

## Mineral content and microbial impurity of *Triphala churna* and its raw materials

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Atomic absorption spectrophotometric study of the powdered fruits of amala, beheda, harda and market samples of triphala churna along with a laboratory preparation indicated that the highly toxic elements such as As, Hg, Co and Cd were absent, Pb being within the limits whereas less toxic or beneficial elements were within the limits specified by American Conference of Governmental Industrial Hygienists (ACGIH). The microbial studies of these samples showed complete absence of pathogens and presence of non-pathogens in amounts lower than the number specified in BP limits. The raw materials and triphala churna samples investigated in this study were considered safe for internal consumption.

**Keywords:** Triphala churna, Atomic Absorption Spectrophotometry, Microbial contamination.

Ayurvedic and Siddha systems specify medicinal plants containing various metal constituents for curing ailments. Minerals and metals present in biological system play a vital role in metabolism. There is a definite relationship between the element content of the plant, or one of its parts, and its nutritional status. Some elements are essential for normal growth and reproduction while others have some beneficial effects<sup>1</sup>. Any element, essential/useful or not, becomes toxic if taken up in excessive amounts. Toxicity of elements such as silver and barium has rarely been reported since they are present in nature in insoluble form, to be taken up by the plant. In contrast, toxic

effects of lead, arsenic, bismuth, bromine, etc. are well documented and the determination of the smallest traces of such elements has been of importance in toxicology for many years. Not only are the plants a source of entry of trace and toxic elements but so also are the various processes that take place right from the collection to the processing, packaging of the finished product. Recently, presence of toxicants, including lead, and microbial contamination in products has been reported in a number of Asian traditional or folklore medicines<sup>2,3</sup>. Awareness regarding the quality and safety of these formulations has led to the legal enforcement of GMP procedures for the manufacture and GLP guidelines for the QC (Quality Control) laboratories by

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FDA with effect from June 2002<sup>4</sup>. Besides, such preparations do not have export potential.

It is, therefore, essential to determine levels of all the elements present in plant parts and their finished products as a Quality Control data especially for preparations, which are extensively used. Triphala churna is one such popular Ayurvedic formulation<sup>5</sup>. Papers published earlier from this laboratory have reported on several QC standards on Triphala churna<sup>6,7</sup>. These are more specific to product and normally define identity, potency and purity of the materials.

This paper reports on the quantitative analysis of twenty inorganic elements along with levels of microbial bioburden and presence/absence of pathogenic microbes.

## Materials and Methods

### A Materials

#### a) Raw materials:

Dried fruits of *Amala* (*Emblica officinalis*), *Beheda* (*Terminalia bellirica*) and *Harda* (*Terminalia chebula*) were procured from Zandu Pharmaceuticals Pvt. Ltd., Mumbai. Samples of Triphala churna (MF1 – MF5) were procured from the market.

#### b) Chemicals:

Triple distilled water (J. K. Labs, Mumbai); HCl, H<sub>2</sub>SO<sub>4</sub> [AR grade – MERCK India]; silver nitrate, aluminium wire, arsenous oxide, barium carbonate, bismuth nitrate, calcium carbonate, cadmium chloride, cobalt nitrate, copper metal, iron wire, lead nitrate, mercury (II) oxide, potassium chloride, lithium

carbonate, magnesium wire, manganese wire, sodium chloride, nickel oxide, stannous chloride, zinc oxide and other chemicals used for preparing standard solutions of metals were of AR/GR grade or had the highest purity.

#### c) Media:

Sterile buffered sodium chloride peptone solution pH 7, Casein soyabean digest agar medium, Sabouraud dextrose agar, Nutrient broth, Sabouraud dextrose broth, MacConkey agar, Bismuth sulphite agar, Cetrimide agar, Vogel Johnson agar and Brain heart infusion agar were purchased from Hi-media.

## B Methods

### (I) Analysis of inorganic elements using Atomic Absorption Spectrophotometry

#### a) Sample Preparation:

Five grams each of the raw materials, laboratory preparation and the market samples were separately incinerated at 450°C in silica crucibles till there was no evolution of smoke. The ash obtained was heated in muffle furnace at 800°C till constant weight (approx. 2.5-3 hrs), post treatment with concentrated H<sub>2</sub>SO<sub>4</sub> and ignition.

#### b) Preparation of Reference Standard Solutions:

Standard solutions of each element were separately prepared using reference standard metals or their salts mentioned under A(b) “chemicals”. The procedure employed was as per the directions given in the Operation Manual<sup>8</sup> of the instrument. The solutions thus prepared were packed in tightly capped plastic bottles and used within 6 hours of their preparation.

c) *Preparation of Sample Solution:*

Samples of ash obtained under B(a) were dissolved in 100ml of dilute hydrochloric acid (1:20). The solutions were transferred to tightly capped plastic bottles and were used for determination of various mineral elements.

d) *Preparation of Reagent Blank Solution:*

0.5 ml conc. sulphuric acid and 5ml conc. hydrochloric acid were quantitatively added in 100ml volumetric flask and diluted with double distilled water to the mark.

e) *Determination of Concentration of Elements:*

Atomic Absorption Spectrophotometer (Perkin Elmer AAS Model – 3100 equipped with MHS –10)<sup>8</sup> was employed for the study. Individual hollow cathode lamps for each element or multiple element hollow cathode lamps were used as energy source. Using reference standards, the calibration curves were obtained for all the elements. The sample solutions were adequately diluted to keep the absorbance in the linear range. Air – Acetylene flame was used for the determination of Ag, As, Bi, Ca, Cd, Co, Cu, Fe, Hg, K, Li, Mg, Mn, Na, Ni, Pb and Zn and Nitrous oxide – Acetylene flame was used for determination of Al, Ba, and Sn.

(II) *Microbial Bioburden Level*<sup>9-12</sup>

(Note: All the operations were carried out in a laminar flow (LF) unit under aseptic conditions).

Total aerobic microbial count (bacterial and fungal) and tests for pathogenic microorganisms were carried out

following procedures described in IP (Pharmacopoeia of India). The pathogenic organisms for detection included *Escherichia coli*, *Salmonella typhi*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and pathogenic fungi.

a) *Sample Handling:*

Samples of raw materials, laboratory preparation and market formulations (MF1 – MF5) of Triphala churna were carefully packed in plastic containers. These were transferred post-disinfection of outside of containers to LF unit. All the instruments such as metal spatula, scissors, glass rods, pipettes, petri-plates etc were sterilized by dry heat at 160°C for 2hrs. These were carefully transferred through the hatch to LF cabinet in aseptic area.

b) *Media Preparation:*

Media specified in A(c) under Materials were prepared and sterilized using procedures described in IP. These were transferred to aseptic area through the hatch.

c) *Media Inoculation:*

The individual samples of powdered raw materials and Triphala churna (about one gram each) were individually transferred to 50ml of sterile media under aseptic conditions in LF unit. After uniform mixing, the inoculated media were incubated for 1-5 days.

## Results and Discussion

The conditions observed for the tests for aerobic bacteria, aerobic fungi and

pathogenic fungi are shown in Table 1. At the end of incubation period, the medium was observed for growth/no growth of organisms. The results obtained are shown in Table 2. As mentioned in the introduction, some elements such as arsenic, lead, bismuth, barium, mercury and germanium are toxic even in small concentrations while some trace

beneficial elements such as cobalt, gold, silver, selenium, etc. assume toxicity when ingested in sufficient amounts. Some of these get stored in liver or kidney and damage the organs, impairing their function. It is, therefore, important to have balance in the mineral intake. Triphala churna being a mixture of fruits from *amala*, *beheda* and *harda* may show

Table 1 — Sterilization and incubation parameters for selective media

| Micro-organism       | Selective Media               | Sterilization conditions | pH        | Incubation                |
|----------------------|-------------------------------|--------------------------|-----------|---------------------------|
| Aerobic bacteria     | Soyabean Casein Digest medium | 115°C, 30 min<br>15 lbs. | 7.0-7.4   | 30°-35°C<br>5 days        |
| Aerobic fungi        | Sabouraud Dextrose            | 115°C, 30 min<br>15 lbs. | 7.0-7.4   | 20°-25°C<br>5 days        |
| <i>S. aureus</i>     | Vogel Johnson Agar            | 115°C, 30 min<br>15 lbs. | 7.2 ± 0.2 | 35° to 37°C<br>24-48 hrs. |
| <i>P. aeruginosa</i> | Cetrimide Agar                | 121°C, 15 min<br>15 lbs. | 7.0-7.4   | 35° to 37°C<br>24-48 hrs. |
| <i>S. typhi</i>      | Bismuth Sulphite Agar         | —                        | 7.6 ± 0.2 | 36° to 38°C<br>48 hrs.    |
| <i>E. coli</i>       | MacConkey Agar                | 121°C, 15 min<br>15 lbs. | 7.1 ± 0.2 | 36° to 38°C<br>48 hrs.    |
| Pathogenic fungi     | Brain Heart Infusion Agar     | 121°C, 15 min<br>15 lbs. | 7.4 ± 0.2 | 20° to 25°C<br>4-7 days   |

Table 2—Microbiological study on Triphala churna and its raw materials

| Samples                                                                                                 | Total Aerobic Plate Count                               |                                                      | Total Aerobic Serial Dilution                           |                                                      | Whether within Limits |
|---------------------------------------------------------------------------------------------------------|---------------------------------------------------------|------------------------------------------------------|---------------------------------------------------------|------------------------------------------------------|-----------------------|
|                                                                                                         | Bacteria<br>[Limit NMT 10 <sup>5</sup><br>organisms/gm] | Fungi<br>[Limit NMT 10 <sup>4</sup><br>organisms/gm] | Bacteria<br>[Limit NMT 10 <sup>5</sup><br>organisms/gm] | Fungi<br>[Limit NMT 10 <sup>4</sup><br>organisms/gm] |                       |
| <i>Amala</i>                                                                                            | 170                                                     | 200                                                  | 180                                                     | 210                                                  | yes                   |
| <i>Beheda</i>                                                                                           | 140                                                     | 165                                                  | 90                                                      | 125                                                  | yes                   |
| <i>Harda</i>                                                                                            | 75                                                      | 110                                                  | 90                                                      | 85                                                   | yes                   |
| Lab. Prepn.                                                                                             | 220                                                     | 360                                                  | 115                                                     | 210                                                  | yes                   |
| MF -1                                                                                                   | 245                                                     | 380                                                  | 150                                                     | 290                                                  | yes                   |
| MF -2                                                                                                   | 300                                                     | 405                                                  | 145                                                     | 200                                                  | yes                   |
| MF -3                                                                                                   | 290                                                     | 335                                                  | 190                                                     | 345                                                  | yes                   |
| MF -4                                                                                                   | 180                                                     | 370                                                  | 135                                                     | 275                                                  | yes                   |
| MF -5                                                                                                   | 275                                                     | 360                                                  | 160                                                     | 300                                                  | yes                   |
| Pathogens                                                                                               |                                                         |                                                      |                                                         |                                                      |                       |
| <i>E. coli</i> , <i>S. typhi</i> , <i>Ps. aeruginosa</i> , <i>S. aureus</i> and <i>pathogenic fungi</i> |                                                         |                                                      |                                                         |                                                      | All absent            |

Table 3 — Quantitative levels of inorganic elements present in Triphala churna and its raw materials

| Sr. Samples No | Average Concentration of Inorganic Elements (ppm) |        |        |       |        |        |        |        |       |        |       |        |        |        |
|----------------|---------------------------------------------------|--------|--------|-------|--------|--------|--------|--------|-------|--------|-------|--------|--------|--------|
|                | Al                                                | Ba     | Bi     | Ca    | Cu     | Fe     | K      | Li     | Mg    | Mn     | Na    | Ni     | Pb     | Zn     |
| 1. Amala       | 0.18                                              | 0.0018 | 0.0016 | 1.91  | 0.059  | 0.0341 | 11.71  | 0.0021 | 1.121 | 0.0008 | 0.327 | 0.0089 | 0.0029 | 0.0481 |
| 2. Beheda      | 0.0204                                            | 0.001  | 0.0006 | 1.739 | 0.0197 | 0.0212 | 7.72   | 0.0068 | 0.777 | 0.0004 | 0.145 | 0.0386 | 0.0011 | 0.009  |
| 3. Harda       | 0.086                                             | 0.0006 | 0.0009 | 5.325 | 0.062  | 0.0633 | 5.19   | 0.0035 | 1.163 | 0.0014 | 0.608 | 0.0289 | 0.0022 | 0.005  |
| 4. Lab. Prepn. | 0.1031                                            | 0.0009 | 0.0008 | 2.858 | 0.0481 | 0.0423 | 9.62   | 0.0046 | 0.958 | 0.0006 | 0.428 | 0.0176 | 0.0016 | 0.006  |
| 5. MF-1        | 0.097                                             | 0.0005 | 0.0007 | 3.279 | 0.0538 | 0.0455 | 11.02  | 0.0019 | 0.862 | 0.0008 | 0.383 | 0.0409 | 0.0012 | 0.005  |
| 6. MF-2        | 0.0828                                            | 0.0008 | 0.0008 | 2.725 | 0.0371 | 0.0369 | 10.695 | 0.0045 | 1.046 | 0.0009 | 0.327 | 0.0228 | 0.0016 | 0.004  |
| 7. MF-3        | 0.121                                             | 0.0007 | 0.0004 | 2.221 | 0.0453 | 0.0347 | 11.731 | 0.0032 | 1.258 | 0.0011 | 0.306 | 0.0371 | 0.0023 | 0.006  |
| 8. MF-4        | 0.1133                                            | 0.0009 | 0.0006 | 2.793 | 0.0441 | 0.0291 | 11.288 | 0.0039 | 1.129 | 0.0007 | 0.307 | 0.0187 | 0.0018 | 0.007  |
| 9. MF-5        | 0.089                                             | 0.0008 | 0.0007 | 3.152 | 0.0541 | 0.0368 | 10.234 | 0.0026 | 1.113 | 0.0010 | 0.333 | 0.0254 | 0.0020 | 0.007  |
| 10. Limits*    | -NA                                               | 0.500  | 0.02   | -NA   | -NA    | -NA    | -NA    | -NA    | -NA   | -NA    | -NA   | 1.50   | 0.05   | 2.00   |

(n = 3): Average of three readings.

Limits\* - indicate Time-weighted average limits established by the American Conference of Governmental Industrial Hygienists<sup>14</sup>.

Sample - 4 refers to Laboratory sample of Triphala churna.

Elements including Ag, As, Co, Cd, and Hg were found to be below the detectable level/ or absent. Their respective reported toxic levels are 0.1 ppm, 0.01 ppm, 0.02 ppm, 0.05 ppm and 0.021 mg/day<sup>13,14</sup>.

variable contents in their elemental composition depending on type of the soil, fertilizer used and other conditions where these fruit-bearing plants are grown. This variation may alter the potency, safety and efficacy of plant products. The results recorded in Table 3 indicate that the levels of elements, viz. Ag, As, Cd, Co and Hg were either below detection level or were absent. As regards the amounts of Ba, Bi, Ni, Pb and Sn, they were found to be below the Time-weighted average limits established by the American Conference of Governmental Industrial Hygienists (ACGIH)<sup>14</sup>.

As regards the microbial contamination, the pathogens (mentioned in IP) were found to be absent. The results from the total aerobic microbial count (bacteria and fungi) indicated that the counts were below the limits prescribed in BP (IP does not specify any limits).

On the basis of the above, the raw materials as well as Triphala churna formulations are considered to be safe for internal use. Formulation manufacturers may specify the levels of both toxic and nontoxic elements in the laboratory Quality Specifications.

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