 L blue** | **0.24 L blue** |
| 0.29 L green | — | — | 0.29 L green | **0.29 F blue** | **0.29 F blue** | — | — | — | — | — | — |
| — | — | — | | **0.32 F L blue** | **0.32 F L blue** | — | — | — | — | — | — |
| 0.37 green | — | — | 0.37 green | | | — | — | — | — | — | — |
| — | — | — | — | — | — | — | — | **0.40 L blue** | **0-.40 L blue** | **0.40 L blue** | **0.40 L blue** |
| **0.44 L green** | **0.44 L green** | — | — | — | — | — | — | — | — | — | — |
| — | — | — | — | — | — | — | — | **0.49 L blue** | **0.49 L blue** | **0.49 L blue** | — |
| **0.54 L green** | **0.54 green** | — | — | **0.54 F L green** | **0.54 F L green** | **0.54 F L blue** | **0.54 F L blue** | — | — | — | — |
| — | — | — | — | — | — | — | — | — | — | 0.58 L blue | — |
| **0.63 L blue** | **0.63 L blue** | — | — | **0.63 F M blue** | **0.63 F M blue** | **0.63 F blue** | **0.63 F blue** | **0.63 L blue** | **0.63 blue** | — | — |
| **0.66 green** | **0.66 green** | — | — | — | — | — | — | — | — | — | — |
| **0.72 green** | **0.72 green** | 0.72 L green | — | — | — | — | — | — | — | **0.72 pink** | **0.72 L pink** |
| — | — | — | — | 0.76 F L blue | — | — | — | 0.76 L blue | — | — | — |
| — | — | — | — | — | — | — | — | **0.84 violet** | **0.84 blue** | — | — |
| — | — | — | — | — | — | — | — | **0.98 violet** | **0.98 violet** | **0.98 violet** | **0.98 violet** |
| **9**[a] | **5** | **1** | **4** | **9** | **7** | **3** | **5** | **8** | **6** | **6** | **5** |

AP = *A. leptophyllum*; D = dark; F = fluorescent; HC = Hutabhugādi cūrṇa; L = light; M = medium; TA = *T. ammi*.
Texts in bold are spots with corresponding $R_f$ values in HC, Ajamoda and Yavani.
[a] Number of spots.




Solvent system: Toluene: Ethyl acetate (10: 1)

Track 1- Hutabhugadi Curna with Ajamoda; Track 2- Ajamoda (*Apium leptiphyllum*); Track 3- Yavani (*Trachyspermum ammi*);

Track 4- Hutabhugādi cūrṇa with Yavani

**Fig. 5.** TLC comparison of HC prepared using Ajamoda (*A. leptophyllum*) and Yavāni (*T. ammi*) (6 μL). HC = Hutabhugādi cūrṇa; TLC = thin-layer chromatography.

0.72 (pink), which is not found in Ajamoda. The proposed chromatogram can be an effective tool to identify the HC prepared using *T. ammi.*

## 4. Conclusion

From the current investigation results a comprehensive monograph on quality standards for Hutabhugadi curna mentioned in Part I of Ayurvedic Formulary of India has been proposed.

### 4.1. Definition

HC is a fine powder preparation made with the ingredients listed in the following formulation composition.

### 4.2. Formulation composition[6]

| | | | | |
|---|---|---|---|---|
| 1. | Hutabhuga (Citraka API) | *P. zeylanica* | Rt. | One part |
| 2. | Ajamoda (Ajamoda API) | *A. leptophyllum* | Fr. | One part |
| 3. | Saindhava lavana | Rock salt | — | One part |
| 4. | Magadha (Pippali API) | *P. longum* | Fr. | One part |
| 5. | Marica API | *P. nigrum* | Fr. | One part |
| 6. | Pathya (Haritaki) API | *T. chebula* | P. | Five parts |

### 4.3. Description

HC is a yellowish-light brown fine powder with a characteristic odor, and has salty, astringent, and pungent taste.

### 4.4. Identification

#### 4.4.1. Microscopy

Microscopic study shows the following results:

Pippali—round, oval to elongated stone cells in groups; cells of pericarp with pink content; fragments or entire pitted vessels; polygonal shiny cells of perisperm; and few thin-walled fibers and starch grains.

Marica—small stone cells in groups, sometimes elongated; entire or fragments of pitted vessels; polygonal shiny cells of perisperm; and few thin-walled fibers and starch grains.

Haritaki—polygonal cells of epicarp with underlying mesocarp cells; group of fibers forming irregular parquetry arrangement; plenty of elongated pitted sclereids and few stone cells; parenchyma of mesocarp with tannin content; thin-walled fibers, which are often pitted; few rosette crystals; and plenty of small and simple starch grains ().

#### 4.4.2. Fluorescence test

The fluorescence characters were detected in chloroform, alcohol, and water extracts of HC under long UV light.

#### 4.4.3. Thin-layer chromatography

Under 254 nm, eight spots with $R_f$ values of 0.25, 0.34, 0.42, 0.57, 0.69, 0.77, 0.82, and 0.98 [all of green color, except the spot with an $R_f$ value of 0.82 (dark blue)] were seen.

Under 366 nm, 11 spots with $R_f$ values of 0.15, 0.21, 0.25, 0.35, 0.49, 0.59, 0.63, 0.70, 0.76, 0.85, and 0.98 [all of fluorescent blue color, except that with an $R_f$ value of 0.76 (fluorescent green)) were seen.

After derivatization with VS, nine spots with $R_f$ values of 0.09 (blue), 0.16 (violet), 0.24 (blue), 0.36 (light green), 0.46 (light green), 0.57 (green), 0.67 (light brown), 0.78 (violet), and 0.98 (violet) were seen.

### 4.5. Physicochemical parameters

| | |
|---|---|
| Loss on drying at 105°C | Not more than 9.22% |
| Total ash | Not more than 13.18% |
| Acid-insoluble ash | Not more than 0.38% |
| Water-insoluble ash | Not more than 11.32% |
| Alcohol-soluble extractive | Not less than 24.67% |
| Water-soluble extractive | Not less than 42.50% |
| pH (10% aqueous solution) | Not more than 3.52 per cent, |
| Assay | |
| Sodium | 3.77% |
| Other requirements | |
| Microbial limits | Within limit |
| Aflatoxin | Within limit |

*Adulterants and substitutes:* Stem of *P. zeylanica* in Citraka; bracts, leaves, stalks, etc. of *A. leptophyllum* in Ajamoda; and fruits of *T. ammi* for Ajamoda can be detected by macro–microscopy, physicochemical tests, and HPTLC tests.

*Storage:* It should be stored in a cool, dry place in tightly closed containers, protected from light and moisture.

*Therapeutic uses:* HC is used to treat *Agnimandya* (digestive impairment), *Pandu* (anemia), *Sopha* (edema), and *Arsa* (piles)[6].

*Dose:* 3–6 g.[6]

*Anupana:* Thin butter milk.[6]

## Conflict of Interest

None declared.

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