Indian Journal of Traditional Knowledge
Vol. 14(4), October 2015, pp. 544-549

# Scientific evaluation and standardization of the *Ayurvedic* compound formulation *Yavānyādi cūrṇa*

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*Received 30 September 2014, revised 17October 2014*

The present study deals with the scientific evaluation and standardization of the Ayurvedic compound formulation *Yavānyādi cūrṇa* following quality control procedures both for the raw materials and the finished product. The obtained values (ranges of physico-chemical parameters) can be adapted to day down new pharmacopoeial standards to be followed for traditional preparation of *Yavānyādi cūrṇa* with batch to batch consistency. The phytochemical constituents found to present in the raw material used for the preparation of *Yavānyādi cūrṇa* facilitate the desirable therapeutic efficacy of the medicinal formulations a whole in elements and also could help in knowing the underlying mechanism of pharmacological action.

**Keywords:** Ayurvedic formulation, *Yavānyādi cūrṇa*, Drug standardization
**IPC Int. Cl.**[8]: A61B 5/117, G06K 9/00, A61K, A01D 20/00

Ayurveda is an indigenous Indian system of medicine that is mainly plant-based and has gained worldwide attention due to safety and efficacy. However, due to lack of proper quality control methods there are variations in the same product obtained from different sources. Standardization is important for insuring of good quality products as standardized drugs of well defined consistent quality are needed for reliable beneficial therapeutic uses. Thus, there is an urgent need to develop parameters for quality control which are cost effective and can be easily adopted by the manufactures. Efforts are being made in this area that have led to the development of analytical protocols both for single herbal drugs as well as for compound herbal formulations[1-8] that can be used as valuable analytical tools in the routine standardization of Ayurvedic drugs and formulations.

*Yavānyādi cūrṇa* is an Ayurvedic polyherbal formulation has been use in asthama, mal absorption syndrome[9], etc. The preparation of *Yavānyādi cūrṇa* is based on traditional methods is accordance with the procedures given in classical texts like Ayurvedic formulary of India I. Due to lack of modern pharmacopoeia standards laid down and followed for processing of *Yavānyādi cūrṇa*, the medicine prepared using traditional methods may not have the desired quality and batch to batch consistency. Hence, there is a need for standardization of Ayurvedic *curna* following scientific parameters including descriptive taxonomic identification of raw drugs, organoleptic characters, phytochemical analysis, chromatographic pattern and microbial screening.

The work was undertaken is the trust as part of a program of testing and validation of traditional practices of using the Ayurvedic medicine. *Yavānyādi cūrṇa*[9] is traditionally used for treatment of *Atīsarta* (diarrhoea), *Ksaya* (pthisis), *Gulma* (abdominai lump), *Udara* (disesses of abdomen), *Kãsa* (cough), *Agnimāndya* (digestive impairment), *Aŕsā* (piles), *Pīnasa* (chronic rhinitis) and *Arucī* (Anorexia). In this connection, standardization of *Yavānyādi cūrṇa* becomes imperative. The current work deals with detailed standardization guidelines involving Good Manufacturing (GMP) prescribed for preparation of Ayurvedic medicines. Standardization guidelines to be followed[10] for herbals products provided by World Health Organization (WHO)[11], and Ayurvedic pharmacopoeia of India have been considered. In the standardization procedures followed in the work, certain in process tests have also been developed and performed.

## Methodology

### Preparation of the *curna*

All the ingredients were used of Pharmacopoeial quality. These were washed, dried and ground

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individually passed through 180 µm separately then weighed separately, mixed in specified ratio and passed through 355 µm to obtain a homogenous blend. It was stored in an airtight container to protect from light and moisture. Three different samples of *Yavānyādi cūrṇa* were studied. Each sample of *Yavānyādi cūrṇa* was formulated using 21 ingredients[9], i.e. *Yavāni* (*Trachyspermu ammi* (L.) Sprague. fruit), *Pippalīmūla* (*Piper longum* L. root), *Tvak* (*Cinnamomum zeylanicum* Blume. stem bark), *Elā* (*Elettaria cardamomum* Maton, seed), *Patra* (*Cinnamomum tamala* Nees & Eberm, leaf), *Nāgakesara* (*Mesua ferrea* L. stamen), *Nāgara* (*Zingiber officinale* Rose. Rhizome), *Marica* (*Piper nigrum* L. fruit), *Agni* (*Plumbago zey lanica* L. root), *Jala* (*Coleus vettiveroides* K.C. Jacos. root), *Ajājī* (*Cuminum cyminum* L. fruit), *Dhānya* (*Corindrum sativum* L. fruit), *Sauvarcala lavaṇa* (black salt), *Vṛkṣāmla* (*Garcinia indica* Choisy. fruit pulp), *Dhātakī* (*Woodfordia fruticosa* Kurz. flower), *Kṛsṇā* (*Piper longum* L. fruit), *Bilva* (*Aegle marmelos* Coor, unripe fruit pulp), *Dāḍima* (*Punica granatum* L. dried seed), *Dīpyaka* (*Apium graveolens* L. fruit), *Sitā* (sugar) and *Kapittha* (*Feronia limonia* (L.) Swingle, unripe fruit pulp) [12-13] prepared as per AFI[9] (Table 1).

**Physico-chemical parameters**

Organoleptic characters, particle size and physico-chemical analysis[10, 14-15] of all the samples were carried out. Quantitative analysis for total ash, acid insoluble ash, water soluble ash, extractive values in water soluble and alcohol soluble extractive, loss on drying at 105°C, volatile oil, total sugar, reducing sugar and *p*H of filtrate of 10% w/v aqueous solution were checked in triplicate according to the prescribed *Standard methods in Indian Pharmacopoeia*[16].

**High pressure thin layer chromatography (HPTLC)**

For HPTLC[11, 14-15, 17], 5 gm of coarsely powdered drug in 250 ml stoppered conical flask and extracted with 100 ml ethanol for 24 hrs by maceration technique with occasional shaking. The extract was extracted and volume was raised up to 100ml in a volumetric flask. Twenty five ml extract was taken from the above stock solution and concentrated on a water bath to similarly, methanol extract was prepared for 3 batches of *Yavānyādi cūrṇa*. TLC of extracts of all the samples was carried out on silica gel 60 $F_{254}$ pre-coated plates (0.2 mm thickness; from Merck India Limited). An Applicator from Camag Linomat-5 (Camag Switzerland: 140443) was used for band application and photo documentation unit (Camag

Table 1—Ingredients of *Yavānyādi cūrṇa*

| S. no. | Sanskrit Name | Botanical name | Parts used | Portion |
|---|---|---|---|---|
| 1. | *Yavāni* | *Trachyspermu ammi* (L.) Sprague | Fruit | 1 Part |
| 2. | *Pippalīmūla (Pippalī )* | *Piper longum* L. | Root | 1 Part |
| 3. | *Tvak* | *Cinnamomum zeylanicum* Blume | Stem bark | 1 Part |
| 4. | *Elā (Suksmailā)* | *Elettaria cardamomum* Maton | Seed | 1 Part |
| 5. | *Patra (Tvakpatra)* | *Cinnamomum tamala* Nees & Eberm | Leaf | 1 Part |
| 6. | *Nāgakesara* | *Mesua ferrea* L. | Stmn. | 1 Part |
| 7. | *Nāgara (Śunṭhī)* | *Zingiber officinale* Rose. | Rhizome | 1 Part |
| 8. | *Marica* | *Piper nigrum* L. | Fruit | 1 Part |
| 9. | *Agni (Citraka)* | *Plumbago zeylanica* L. | Root | 1 Part |
| 10. | *Jala (Hrībera)* | *Coleus vettiveroides* K.C. Jacos. | Root | 1 Part |
| 11. | *Ajājī (Śveta jīraka)* | *Cuminum cyminum* L. | Fruit | 1 Part |
| 12. | *Dhānya (Dhānyaka)* | *Corindrum sativum* L. | Fruit | 1 Part |
| 13. | *Sauvarcala lavaṇa* | Black Salt | - | 3 Parts |
| 14. | *Vṛkṣāmla* | *Garcinia indica* Choisy. | Fruit Pulp | 3 Parts |
| 15. | *Dhātakī* | *Woodfordia fruticosa* Kurz. | Flower | 3 Parts |
| 16. | *Kṛsṇā (Pippalī)* | *Piper longum* L. | Fruit | 3 Parts |
| 17. | *Bilva* | *Aegle marmelos* Coor | Unripe Fruit Pulp | 3 Parts |
| 18. | *Dāḍima* | *Punica granatum* L. | Dried seed | 3 Parts |
| 19. | *Dīpyaka (Ajamodā)* | *Apium graveolens* L. | Fruit | 3 Parts |
| 20. | *Sitā (Śarkarā)* | Sugar | - | 6 Parts |
| 21. | *Kapittha* | *Feronia limonia* (L.) Swingle | Unripe Fruit Pulp | 8 Parts |

Reprostar-3: 140604) was used for documentation of chromatographic fingerprints. The mobile phase used was Toluene: Ethyl acetate (7: 3). The plate was developed over a distance of 9 cm in a saturated development chamber (Twin trough chamber (10×10 c with SS lid, and visualized under visible light, 254nm and 366nm. After spraying with anisaldehyde-sulphuric acid is followed by heating at 105°C for 5 min.

**Test for aflatoxin**

The 3 sample of *Yavānyādi cūrṇa* were also checked for mycotoxin, i.e., Aflatoxin with standard markers $B_1$, $B_2$, $G_1$ & $G_2$[14-15].

**Test for microbial limits**

Following tests were carry out as per standard methods[11,14] to determine the microbial load in 3 batches of *Yavānyādi cūrṇa*, a formulated compound drug powder of pharmaceutical substances

1. Enumeration of *Staphylococcus aureus* /gm
2. Enumeration of *Salmonella* sp./gm
3. Enumeration of *Pseudomonas aeruginosa*/gm
4. Determination of *E.coli*
5. Determination of total microbial plate count (TMC)
6. Determination of yeast and mould.

The microbiological tests were determined using specified agar and enrichment media from Himedia Pvt. Ltd., Mumbai.

**Heavy metal**

Heavy metal analysis[11,14-15,18] (lead, cadmium, arsenic and mercury) were carried out using Atomic absorption spectrophotometry (Shimadzu-Model-A-7000). All samples are digested with concentrated $HNO_3$: $HClO_4$ (4:1). Standards solutions are made for different dilution to get linear calibration (Merck). Pb and Cd were performed using graphite oven method, while As (Arsenic) were determined as hydride method and Hg were determined using cold absorption method.

**Assay of sodium and potassium**

Sodium (Na)[18] and Potassium (K)[18] were carried out using Flame photometer (ELCO-Model-CL361) and results are presented in per cent.

## Results and discussion

A brownish, fine powder with a spicy odour is with salty taste. The powder completely passes through 355 µm and not less than 50 % through 180 µm.

Physico-chemical data was subjected to various analytical parameters average value of loss and drying at 105°C 8.15.15% total ash content 5.13%, acid insoluble ash 3.36%, alcohol soluble extractive 12.35%, water soluble extractive 40.20%, total sugar 7.45%, reducing sugar 0.58% volatile oil 1.5%, and *p*H 4.77 (Tables 2-3).

The TLC plate were examine under ultra violet light at 254 nm; at 366 nm; at visible for both before and after derivetisation with anisaldehyde-sulphuric acid reagent (Figs. 1-4). The $R_f$ values and colours of the bands obtained were recorded. It shows major spot under ultraviolet light (254nm) $R_f$ 0.36, 0.42, 0.50, 0.57, 0.68, 0.84 and 0.94 (all spots black), It shows major spot under ultraviolet light (366 nm) $R_f$ 0.05 (red), 0.18, 0.36, 0.47 (blue), 0.51 (red), 0.71(fluorescent), 0.82 (red) and 0.89(blue). Spray the plate with anisaldehyde-sulphuric acid reagent followed by heating at 105°C for about 5 min and observes under ultraviolet light. It shows major $R_f$ under ultraviolet light (366nm) $R_f$ 0.08 (pink), 0.35, 0.44 (both spots sky blue), 0.56, 0.68 (brown), 0.71 (sky blue), 0.85 and 0.89 (red), It shows major spot

Table 2—Physicochemical analysis of different batches of *Yavānyādi cūrṇa*

| Parameters | *Yavānyādi cūrṇa* | | | Average values |
|---|---|---|---|---|
| | Batch 01 | Batch 02 | Batch 03 | |
| Loss on drying at 105°C (%w/w) | 15.15 | 15.17 | 15.13 | 15.15 |
| Total ash value (%w/w) | 5.14 | 5.14 | 5.13 | 5.13 |
| Acid-insoluble ash value (%w/w) | 3.3 | 3.5 | 3.3 | 3.36 |
| Water soluble extractive value (%w/w) | 40.14 | 40.31 | 40.15 | 40.2 |
| Alcohol soluble extractive value (%w/w) | 12.29 | 12.42 | 12.34 | 12.35 |
| Total Sugar (%w/w) | 7.50 | 7.00 | 7.85 | 7.45 |
| Reducing Sugar (%w/w) | 0.5 | 0.65 | 0.60 | 0.58 |
| Volatile oil (%v/w) | 1.50 | 1.50 | 1.50 | 1.50 |
| pH (Filter of 10% w/v aqueous solution) | 4.5 | 4.9 | 4.9 | 4.77 |

Table 3—Physicochemical analysis of *Yavānyādi cūrṇa* ingredients

| Name of ingredients | Loss on drying at 105°C (%w/w) | Total ash value (%w/w) | Acid-insoluble ash value (%w/w) | Alcohol soluble extractive value (%w/w) | Water soluble extractive value (%w/w) | Volatile oil (% v/w) |
|---|---|---|---|---|---|---|
| *Yavāni* | 8.49 | 5.56 | 0.14 | 4.22 | 18.78 | - |
| *Pippalīmūla* | 12.91 | 4.87 | 0.13 | 5.30 | 17.92 | - |
| *Tvak* | 8.15 | 2.90 | 1.80 | 3.96 | 4.73 | 1.3 |
| *Elā* | 6.35 | 4.10 | 3.61 | 10.37 | 15.11 | 7.11 |
| *Patra* | 6.95 | 4.60 | 0.97 | 6.14 | 15.45 | 1.4 |
| *Nāgakesara* | 7.14 | 5.12 | 2.63 | 16.86 | 14.16 | - |
| *Nāgara* | 8.68 | 5.84 | 0.97 | 4.14 | 15.45 | - |
| *Marica* | 6.95 | 4.28 | 0.43 | 7.14 | 11.45 | - |
| *Agni* | 7.78 | 2.14 | 1.18 | 13.96 | 15.42 | - |
| *Jala* | 6.44 | 7.92 | 2.36 | 30.36 | 23.02 | - |
| *Ajājī* | 8.98 | 6.26 | 0.65 | 17.46 | 31.79 | - |
| *Dhānya* | 5.79 | 4.69 | 0.94 | 23.55 | 22.22 | - |
| *Vṛkṣāmla* | 15.92 | 1.88 | 1.78 | 32.98 | 36.12 | - |
| *Dhātakī* | 6.95 | 8.04 | 0.85 | 12.54 | 32.55 | - |
| *Kṛsṇā* | 11.25 | 6.61 | 0.36 | 7.85 | 19.50 | - |
| *Bilva* | 8.17 | 1.78 | 0.79 | 18.66 | 73.22 | - |
| *Dāḍima* | 7.01 | 3.32 | 0.31 | 11.17 | 22.44 | - |
| *Dīpyaka* | 8.98 | 6.26 | 0.65 | 17.46 | 31.79 | - |
| *Kapittha* | 9.0 | 5.50 | 0.80 | 14.0 | 35.0 | - |

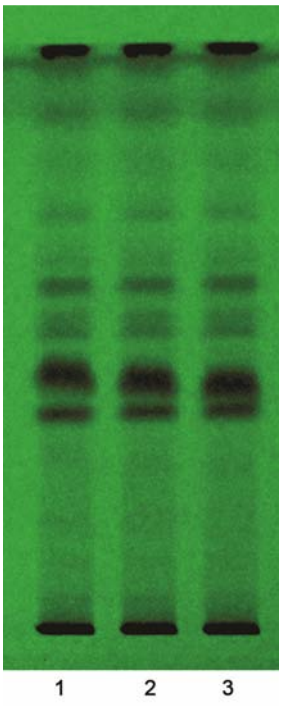



Fig.1—TLC profile of Yavānyādi cūrṇa observed under 254 nm; Track 1: Batch 01, Track 2: Batch 02, Track 3: Batch 03

under visible light 0.36, 0.42 (both spots brown), 0.76 (pink) and 0.85 (red).

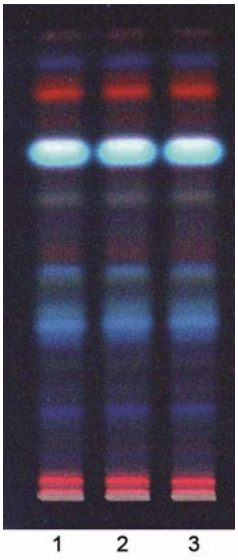



Fig. 2—TLC profile of Yavānyādi cūrṇa observed under 366 nm; Track 1: Batch 01, Track 2: Batch 02, Track 3: Batch 03

The Aflatoxin was absent in the formulated *Yavānyādi cūrṇa* (Fig. 5). The microbial profile of the *Yavānyādi cūrṇa* was found satisfactory. Total microbial plate count (average 200 cfu/gm), Yeast and Moulds (average 30 cfu/gm) counts were reported less than the limit set by API[9] and pathogenic bacteria,

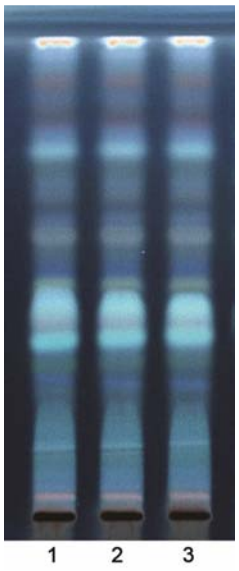



Fig. 3—TLC profile of Yavānyādi cūrṇa after spraying with *Anisaldehyde-sulphuric acid* reagent observed under 366 nm; Track 1: Batch 01, Track 2: Batch 02, Track 3: Batch 03

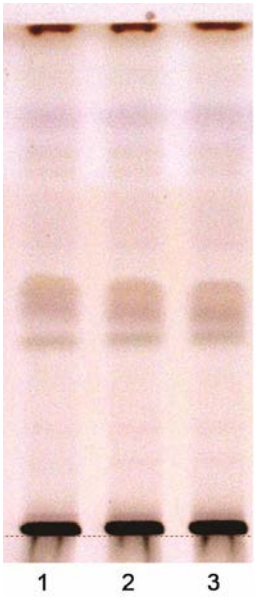



Fig. 4—TLC profile of Yavānyādi cūrṇa after spraying with *Anisaldehyde-sulphuric acid* reagent observed under visible light; Track 1: Batch 01, Track 2: Batch 02, Track 3: Batch 03

i.e. *Salmonella, Pesudomonas, Staphyllococcus* and *E.coli* were found to be absent (Figs. 6).

In the present study the level of heavy metals are within limit set by API (Table 4), thus the results reveal that the formulation are safe for consumption.

Na and K are detected and tabulated in Table 4, thus showing the purity of the raw drugs and also the finished product. This also indicates quality control maintenance during preparation.

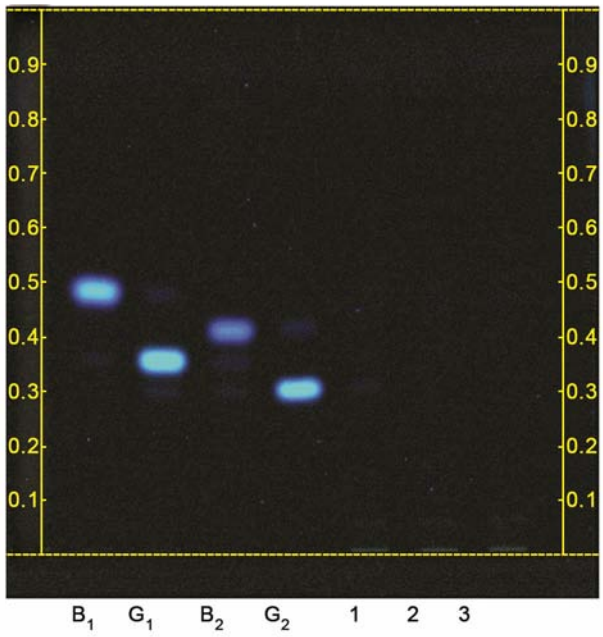
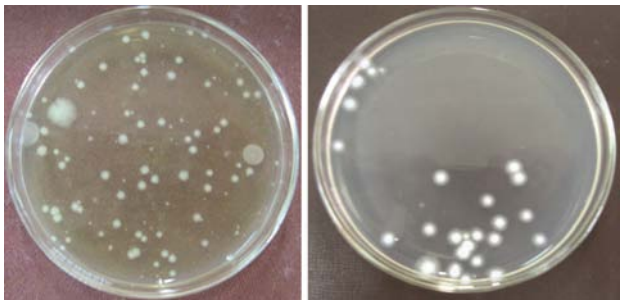



Fig. 5—TLC finger prints in test solution for Aflatoxin of *Yavānyādi cūrṇa* at 366 nm(Before derivatization), Standard markers (Aflatoxin)- $B_1$, $G_1$, $B_2$ and $G_2$ , Test Solutions of Yavānyādi cūrṇa for Track 1- Batch01, Track 2-Batch 02, Track 3- Batch 03



Figs. 6—Photographs of Microbiological limit test in *Yavānyādi cūrṇa*

Table 4—Heavy metals and Na & K assay of *Yavānyādi cūrṇa*

| S. no. | Parameters | *Yavānyādi cūrṇa* Batch 01 | Batch 02 | Batch 03 | Actual concentration unit | API Limits |
|---|---|---|---|---|---|---|
| 1. | Lead (Pb) | 5.8718 | 5.0256 | 5.6410 | ppm | 10 ppm |
| 2. | Cadmium (Cd) | 0.0639 | 0.0638 | 0.0657 | ppm | 0.3 ppm |
| 3. | Arsenic (As) | 11.6927 | 11.6543 | 0.0128 | ppm | 03 ppm |
| 4. | Mercury (Hg) | 19.4040 | 19.6654 | 0.0207 | ppm | 01 ppm |
| 5. | Total Sodium | 507.1 | 491.2 | 505.5 | ppm | — |
| 6. | Total Potassium | 304.0 | 297.0 | 320.2 | ppm | — |

## Conclusion

The *Ayurvedic* system of medicines is prevalent in India since the *Vedic* period and as early as the dawn of human civilization. Though, *Ayurveda* has undergone many changes in the course of its long history, it still remains the mainstay of medical relief to a large section of population of the nation. Due to urbanization and dwindling of forests, the *Vaidya* by and large is no longer a self contained unit collecting and preparing his own medicines as before. He has now to depend on the newly developed agencies like one collecting and supplying the crude drugs and the other undertaking mass production of medicines in the *Ayurvedic* pharmaceutical units run on the commercial scale[11]. Thus, the present study revealed that the microbiological features and the distinguished HPTLC fingerprints profile may be used as marker parameters for monitoring the quality of the formulation. Hence, the physicochemical parameters, biochemical analysis and HPTLC fingerprints profile can be used for quality evaluation and the standardization of compound formulations. Spiking of the formulations with the different genuine ingredients further confirms the presence of individual components in them.

## Acknowledgement

Authors are grateful to Dr Bharat Pathak, General Secretary, Deendayal Research Institute, Arogyadham, Chitrakoot, for providing the infrastructure and thankful to Department of AYUSH, Ministry of Health and Family Welfare, Government of India, for financial support under the scheme Centre of Excellence.

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