Physicochemical, Preliminary Phytochemical and Powder Microscopical studies of “M-Swadishta Virechana Churna” – An Ayurvedic Polyherbal Formulation

Venkateshwarlu G*1, Shantha T R1, Venakata Narasimhaji CH1, Shubhashree M N1, S.K.Giri1, Sridhar B N1, Ramesh Babu D2

1. National Ayurveda Dietetics Research Institute, Jayanagar, Bangalore-560 011.
2. Central Council for Research in Ayurveda and Siddha, Janakpuri, New Delhi.

Abstract

Ayurvedic formulation M-Swadista virechana churna is known to be an effective laxative in diseases like Arsha (heamorrhoids), Ama-sangraha (accumulation of Ama-undigested toxic substance) and Vibandha (constipation). Literature reviews revealed that there are no reports available on the physicochemical standardization & Powder microscopy of “M-Swadishta virechana churna. Hence, analytical studies have been taken up to derive the Pharmacopoeial standards for quality control. The ayurvedic compound formulation, M-Swadishta virechana churna contains Swarnapatri (Cassia angustifolia), Madhuka (Glycerrhiza glabra) and Ikshu Sarkara (cane sugar- Saccharum officinarum). The present communication deals with the physico chemical, preliminary phyto chemical and chromatographic with powder microscopical studies gave valuable parameters for laying down Pharmacopoeial standards of the compound formulation.

Keywords: Ayurvedic formulation, M-Swadishta virechana churna, physico chemical, phytochemical studies, chromatographic, powder microscopic studies. Pharmacopoeal standards

Introduction

Ayurveda is an ancient medical science for the restoration of health and prevention of diseases. Swadista virechana churna is one among the popularly used Ayurvedic compound formulation administered as a laxative in diseases like malavaroda (constipation), amavruddhi(increase of undigested toxic substance), shiroroja(headache), arsha (haemorrhoids), raktabikara(blood disorders), charmaroga (skin diseases), pama(scabies), udara shodanartha (therapeutic purgation) (1).

M-Swadishta Virechana churna is a variant of Swadishta virechana churna containing Swarnapatri (Cassia angustifolia), Madhuka (Glycerrhiza glabra) and Ikshu sarkara (cane sugar- Saccharum officinarum) primarily indicated in arsha(haemorrhoids), aamasangraha (accumulation of undigested toxic substance) and vibandha(constipation).

The main ingredient of M-Swadishta Virechana churna is Swarnapatri(Cassia angustifolia), also known as senna. The leaves contain flavanols, isorhamnetin, kaempferol, rhein, emodin and anthraquinone glycosides i.e sennosides A, B, C & D, which are laxatives and a popular remedy for constipation because of its stool-softening

*Corresponding author:
Assistant Director (Ay.)
National Ayurveda Dietetics Research Institute, GCP Annexe, Ashoka pillar, Jayanagar, Bangalore-560 011.
Email ID: drvenkatg@gmail.com
action and frequently used in Indian systems of medicine (2).

The active component of senna leaves Anthraquinone glycoside molecules are converted by the normal bacteria in the colon into rhein-anthrone, which in turn has two effects. It first stimulates colon activity and thus speeds bowel movements. Second, it increases fluid secretion by the colon. Together, these actions work to get a sluggish colon functional again. Several controlled studies have confirmed the benefit of senna in treating constipation (3).

The other ingredient of formulation, Madhuka (Glycyrrhiza glabra Linn) is commonly known as liquorice and sweet wood belonging to leguminosae family. The liquorice roots are sweet, refrigerant, tonic, mild laxative, aphrodisiac, haemostatic and an active anti-allergic, anti-inflammatory, spasmolytic, antiulcer and liver protective herb is widely used Ayurveda. Glycerrhiza glabra Linn contains tri terpene, saponins, flavonoides, polysaccharides, pectines, simple sugars, amino acids, mineral salts and various other substances(4). Glycyrrhizin, a triterpenoid compound accounts for the sweet taste of Glycyrrhiza glabra Linn. roots (Madhuka). The isoflavones, glabridin and hispaglabridines A and B have significant antioxidant activity (5).In vitro studies have demonstrated liquorice isoflavones, hispaglabridines A and B inhibit Fe^{3+} induced mitochondrial lipid peroxidation in rat liver cells (6).

Another adjuvant ingredient of the compound formulation, Ikshu sarkara (cane sugar- Saccharam officinarum) have the properties of sweet, unctuous, coolant, nourishing, aphrodisiac, mild laxative, antiemetic (7). It offers a host of essential nutrients and minerals, which contains magnesium, phosphorus, calcium, potassium and iron. Cane sugar also have mild laxative action, thus helps in prevent cancers of colon, prostate and breast cancer and also helps revitalize, energize and hydrate the body (8).

Though, the leaves of Swarnapatri or Senna (Cassia angustifolia) have an excellent laxative quality, but afterwards have a binding effect. It can also cause nausea and gripping pains when used alone due to inhibition of water and electrolyte absorption from the large intestine, which increases volume and pressure of the intestinal contents and thus stimulate the colon motility resulting in propulsive contractions (9). However, the adverse effects of the senna can be counter balanced by using Madhuka (Glycerrhiza glabra Linn.) and Ikshu sarkara (Cane sugar- Saccharam officinarum).

**Methodology**

M-Swadisht Virechana churna is a polyherbal formulation, a variant of Swadishta virechana churna consisting of 3 ingredients viz. Swarnapatri (Cassia angustifolia) and Madhuka (Glycerrhiza glabra) along with Ikshu sarkara (cane sugar- Saccharam officinarum) (Table.1).

The drug sample (batch 313) was procured from “Indian Medical Pharmaceutical Company Ltd.(IMPCL)” Almora, India.

Organoleptic evaluation was used for identification of sensory characteristics powder like colour, odour (smell), touch and taste.

For powder microscopic analysis, about 1 to 3 pinch of the sample was warmed with 2 to 4 drops of chloral hydrate solution, water and little safranin stain and observed under the microscope to identify the diagnostic features of the compound formulation for the presence of different fragments of tissues in curma. It was also observed by putting a pinch of phloroglucinol, 2 drops of concentrated hydrochloric acid and few drops of alcohol for the presence of lignin containing tissues.(10)

Fluorescence analysis of the powder was observed under UV light according to Chase and Pratt et al.(11)
Physicochemical analysis like determination of percentage of moisture content (loss on drying at 105°C), Ash value, water soluble ash, total insoluble ash in various solvent, sulphated ash, percentage of soluble extractives in methanol, ethanol & ether. pH of filtrate of 10% w/v aqueous solution was carried out as per methods described in Quality control methods for medicinal plants (WHO, 1998) (12)

TLC studies have been carried out according to Igon Stahl et al.(13) and preliminary phytochemical studies were carried out according to Kokate.C.K et al. (14)

Phyto chemical constituents of the ingredients of M-Swadista virechana curna has been reviewed and preliminary phytochemicals of the compound formulation have been screened.

Observations and Result

Macroscopic (organoleptic) characters of the powder [Fig.II(1)]

Powder is light green in colour, slightly pleasant odour, smooth and fine in touch, sweet, & bitter in taste.

Microscopic characters [Fig.II(1a-20)]

The polyherbal powder was treated with chloral hydrate, water & glycerin and microscopical examination was carried out for the presence of following different fragments of tissues.

- Abundant different kinds of tissues like Sclerenchymatous fibers,
- Parenchymatous cells containing Prisms of Calcium oxalate crystals,
- Unicellular warty trichomes.
- Polygonal epidermal cells in surface view with Paracytic stomata,
- Clusters of calcium oxalate and prism shaped crystals,
- Crystal fibers and epidermal cells with cuticle, Simple starch grains,
- Groups of lignified fibers, Pitted xylem vessel,
- Epidermal cells and parenchyma cells, thin walled parenchymatous cells,
- Rectangular to polygonal stone cells with broad lumen,
- Pitted xylem vessels, lignified fibers,
- Thin walled parenchymatous cells,
- Abundant Prisms of calcium oxalate crystals.

Physicochemical and Preliminary phytochemical studies

The physicochemical studies were carried out and the observations are given in Table 3. The test for percentage of moisture content (loss on drying) determines both water and volatile matter. Total ash measures the amount of materials remaining after ignition. Acid insoluble ash measures the amount of silica present especially sand and siliceous matter. Extractive values were examined which are useful for evaluation of nature of chemical constituents present in drug (Table 3).

Active phytochemical constituents in each of the ingredient of the formulation has been reviewed (Table 2) and Preliminary phytochemical screening of compound formulation was identified through qualitative chemical analysis indicated the presence of alkaloids, carbohydrates, flavonoids, terpenoids, resins, saponins, steroid and tannins etc. (Table 4).

Fluorescence analysis

The fluorescence behavior of the powdered drug in different solutions towards ordinary light and Ultra Violet light (both long 365nm & short 254nm wavelengths) were observed (Table 5).

Thin Layer Chromatographic studies (TLC)
TLC studies of the chloroform and methanol extract (8:2) was carried out in various solvent systems at 30°C using Silica gel GF$_{254}$ precoated sheets of thickness 2mm. The chloroform and methanol extract (8:2) was found to be the best suitable mobile phase for the extract.

The TLC pattern obtained by the capillary action of mobile phase through the stationary phase. Thin layer chromatography pattern obtained by the methanol extract of curna showed under different light include UV (short&long wave) developed in Iodine chamber gave 10 spots of Rf values (Table 6).

Table 1. Ingredients of M-Swadista virechana curna

| Botanical name of the ingredient | Sanskrit name | Parts used | Quantity (per 100gm) |
|---------------------------------|---------------|------------|---------------------|
| Cassia angustifolia Vahl.       | Swarnapatri   | Leaves     | 42.3 gm             |
| Glycrrhiza glabra Linn.         | Madhuka       | Roots      | 21.16 gm            |
| Saccharum officinarum Linn.     | Ikshu sarkara | Cane sugar | 36.54gm             |

Table 2. Phyto chemical constituents of the ingredients of M-Swadista virechana curna

| Ingredient | Bot.Name | Family | Parts used | Phytochemical constituents |
|------------|----------|--------|------------|---------------------------|
| Swarnapatri| Cassia angustifolia Vahl. | Caesalpininiace | Leaves | Anthraquinone glycosides (+ i.e.sennosides A,B,C), Flavonoides+, Polyol+, sugars+, Free acids+, Leucanthocyanines+, Phytosterols+, Free amino acids+, Mucilage+, Resins + Tannins+, volatile oil+ |
| Madhuka    | Glycerrhiza glabra Linn. | Leguminseae | Roots | Glycosides(glycyrrhizin)+, Saponin+ Flavonoides++ Isoflavonoides+ Volatile oil+ Amino acids+ Beta-sitosterol+ Glucose and sucrose+ Tannins (traces)+ Gum+ Vitamins: A, B1, B2, B6, |
Table 3. Physico-chemical studies of M-Swadista virechana curma:

| Sl.No | Parameters                                    | Values              |
|-------|-----------------------------------------------|---------------------|
| 1     | Loss on drying at 105° c {or} Moisture content (%) | 5.29                |
| 2     | Water-soluble extractive (%)                  | 62.85               |
| 3     | Methanol soluble extractive (%)               | 41.78               |
| 4     | Ash content (%) (Total)                       | 4.14                |
| 5     | Acid-insoluble ash                            | 0.318               |
| 6     | pH                                            | 4.18                |
| 7     | Ether soluble extractive (%)                  | 8.18                |
| 8     | Sulphated ash (%)                             | 7.99                |
| 9     | Ethanol soluble extractive (%)                | 14.18               |
| 10    | Particle size (80 mesh)                       | Moderately fine powder |
| 11    | TLC (observed no. of spots)                   | 10                  |
| 12    | Rf Values (calculated)                        | 0.047;0.154;0.369;0.392;0.52;0.61;0.702;0.809;0.857;0.928 |

Table 4. Preliminary phytochemical constituents of the finished product of M-Swadista virechana curma

| Sl.No | Phytochemical constituent | Methanol extract |
|-------|---------------------------|------------------|
| 1     | Alkaloids                 | +ve              |
| 2     | Carbohydrates             | +ve              |
| 3     | Flavonoids                | +ve              |
| 4     | Terpenoids                | +ve              |
| 5     | Resins                    | +ve              |
| 6     | Saponins                  | +ve              |
| 7     | Steroids                  | +ve              |
| 8     | Tannins                   | +ve              |
| 9     | Starch                    | -ve              |

Table 5. Fluorescence analysis

| Sample + Reagent | Observation under Ordinary light | U.V. Long wave 365 nm | U.V. Short wave 254 nm |
|------------------|----------------------------------|-----------------------|------------------------|
| Powder as such   | Light green                      | Fluorescent green      | Yellowish green         |
| Powder +         |                                  |                       |                        |
| Extractive          | Adsorbent       | Solvent system                  | Rf values (viewed in iodine chamber) |
|---------------------|-----------------|---------------------------------|-------------------------------------|
| Methanol            | Silica gel GF254 precoated sheets | Chloroform: Methanol; 8:2 | 0.047; 0.154; 0.369; 0.392; 0.52; 0.619; 0.702; 0.809; 0.857; 0.928 |

Table 6. TLC Studies

Fig.I (Plates 1-3). T.L.C of M-Swadista virechana curma in Methanol extract

Chloroform: Methanol 8:2 Under UV 254 nm

Chloroform: Methanol 8:2 Under UV 366 nm

Chloroform: Methanol 8:2 In Iodine vapour

Fig.II (1-20). Macroscopical and Microscopical characters of M-Swadista virechana Churna
| Fig. 1 | Fig. 1a | Fig. 2 |
|-------|--------|--------|
| Macroscopy of the churna | Different fragments of tissues. 10xX10x | Sclerenchymatous fiber 10xX10x |

| Fig. 3 | Fig. 4 | Fig. 5 |
|-------|--------|--------|
| Parenchymatous cells containing Prisms of Calcium oxalate crystals 10x X 10x | Unicellular trichome. 10xX10x | Polygonal epidermal cells surface view. 10xX10x |

| Fig. 6 | Fig. 7 | Fig. 8 |
|-------|--------|--------|
| Paracytic stomata 10xX10x | Clusters of calcium oxalate and prism shaped crystals. | Crystal fibers and epidermal cells. With cuticle 10xX40x |

| Fig. 9 | Fig. 10 | Fig. 11 |
|-------|--------|--------|
| Simple starch grains.10xX40x | Groups of lignified fibers. 10xX40x | Pitted xylem vessel, epidermal cells and parenchyma cells. 10xX40x |
Discussion and Conclusion

The sample of M.Swadista virechana churna was found to be light green in colour moderately fine powder with pleasant smell and sweet & bitter in taste. The churna did not show any fungus or objectionable matter when spread out on the petridish. It was observed that the sample passed through 60-mesh sieve and not less than 50% passed through 80-mesh sieve.

Microscopic characterization of powder revealed abundant different kinds of tissues like Sclerenchymatous fibers, parenchymatous cells containing prism shaped and clusters of calcium oxalate crystals, unicellular warty trichomes. It was also observed polygonal epidermal cells in surface view with paracytic stomata, crystal fibers and simple starch grains and group of lignified fibers, pitted xylem vessels [Fig.II (1a to 20)].

The physicochemical analysis was carried out and observations are presented in table 3. TLC profile of the sample was depicted in table 6 and the chromatogram was observed (fig.1). TLC of methanol extract curve using chloroform: methanol.
(8:2) w/v shows under UV light (short&long wave) developed in Iodine chamber gave 10 spots of RF values 0.047;0.154;0.369;0.392;0.52;0.619;0.702; 0.809;0.857;0.928.

Preliminary phytochemical constituents in M-Swadista virechana churna like alkaloids, carbohydrates, flavonoids, terpenoids, resins, saponins, steroids and tannins could have pharmacological action on their own or in conjunction with body fluids in terms of efficacy to possibly help the body to reckon with ailments.

The results obtained could be immense useful in laying down the pharmacopeial standards of M.Swadista virechana churna.

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