Physico-chemical investigation of a polyherbal formulation - Vidangatandulaadi choorna

Amrutha S. Babu¹, Mahadevan Subramaniam², Mahesh C. Kundagol¹, James Chacko¹, Devipriya Soman*¹

¹Department of Kayachikitsa (General Medicine), Amrita School of Ayurveda, Amritapuri, Amrita Vishwa Vidyapeetham, Kollam-690525, Kerala, India
²ACARA Research Lab, Amrita School of Ayurveda, Amritapuri, Amrita Vishwa Vidyapeetham, Kollam-690525, Kerala, India

Article History:
Received on: 15 Aug 2020
Revised on: 16 Sep 2020
Accepted on: 17 Sep 2020

Keywords:
High-performance thin-layer chromatography, Inductively Coupled Plasma - Mass Spectrometry, Physico-chemical, Vidangatandulaadi Choorna

Vidangatandulaadi Choorna is a polyherbal formulation consisting of seven ingredients. Trivirth (Operculina turpethum (Linn.) Silva Manso) is the chief ingredient responsible for the purgative action of the formulation. This yoga is mentioned in Kalpasthana of Ashtangahridaya, intended for virechana (Purgation). It is useful in Kapha-vatha disorders. Even though many kinds of research have been done to identify the physicochemical constituents of individual drugs in the formulation, no studies were done to identify the physicochemical properties of the formulation. This analysis helps in understanding the mechanism for different pharmacological actions of the formulation. Hence, Physico-chemical study of Vidangatandulaadi Choorna along with high-performance thin-layer chromatography (HPTLC) fingerprinting is done to fix the standards. All the drugs included in the formulation is identified by the botanist and is prepared according to Standards mentioned for the preparation of Choorna mentioned in Ayurvedic Pharmacopoeia of India. The formulation is least encountered, but it has shown its significant action in Dyslipidemia in folklore practices. As there are no Standards mentioned for this formulation, the result observed in the present study may be considered suitable. The data obtained from Physicochemical investigation, high-performance thin-layer chromatography profile and ICP-MS (Inductively Coupled Plasma - Mass Spectrometry) could be used as the standards for the present formulation under study.

INTRODUCTION

Vidangatandulaadi Choorna is one of the clinically significant formulations used in the management of Dyslipidemia. It is mentioned in Ashtangahridaya, kalpasthana as a Nityavirechaka (mild laxative) (Ashtangahridaya of Vagbhata, 2002). It is also indicated in the management of Gulma (abdominal distention), Pleeha (Diseases related to the spleen), Udara (ascites), Kasa (cough), Haleemaka (Jaundice), Arochaka (loss of taste) and all Kapha-Vatha diseases. It contains seven ingredients like Vidanga (Embelia ribes Burm. f.), Harithaki (Terminalia chebula Retz.), Vibhitaki (Ter-
minalia belerica Roxb.), Amalaki (Emblica officinalis Gaertn.), Yavakshara (Hordernium vulgare Linn.), Pippali (Piper longum Linn.), Trivrit (Orectolina turpethum (Linn.) Silva Manso). All the six drugs except Trivrit are taken in equal quantity, and Trivrit, half of the amount of the total other six ingredients. Dravyaguna experts identified all the drugs, and Choorna was prepared according to the Standards mentioned for Choorna preparation (Department Of Ayush, 2007a).

The said medicine is used as a traditional method in managing Dyslipidemia. No studies have been published related to Vidangatandulaadi Choorna exploring its Physico- Chemical properties and Pharmacological effects. So an attempt is made to understand the Physico-Chemical properties of the drug along with HPTLC profile to fix the standards of the drug, which may lay a future scope for further studies related to this drug. The result of the study can be used to explain the therapeutic benefits of the medicinal formulation.

MATERIALS AND METHODS

All the drugs were procured from Amrita Life, the manufacturing unit under Amrita School of Ayurveda, Vallikavu, Kollam. The authenticity of the drug was confirmed by botanist and experts in Department of Dravyaguna, Amrita School of Ayurveda, Vallikavu, Kollam. Choorna was prepared based on API. Ingredients of the Choorna is mentioned in Table 1. Physico-Chemical parameters like a loss on drying, Water-soluble extractive, Alcohol soluble extractive, pH of 10% solution of Total ash, Acid insoluble ash were done as per API standard guidelines (Department Of Ayush, 2007b). HPTLC fingerprinting was done. ICP-MS was used to estimate the heavy metal contents in the prepared drug.

Materials

Physico-chemical study

Vidangatandulaadi Choorna, Beaker, Crucible, Demineralised water, Digital pH meter, Round bottom flask, Chloroform, Electric incinerator, Desiccator, Diluted HCl and Filter paper was used.

HPTLC Analysis

Test solution used is 1 gram Vidangatandulaadi Choorna and 10ml Methanol. Stationary Phase is Merk, 1,0554.0001. TLC Silica gel 60 F254,10 × 10 Aluminium sheet. The mobile phase is Toluene: Ethyl acetate: Formic acid: Methanol (7:5:1:0.5). Development with CAMAG 10 × 10cm Twin trough chamber. HPTLC instrumentation used is CAMAG Linomat 5, CAMAG TLC Scanner 3, CAMAG Reprostar 3. Derivatisation with iodine vapour.

ICP-MS (Inductively Coupled Plasma – Mass Spectrometry)

Instrument used is ICP – MS (Make: Agilent, 7800, Japan) and the materials used are Microwave digesting system (MDS) tube, Con.HNO3, Con. HCl, H2O2 and Standard flask.

Methods

Physicochemical investigation

Determination of pH

Procedure - Ten gram of total ash was dissolved in 100ml of demineralised water. The pH of this 10% solution was measured with a digital pH meter.

Determination of Water-soluble extractive

Procedure - Five gram powdered drug was placed in a round bottom flask and mixed with 100 ml chloroform water. It is kept for 24 hrs with occasional shaking. After that, it was filtered and the filtrate collected in a tared clean beaker. The residue was weighed after evaporating to dryness.

Determination of Alcohol soluble extractive

Procedure - Five gram powdered drug was placed in a round bottom flask. It was mixed with 100ml alcohol. It was kept for 24 hours with occasional shaking. It was filtered and the filtrate collected in a tared clean beaker. The filtrate was evaporated to complete dryness, and then the remnant was weighed.

Determination of Total ash

Procedure - Two-gram air-dried drug was weighed with accuracy. It was placed in a tared crucible. It was heated in an incinerator gently, and then the drug was incinerated to ash until it was free from any organic matter. The crucible was kept in a desiccator and cooled and weighed with the contents. Percentage of ash concerning the air-dried drug was calculated.

Determination of Acid insoluble ash

Procedure - Total ash was prepared with two gram dried drug. The whole total ash was dissolved in 25ml diluted HCl. It was boiled for 5 minutes. The insoluble portion was filtered through an ashless filter paper. It was washed with demineralised water. Dried filter paper with the contents was selected. It was incinerated in a tared silica crucible with the contents. The incinerated content with the crucible was weighed. Percentage of acid-insoluble ash was calculated.
**HPTLC analysis of Vidangatandulaadi Choorna**

Procedure - One gram *Vidangatandulaadi Choorna* sample was taken, extracted with 10ml Methanol, and spotted as ten microliters. The stationary phase is Merk, 1.0554.0007, TLC Silica gel 60 F$_{254}$,10×10 Aluminium sheet. Mobile phase taken is Toluene: Ethyl acetate: Formic acid: Methanol (7:5:1:0.5). The plate was development in CAMAG 10×10cm Twin trough chamber. HPTLC instrumentation used is CAMAG Linomat 5, CAMAG TLC Scanner 3, CAMAG Reprostar 3. Derivatisation is done in Iodine vapour (Evans, 2009).

**ICP-MS (Inductively Coupled Plasma – Mass Spectrometry)**

Procedure - About 200-500 mg of the sample was accurately weighed and transformed in a cleaned microwave digesting system(MDS)tube. An adequate amount of Con.HNO$_3$, Con. HCl and a few drops of H$_2$O$_2$ were added to the MDS tube. The MDS tube was further kept in microwave digestive system for complete digestion of solid samples to liquid for one hour at a temperature of 180°C. The resultant liquid sample was carefully transferred to a 50ml standard flask and diluted to 50ml. The diluted sample was directly aspirated into the ICP-MS instrument, and the result was obtained (Wilschefski and Baxter, 2019).

**OBSERVATIONS**

**Observation on Physicochemical parameters of Vidangatandulaadi Choorna**

The analysis of Physicochemical parameters of *Vidangatandulaadi Choorna* revealed the following observations. It appears as a dark brown powder. The loss on drying is estimated to 3%w/w. The pH of 10% solution of content is 5.23. The water-soluble extractive is 28.23% w/w, Alcohol soluble extractive is 31.58% w/w, Ash value is 5.80% w/w and acid insoluble ash is below the detection limit.

**Observation on HPTLC analysis of Vidangatandulaadi Choorna**

Peak display (Densitogram) of *Vidangatandulaadi Choorna* sample at 254nm is shown in Figure 1. Peak display (Densitogram) of *Vidangatandulaadi Choorna* sample at 366nm is shown in Figure 2. Fingerprint profile (Chromatogram) of *Vidangatandulaadi Choorna* sample at 254 nm, at 366 nm and White light is shown in Figure 3. Derivatised TLC plate views of *Vidangatandulaadi Choorna* sample at 254 nm, at 366 nm and at White light, is shown in
Table 1: Ingredients of *Vidangatandulaadi Choorna*

| Sanskrit Name | Scientific Name       | Parts Used | Quantity |
|---------------|-----------------------|------------|----------|
| 1. Vidanga    | *Embelia ribes* Burm. f. | Seed       | 1 Part   |
| 2. Harithaki  | *Terminalia chebula* Retz. | Fruit rind | 1 Part   |
| 3. Vibhitaki  | *Terminalia belerica* Roxb. | Fruit rind | 1 Part   |
| 4. Amalaki    | *Emblira officinalis* Gaertn. | Fruit rind | 1 Part   |
| 5. Yavakshara | *Hordeum vulgare* Linn. | Whole plant | 1 Part  |
| 6. Pippali    | *Piper longum* Linn.    | Fruit      | 1 Part   |
| 7. Trivirth   | *Operculina turpethum* (Linn.) Silva Manso | Root      | 3 Part   |

Table 2: RF value & % area of *Vidangatandulaadi Choorna* sample at 254nm

| Peak No | RF Value | Area (AU) | % Area (AU) |
|---------|----------|-----------|-------------|
| 1       | 0.08     | 4356.9    | 6.11        |
| 2       | 0.14     | 4287.4    | 6.01        |
| 3       | 0.17     | 3354.3    | 4.70        |
| 4       | 0.23     | 9496.7    | 13.31       |
| 5       | 0.33     | 1210.8    | 1.70        |
| 6       | 0.36     | 574.5     | 0.81        |
| 7       | 0.42     | 7531.4    | 10.56       |
| 8       | 0.57     | 20039.1   | 28.09       |
| 9       | 0.71     | 9738.7    | 13.65       |
| 10      | 0.78     | 10745.1   | 15.06       |

Table 3: RF value & % area of *Vidangatandulaadi Choorna* sample at 366nm

| Peak No | RF Value | Area (AU) | % Area (AU) |
|---------|----------|-----------|-------------|
| 1       | 0.06     | 1627.5    | 3.17        |
| 2       | 0.07     | 3365.7    | 6.55        |
| 3       | 0.13     | 2260.2    | 4.40        |
| 4       | 0.16     | 2253.5    | 4.39        |
| 5       | 0.23     | 736.3     | 1.43        |
| 6       | 0.42     | 35631.8   | 7.66        |
| 7       | 0.69     | 3936.4    | 7.66        |
| 8       | 0.83     | 1554.3    | 3.03        |

Figure 4. RF value & % area of *Vidangatandulaadi Choorna* sample at 254nm with Total peak no:10 and Total area 71334.9 (AU) is given in Table 2. RF value & % area of *Vidangatandulaadi Choorna* sample at 366nm with Total peak no:8 and Total area 51365.7 (AU) is given in Table 3.

**Observations on ICP-MS (Inductively Coupled Plasma – Mass Spectrometer)**

The presence of heavy metals observed by Inductively Coupled Plasma – Mass Spectrometer is given as follows. Lead accounts the maximum value reaching about 1.36mg/kg and Mercury about 0.65mg/kg. The presence of Arsenic is 0.18mg/kg and Cadmium is 0.12mg/kg.

**RESULTS AND DISCUSSION**

**Physico- Chemical study**

Degradation time of the plant material indicates the quantity of moisture content in it. The powdered plant material degrades quickly due to the growth of microbes and fungus if the moisture content is high. The loss on drying was only 3% for the present sample, which ensure a reasonable period of shelf life for it. Presence of minerals and silica in the plant material was indicated by the Total ash value, which was obtained as 5.80%. The amount of acid-insoluble siliceous matter present in the plant was under the detectable limit. 28.23% w/w water-soluble extractive value indicated that *Vidangatandulaadi Choorna*...
contains sugar, acids and inorganic compounds. The alcohol-soluble extractive indicated the presence of polar constituents like alkaloids, glycosides, phenols etc.

**HPTLC Analysis**

HPTLC fingerprinting profile of *Vidangatandulaadi Choorna* was developed in Toluene: Ethyl acetate: Formic acid: Methanol (7:5:1:0.5) solvent system. This was taken as the standard Densitogram of *Vidangatandulaadi Choorna*. Densitogram showed ten peaks at 254nm and eight peaks at 366nm. Each peak represents a chemical entity. Almost four spots are seen repeated in 254 and 366nm. There is a scope for further analysis for finding out the chemical compounds represented by the peaks. To find out the chemical nature represented by the peaks, TLC-MS may be utilised, in the present solvent system, Toluene: Ethyl acetate: Formic acid: Methanol (7:5:1:0.5). At 366nm there are fluorescent spots with green, blue, red and pink colours. Three distinct brown spots are there; it may indicate compound with unsaturation.

**ICP-MS (Inductively Coupled Plasma – Mass Spectrometer)**

The procedure estimates the number of heavy metals present in the given sample. As per WHO permissible limit of heavy metals like Mercury is 1ppm, lead is 10 ppm, Cadmium is 0.3 ppm, and arsenic is 3 ppm (*Department Of Ayush, 2007c*). Here in our study, we got all these values under the normal limit, which increases the authenticity of the sample.

**CONCLUSION**

*Vidangatandulaadi Choorna* was studied for understanding its physicochemical parameters and HPTLC profile. As there are no standards mentioned for this formulation, the physicochemical data and HPTLC profile evolved from the present study could be used as standardisation parameters of the formulation.

**ACKNOWLEDGEMENT**

Authors are grateful to Amrita School of Ayurveda, Kollam, India for providing facilities for conducting this study.

**Funding source**

The authors declare that they have no funding support for this study.

**Conflict of interest**

There is no conflict of interests as declared by authors.

**REFERENCES**

Ashtangahridaya of Vagbhata 2002. Hari Sadashiva Sastri Paradakara, kalpasthana. verse-2/15-16, Chowkamba Sanskrit Sansthana, Varanasi, Reprint.

Department Of Ayush 2007a. The Ayurvedic Pharmacopoeia of India. Government of India, Ministry of Health and Family Welfare, New Delhi. First Edition, Part II, Volume I, Page-140.

Department Of Ayush 2007b. The Ayurvedic Pharmacopoeia of India. Government of India, Ministry of Health and Family Welfare, New Delhi. First Edition, Part II, Volume I, Page 147.

Department Of Ayush 2007c. The Ayurvedic Pharmacopoeia of India. Government of India, Ministry of Health and Family Welfare, New Delhi. First Edition, Part II, Volume I, Page 248.

Evans, W. 2009. Trease and Evans Pharmacognosy. pages 139–139. Saunders Ltd. Sixteenth Edition, Part IV, Chapter 17, Page 139. ISBN: 9780702029349.

Wilschefski, S., Baxter, M. 2019. Inductively Coupled Plasma Mass Spectrometry: Introduction to Analytical Aspects. *Clinical Biochemist Reviews*, 40(3):115–133.