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# Pharmaceutical Analysis of ASHWAGANDHADI AVALEH: An Ayurved Formulation for Malnutrition

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## ABSTRACT

Ayurveda is one of the most ancient medical science of the world . The purpose of *Ayurveda* is to protect the health of the healthy and to alleviate disorders of the diseased. There are mainly eight branches of *Ayurveda* out of which one among them deals with preparation of formulations.*Ashwagandhadi Avaleha* drug specially use in the treatment of karshya which mentioned in *Sahasrayoga.* Since the therapeutic values and efficacy of the formulation depends on many factors, a physicochemical assay and HPTLC analysis of the above formulation has been taken up for the present study.

## Introduction:

*Ayurveda* is considered as the science of life. The ultimate aim of *Ayurveda* is to guide every human being to maintain and promote health, and prevent ailments, which is the main hindrance to achieve dharma. *Ashwagandhadi Avaleha* drug specially use in the treatment of *karshya* which mentioned in *Sahasrayogam.*

This formulation in present era needs the standardization. In this study *Ashwagandhadi Avaleh* is prepared as per the quotations explained in the classics. The *Ashwagandhadi Avaleh* is herbal preparation. *Gutika*, *Modaka*, *Pindi* and *Vati* are synonymous terms used in classics for *Avaleh*. The analytical study of *Avaleh* is performed with following parameters: physico- chemical parameters i.e. colour, oder, taste, PH, Loss on Drying, total ash, water soluble extractive and alcohol soluble extractive, are performed. HPTLC , total reducing sugar, Total non-reducing sugar and total protein analysis are performed for identification of chemical constituents and respectively.

## MATERIALS AND METHODS

### Aim and objectives

- Identification and authentication of raw drugs used for *Ashwagandhadi avaleh.*

- Preparation of *Ashwagandhadi avaleh*at GMP certified pharmacy as per classical explanation.
- Physicochemical, phytochemical of *Ashwagandhadi Avaleh.*

**Drug review**

- The name of the drug, parts used and its quantity were mentioned in TABLE 1.

**Collection, Identification and Authentication of Raw Drugs**

- The raw drugs of the *Ashwagandhadi Avaleh* were procured fom G.Y. Hakim & Sons opp. GPO, Raopura Road, Vadodara.
- Raw drugs identification and authentication was done by the Department of *Dravya guna* and preaparation of *Avaleha* was carried out in GMP certified pharmacy of Parul Institute of Ayurveda, Parul University, Vadodara.

**Classical method of *Ashwagandhadi Avaleh* Preparation :**

The preparation of the drug was done as follows:

**INGREDIENT-**

**A) Churna dravyas- 1 part each**

| Drug name | Botanical name | Part used | Ratio |
|---|---|---|---|
| *Ashwagandha* | *Withania somnifera* | Root | 1 Part |
| *Tila* | *Sesamum indicum* | Seeds | 1 Part |
| *Masha* | *Phaseolus mungo* | Seeds | 1 Part |
| *Pippali* | *Piper longum Linn.* | Fruit | 1 Part |

**(B) Paka Dravyas:**

(1) Guda (Jaggery)-------- 8 part.

(2) Goghrita (Cow‘s ghee) --- 2 part

**DRUG PREPARATION** :

1) Initally fine powder of all the four constituents of *ASHWAGANDHADI AVALEHA* i.e. *Ashwagandha, Tila, Masha,* and *Pippali* ,was prepared.
2) Then jaggary in given quantity was heated till one tara chasni (sugar syrup).
3) Then powder of the drugs added into chasni and stir them properly on low flame heat.
4) Goghrita was added into it and kept for cooling.
5) 0.02% sodium benzoate was added as a preservative in *avaleha*.
6) Finally the drug was stored in air tight containers as per requirement.

- **Methods of physicochemical evaluation** : *Ashwagandhadi Avaleh* was analyzed by using standard qualitative and quantitative parameters. All the procedures were conducted at G.M.P certified . The physico- chemical parameters i.e. colour, odour, taste, pH, Loss on Drying, total ash, water soluble extractive, alcohol soluble extractive was analyzed in Quality Control & Analytical Study laboratory of Parul Institute of Ayurveda, Parul University and HPTLC , Total Reducing sugar, Total Non- reducing sugar and Total protein was analysed at VASU RESEARCH CENTRE, Vadodara.

- **Chromatography**

  HPTLC (high-performance thin layer chromatography) is a sophisticated form of TLC, which provides superior separation efficiency. The HPTLC concept includes validated methods for qualitative and quantitative analysis, and fulfils all quality requirements for use in fully regulated environments. In this study HPTLC, Total Reducing sugar, Total Non- reducing sugar and Total protein has been performed for drug analysis. It is an enhanced form of TLC. A number of enhancements can be made to the basic methods of TLC to automate the different steps, to increase the evolution achieved and to allow more accurate quantitative measurements. HPTLC as shown in IMAGE

**RESULTS AND DISCUSSION**

**(1) Organoleptic evaluation:** Organoleptic Characteristics of Powder drugs details are mentioned in the TABLE 1

**(2) Physico-Chemical Parameters**:

- Details of physico-chemicals values are mentioned in TABLE 7
- Loss on drying: On drying the samples indicate that the samples were devoid of excess
  water content and there was no microbial overgrowth or insect infestation present. In this sample loss on drying is 7 %, it indicates the samples may have good shelf-life and may not decayon storage.

➢ **Total ash :**

- It indicates of contamination, substitution, adulteration.
- The Low total ash signifying low levels of inorganic matter and silica content.
- In this Total Ash 4.3%. In this sample it is lightly more.

- Water soluble extract and Alcohol soluble: Water soluble extract and Alcohol soluble extract are 40% and 7.58% respectively.
- The high solubility of the sample in water denotes that drug is best suited for extraction with water or water based preparations.

➢ **PH**:

- The pH was measured to note the acidity or alkalinity of the aqueous solution of the drug.
- This helps in understanding the pharmacological basis of drug absorption and metabolism.
- In this sample pH is 6% .

**(3) High-performance Thin Layer Chromatography study:**

**Preparation of test solution ::** Weigh 5 g of sample in a beaker and to it add 10 mL of Water. Sonicate for 30 Minutes, filter and transfer the filtrate to a separating funnel. Partition the filtrate with 20 mL Ethyl Acetate and collect the Ethyl Acetate layer in a separate beaker. Repeat the procedure twice with 15 mL of Ethyl Acetate. Pool the Ethyl acetate layers together and evaporate to dryness. Thereafter, reconstitute the sample with 2 mL of Ethyl Acetate and filter with 0.22 µm syringe filter. Use the Test solution thus obtained for HPTLC fingerprinting.

The results are tabulated as under. (IMAGE 3)

**Preparation of spray reagent[Vanillin – sulphuric acid reagent]:** Dissolve 50 mg Vanillin in 2 mL Methanol and 8 mL Sulphuric acid (98 %). From this stock solution prepare 10 % solution in Methanol. Image 3

**Details of HPTLC profile of all tracks at 254 nm.** Under the 254 nm wavelength-Track - 1of Ashwagandhadi Avaleh(5µL) - 4 spots were detected and starts with respect to retardation factor 0.34, 0.53, 0.64, and 0.78 (IMAGE 4)

**Details of HPTLC profile of all tracks at 366 nm.** Under the 366 nm wavelength-Track - 1of Ashwagandhadi Avaleh (5µL) - 7 spots were detected and starts with respect to retardation factor 0.15, 0.20, 0.40, 0.53, 0.64, 0.78, and 0.90 . (IMAGE 5)

**Details of HPTLC profile of all tracks at 540 nm.** Under the 540 nm wavelength-Track - 1of *Ashwagandhadi Avaleh*(5µL) – 10 spots were detected and starts with respect to retardation factor 0.15, 0.34, 0.40, 0.45, 0.48, 0.53, 0.59, 0.64, 0.78, and 0.90.

## CONCLUSION

Any plant or formulation which is used medicinally requires detail study prior to its use because the therapeutic efficacy is depends on the quality of ingredients used for the medicine preparation. In this study, *Ashwagandhadi Avaleh* was prepared according to the classical textual standard operative procedure mentioned in classic. The raw drugs were indentified and authenticated before using for preparation. The prepared drug, *Ashwagandhadi Avaleh*was pharmacologically subjected for physicochemical analysis, HPTLC, . In future, this study will be helpful for standardization of *Ashwagandhadi*

*Avaleha*and for the preparation of the monography of *Ashwagandhadi Avaleha*in the Ayurvedic Formulary of India (AFI).

**Conflict of Interest: None**

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**Table 1 Ingredient of Ashawgandhadi Avaleha**

**INGREDIENT-**

**A) Churna dravyas- 1 part each**

| Drug name | Botanical name | Part used | Ratio |
|---|---|---|---|
| **Ashwagandha** | ***Withania somnifera*** | **Root** | **1 Part** |
| **Tila** | ***Sesamum indicum*** | **Seeds** | **1 Part** |
| **Masha** | ***Phaseolus mungo*** | **Seeds** | **1 Part** |
| **Pippali** | ***Piper longum Linn.*** | **Fruit** | **1 Part** |

**(B) Paka Dravyas:**

**(1) Guda (Jaggery)-------- 8 part.**

**(2) Goghrita (Cow‘s ghee) --- 2 part**

**Table 2**

**ORGANOLEPTIC CHARACTERSTICS**

| Sr. No. | Parameter | Observation |
|---|---|---|
| 1 | colour | Greenish |
| 2 | Odour | Aromatic |
| 3 | Touch | Soft |
| 4 | Consistency | Semisolid |
| 5 | Taste | Sweet & Bitter |

**Table 3**

**PHYSICO-CHEMICAL PARAMETERS**

| Sr. No | Parameter | Observation |
|---|---|---|
| 1 | Loss on Drying | 7 |
| 2 | Total Ash | 4.3 |
| 3 | Water Soluble Extractives | 19 |
| 4 | Alcohal Soluble Extractives | 7.58 |
| 5 | PH | 6 |

**Table 4**

**PHYSICOCHEMICAL ANALYSIS-**

| Sr. No | Parameters | Result |
|---|---|---|
| 1 | Total Reducing Sugar (%) | 23.22 |
| 2 | Total Non-Reducing Sugar (%) | 41.14 |
| 3 | Total Protein (%) | 4.08 |
| 4 | HPTLC Fingerprinting | Reports attached |

**Table 5**

| **HPTLC FINGERPRINTING REPORT** | | |
|---|---|---|
| **Sample** | **:** | **Ashwagandhadi Avaleha** |
| **Name of Scholar** | **:** | Dr. Lakshmi Devi Chauhan, Parul Institute of Ayurved, Vadodara |
| **Sample ID** | **:** | AD/20/156 |
| **Date of Report** | **:** | 17/09/2020 |

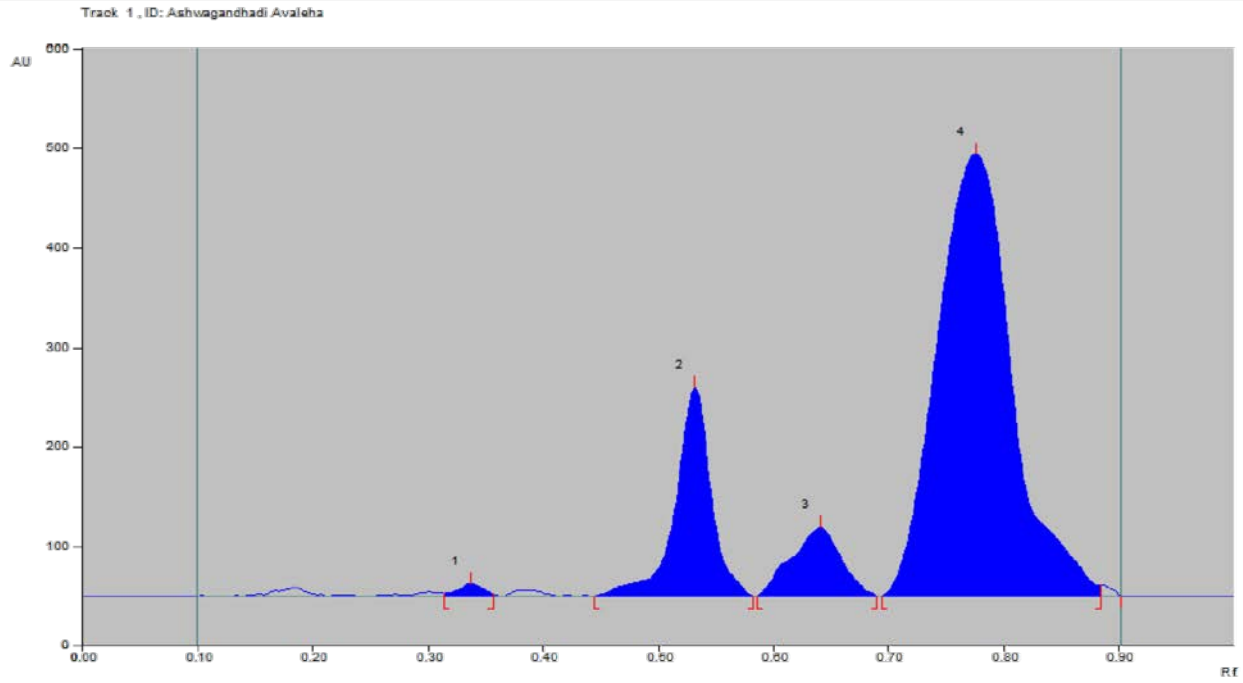
**Preparation of Test solution:** Weigh 5 g of sample in a beaker and to it add 10 mL of Water. Sonicate for 30 Minutes, filter and transfer the filtrate to a separating funnel. Partition the filtrate with 20 mL Ethyl Acetate and collect the Ethyl Acetate layer in a separate beaker. Repeat the procedure twice with 15 mL of Ethyl Acetate. Pool the Ethyl acetate layers together and evaporate to dryness. Thereafter, reconstitute the sample with 2 mL of Ethyl Acetate and filter with 0.22 µm syringe filter. Use the Test solution thus obtained for HPTLC fingerprinting.

**Preparation of Spray reagent [Vanillin – sulphuric acid reagent]:** Dissolve 50 mg Vanillin in 2 mL Methanol and 8 mL Sulphuric acid (98 %). From this stock solution prepare 10 % solution in Methanol.

**Chromatographic Conditions:**

| | |
|---|---|
| Application Mode | CAMAG Linomat 5 - Applicator |
| Filtering System | Whatman filter paper No. 1 |
| Stationary Phase | MERCK - TLC / HPTLC Silica gel 60 $F_{254}$ on Aluminum sheets |
| Application (Y axis) Start Position | 10 mm |
| Development End Position | 80 mm from plate base |
| Sample Application Volume | 4 µL |
| Development Mode | CAMAG TLC Twin Trough Chamber |
| Chamber Saturation Time | 30 minutes |
| Mobile Phase (MP) | Toluene : Ethyl Acetate : Formic acid : Methanol (6 : 3 : 0.1 : 1 v/v) |
| Visualization | @ 254 nm, @ 366 nm and @ 540 nm (after derivatization) |
| Spray reagent | Vanillin Sulphuric acid reagent |
| Derivatization mode | CAMAG – Dip tank for about 1 minute |
| Drying Mode, Temp. & Time | TLC Plate Heater Preheated at 100± $5^0$C for 3 minutes |

## HPTLC Chromatogram @ 254 nm



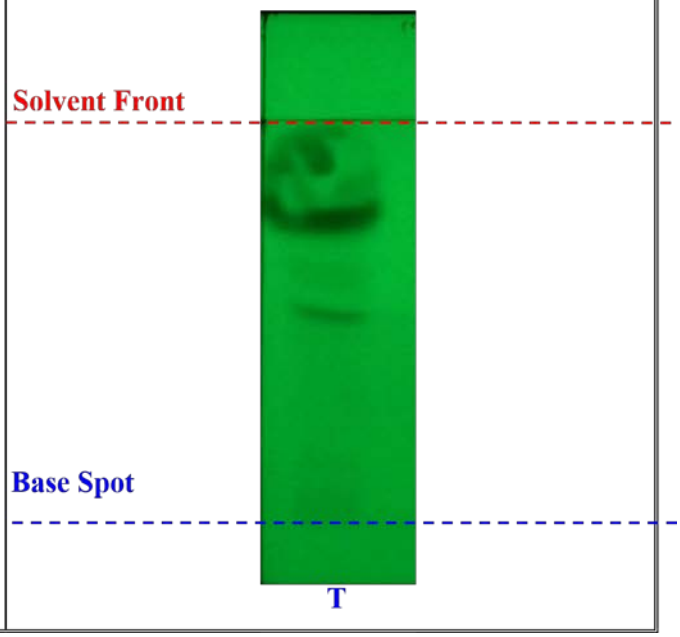

**2D chromatogram**

### $R_f$ value

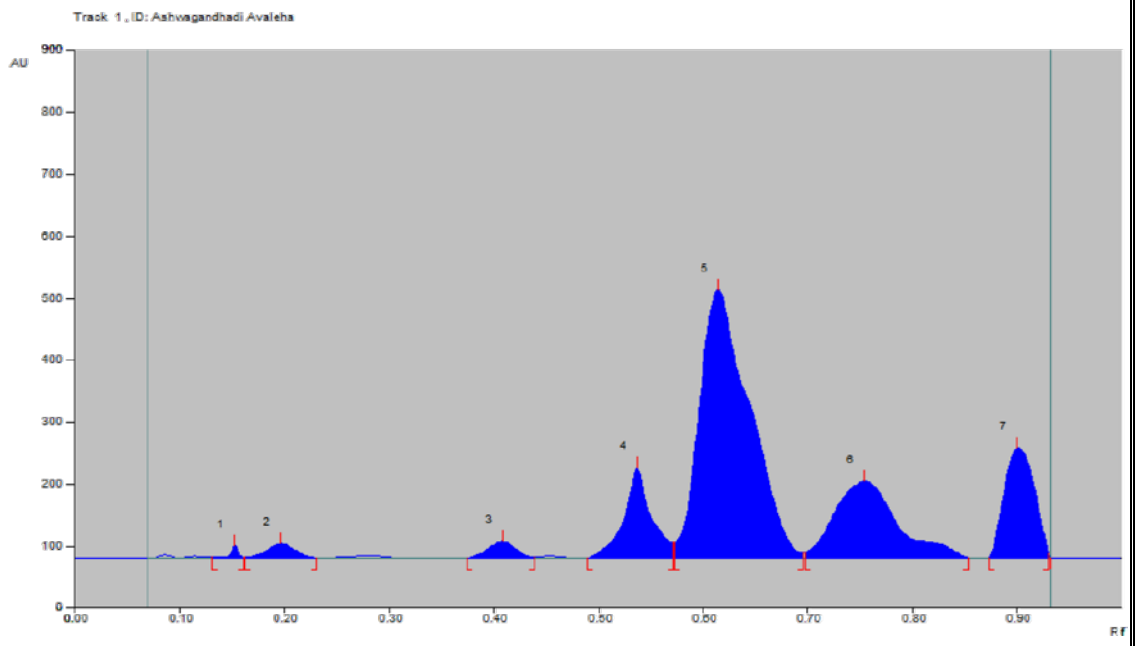
| Spot No. | Track T |
|---|---|
| 1 | 0.34 |
| 2 | 0.53 |
| 3 | 0.64 |
| 4 | 0.78 |

**Track T**: Ashwagandhadi Avaleha

### HPTLC Plate



## HPTLC Chromatogram @ 366 nm



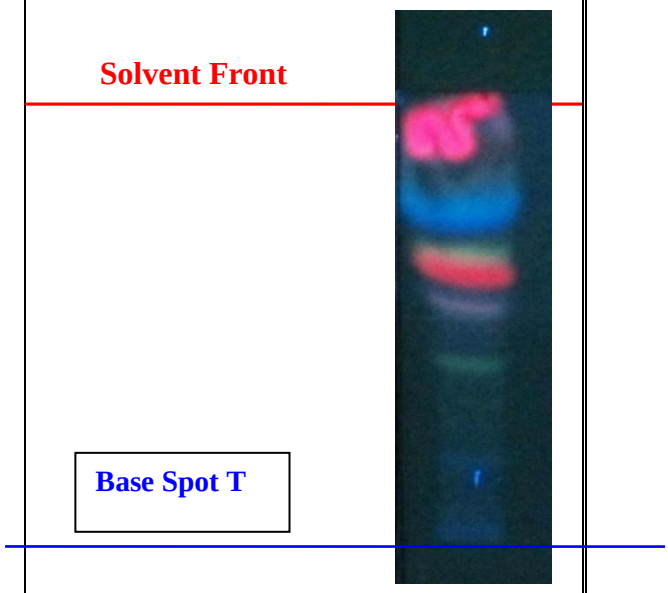

## 2D chromatogram

### $R_f$ value

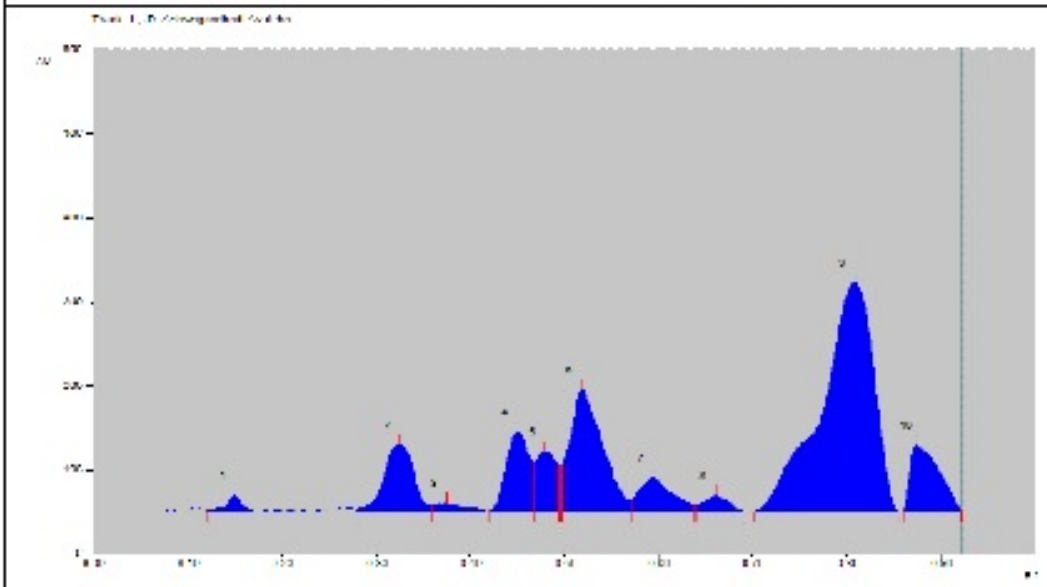
| Spot No. | Track T1 |
|---|---|
| 1 | 0.15 |
| 2 | 0.20 |
| 3 | 0.40 |
| 4 | 0.53 |
| 5 | 0.64 |
| 6 | 0.78 |
| 7 | 0.90 |

**Track T**: Ashwagandhadi Avaleha

### HPTLC Plate



## HPTLC Chromatogram @ 540 nm



2D chromatogram

**$R_f$ value**

| Spot No. | Track T |
|---|---|
| 1 | 0.15 |
| 2 | 0.34 |
| 3 | 0.40 |
| 4 | 0.45 |
| 5 | 0.48 |
| 6 | 0.53 |
| 7 | 0.59 |
| 8 | 0.64 |
| 9 | 0.78 |
| 10 | 0.90 |

**Track T**: Ashwagandhadi Avaleha

**HPTLC Plate**