



## Phyto and Physicochemical screening of Methi Ajwain Churna

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### Abstract

Ayurvedic formulations are highly effective and known to have minimal or no side effect, but due to lack of validation parameters for identification and quality control, there is a lacuna in demand of Ayurvedic formulations at international level. Methi ajwain churna is ayurvedic preparation used in the treatment of digestive disorder, to control cholesterol and blood sugar level and also effective in reducing joint pain. Present study is initiated to establish standard methods and quality parameters for Methi Ajwain Churna. The objective of the work is to formulate and authenticate the Methi Ajwain Churna according to World Health Organization (WHO), GMP guidelines which are the first available report so far. It includes Organoleptic properties, physicochemical parameters, phytochemical analysis, heavy metal analysis, microbial load and HPTLC Fingerprinting. The parameters were found to be comparable and sufficient for evaluation of the churna. The obtained values should be helpful to develop pharmacopoeial standards of Methi Ajwain Churna. This data will be helpful to overcome batch to batch variations in traditional preparation of Methi Ajwain Churna.

**Keywords:** Ayurvedic formulation, Organoleptic, Phytochemical, Physicochemical, HPTLC Fingerprinting

### Introduction

Ayurveda is frequently practiced form of traditional medicine in India. The use of ayurvedic medicines is common in both adults and children and is increasing in many areas of the world. Ayurveda is concerned with healthy living along with curative measures that synchronize an individual physically, mentally and spiritually. Ayurveda serve to maintain health of human being either by optimizing life style or medication with miraculous formulations. Ayurvedic system of medicine develops enormous formulations of which churna is one of the products. Powder form of drug is mostly preferred and churna being very fine in particle size, they have better absorption and hence good bioavailability. Safety and efficacy of these Ayurvedic preparations are the main parameters to be estimated to ensure the quality of the drug used in the formulation. During the last decade, it has become so vital within the scientific community to standardize

the quality of herbal formulations. (Varun Kumar Singh, 2013).

Methi ajwain is an Ayurvedic formulation available in the form of churna Powder. This formulation is combination of Methi (fenugreek seeds), Ajwain (*Trachyspermum ammi*), Kalijiri (*Centratherum anthelminticum*), Sunthi (*Centratherum anthelminticum*), Ginger (*Zingiber officinale*). Methi Ajwain churna is Ayurvedic Preparation Used in the treatment of digestive disorder, to control cholesterol and also effective in reducing joint Pain. Methi Ajwain Churna is an anti-inflammatory, antioxidant, antibacterial, antiviral, anticancer, diuretic, wound healing. It has a significant antidiabetic effect, and can be used to manage the blood sugar level. The use of this Churna increase the rate of urine flow, and sodium excretion which help the body to get rid of fluid retention. (G Lal, 2018).



The Methi ajwain churna possess unique medicinal benefits but still confined as alternative medicine because of raw knowledge and absence of proper scientific standardization. For useful application of this churna in modern medicine, phytochemical and physiochemical evaluation is important. In this present research work we establish parameter for quality control of Methi ajwain churna.

## Materials and Methods

### 1. Collection of raw materials

Raw materials were collected from local Ayurveda store which is located at Dombivli (E).

**Table 1 - Composition of Churna/ Ingredients Used**

Name of Ingredient	Botanical Name	Part Used	Image
1.Methi	<i>Trigonella foenum-graecum</i>	Seed	
2.Ajwain	<i>Trachyspermum ammi</i>	Fruit	
3. Kalijiri	<i>Centratherum anthelminticum</i>	Seed	
4. Sunthi	<i>Zingiber officinale</i>	Dried Rhizome	
5. Hing	<i>Ferula asafoetida</i>	Oleo-resin	

## 2. Preparation of churna

The Methi ajwain churna is prepared by mixing the methi seeds (*Trigonella foenum-graecum*), Ajwain

(*Trachyspermum ammi*), Kalijiri (*Centratherum anthelminticum*), Hing (*Ferula asafoetida*), dry ginger powder (*Zingiber officinale*) in following quantity.

**Table 2- Ingredients quantity & it uses**

Name of Ingredients	Botanical Name	Quantity (gms)	Uses
1.Methi	<i>Trigonella foenum-graecum</i>	250	Methi has benifites for lowering blood sugar levels, also reduce cholesterol levels, treatment of digestive disorder
2.Ajwain	<i>Trachyspermum ammi</i>	100	Ajwain Contains a high level of antioxidant which help in detoxification of the body. Removal of toxin from the body also help in quicker results for losing weight
3. Kalijiri	<i>Centratherum anthelminticum</i>	100	Kalijiri helps in releasing insulin, which ultimately lowers blood sugar level, Kalijiri has property of reducing bad cholesterol and reduces the risk of heart problem
4. Sunthi	<i>Zingiber officinale</i>	3 tbsp	Sunti has a unique flavour and helps manage gastric discomfort. Improves Metabolism, Gingerol, an important ingredient found in sunthi, helps overcome nausea and vomiting.
5. Hing	<i>Ferula asafoetida</i>	2 tbsp	Hing is highly beneficial in curing digestive disorder and flatulence

## 3. Evaluation of Methi Ajwain Churna

### 3.1 Macroscopic Evaluations

Methi Ajwain Churna was evaluated for organoleptic parameters such as color, taste, odor, touch, and texture. (Siddiqui, 1995).

### 3.2 Preliminary phytochemical analysis

The aqueous and alcoholic extracts of the Churna and its ingredients were prepared and were analysed for the phytoconstituents present in the test for alkaloids, flavonoids, carbohydrates, tannin, saponins, glycosides, phenolic content were performed. (O.Sita Kumari, 2016).

### 3.3 Physicochemical Investigation and Flow Properties

Different physicochemical investigation of the churna were carried out using standard pharmacopoeial

methods, including determination of alcohol soluble extractives, water soluble extractives, total ash, loss on drying. Flowability parameter like bulk density, tap density and Hausner's ratio were determined for the Churna formulation. (Princy Agrwal, 2018).

### 3.4 Heavy Metal Determination

Heavy Metal analysis of formulation was performed to check if any heavy metals are present in the formulation. (Indra it Sen, et al., 2018).

### 3.5 Microscopic Evaluation

Methi Ajwain Churna was evaluated for microscopic features using compound microscope at various magnifications. The powder was taken on slide mounted in glycerine and observed for specific characters. Staining with safranin and iodine was also employed to get resolved images. (Indian Pharmacopeia 1996).

### 3.6 Total Viable Count

Total viable count is most widely accepted technique recommended by WHO for total count of microorganisms in plant materials and herbal formulations. All of the various pharmacopoeia the total viable count has range from  $10^5$ -  $10^7$  cfu/g. Total aerobic and anaerobic bacteria count is done by spread plate technique and then incubate at  $30-35^{\circ}\text{C}$  for 24hrs. To count yeast and mould the technique employed spread plate technique in saboraud dextrose agar is used and incubate at  $30-35^{\circ}\text{C}$  for 24 hours. The specification of WHO for total aerobic microorganism is not more than  $10^7$  cfu/g and for fungi and mould  $10^4$  cfu/g for plant materials. High counts of fungi are risk because of the possibility to produce mycotoxin such as aflatoxin which are carcinogenic. Based on OSP3 the total microbial count dried or powdered herbal materials and product was not more than 105 cfu/g. (Mukund Nagarnaik, 2014).

### 3.7 UV Spectrophotometry

The methanolic extract of formulation was analysed under UV Spectrometer ranging on various

wavelength from 200nm up to 700nm. The UV was analysed twice once full scan and then 210nm to 270nm to get exact maximum absorbance. (H. Joshi, 2013).

### 3.8 Preparation of Sample

All the raw materials and prepared formulation powders were dissolved in Methanol and kept overnight. Next day all the solutions were filtered through whattman filter paper to obtain clear extracts.

### 3.9 High Performance Thin Layer Chromatography

The fingerprinting method for Methi Ajwain Churna was performed by simple high-performance thin layer chromatography (HPTLC) determination using Tannic acid as a standard, which is important and major content in raw material. 10 $\mu$ l of the filtered solution of formulation extract and standard was applied on the HPTLC plate as per conditions mentioned. (Garg S, et al., 2013)

**Table 3 Conditions for HPTLC Fingerprinting**

Stationary Phase	HPTLC plates silica gel
Plate Size	10.0 x 10.0 cm
Mobile Phase	Chloroform: Methanol: Glacial acetic acid (7: 3: 0.2 v/v )
Saturation Time	20 mins
Standard Used	10 ppm Tannic acid
Spot Volume	10 $\mu$ l
Band Length	8.0 mm
Solvent Front	80mm
Wavelength and Lamp	Ultra Violet Lamp at 254nm
Sample Applicator	CAMAG Linomat 5
Sample Detection	CAMAG Visualizer: 200480
Number of Tracks	7

## Results and Discussion

The Methi Ajwain Churna was prepared using the prescribed plant part of the authenticated herbs as per the standard procedure mentioned in the Ayurvedic Formulary of India. In Macroscopic evaluation, the prepared Methi Ajwain Churna was found to be light brown in colour with characteristic odour and tasted high bitter. (Table 4). The phytochemical analysis of the churna was performed and the results have been tabulated in (Table 5). The preliminary phytochemical screening of extracts revealed the presence of phytoconstituents like alkaloid, tannins, steroids, flavonoids, saponin, carbohydrates. The sample was analyzed for all the above mentioned heavy metals and the results were found to be Negative (Table 7). Thus, this churna has been quantitatively estimated for its safety related to the heavy metal content. The microscopic evaluation of formulation when observed under 10X and 1000X microscopy it showed presence of endosperm, Palisade cells, Parenchyma cells, Trichome, Vascular strand, pitted cells. (Fig. 1-9). Physicochemical Investigation and Flow Properties such as Bulk density, Tap Density,

Compressibility Index, Hausner Ratio and Ash Value were determined and the values are presented in (Table 6).

The Methanolic extract of Methi Ajwain Churna was analysed under UV spectrometry. It was first full scan from 200-700nm. (Table 9) (Fig. 10). It was observed that maximum UV absorbance lied between 210-270nm (table 10). (Fig.11). The UV absorbance was done for a specific range and it was found at wavelength 260 nm with absorbance 2.439. The results of UV can be used to determine the concentration of formulation. HPTLC profiling was done by using Tannic acid as it as active component (Table 12) (Fig. 12). HPTLC fingerprinting results can be further used for identification and quantification of individual components present in the formulation. Microbial analysis involving total viable count showed that there was minimal or under limit growth of bacteria in anaerobic and aerobic condition while, the growth of fungi, was not seen, hence, it can be stated as the formulation is of good quality and is safe. (Table 8).

**Table 4 Organoleptic Characteristics of Methi Ajwain Churna**

Parameters	Results
Odour	Sweet and aromatic
Taste	High bitter
Touch	Rough
Colour	Light brown
Appearance	Powder

**Table 5 Phytochemical Evaluation of Methi Ajwain Churna**

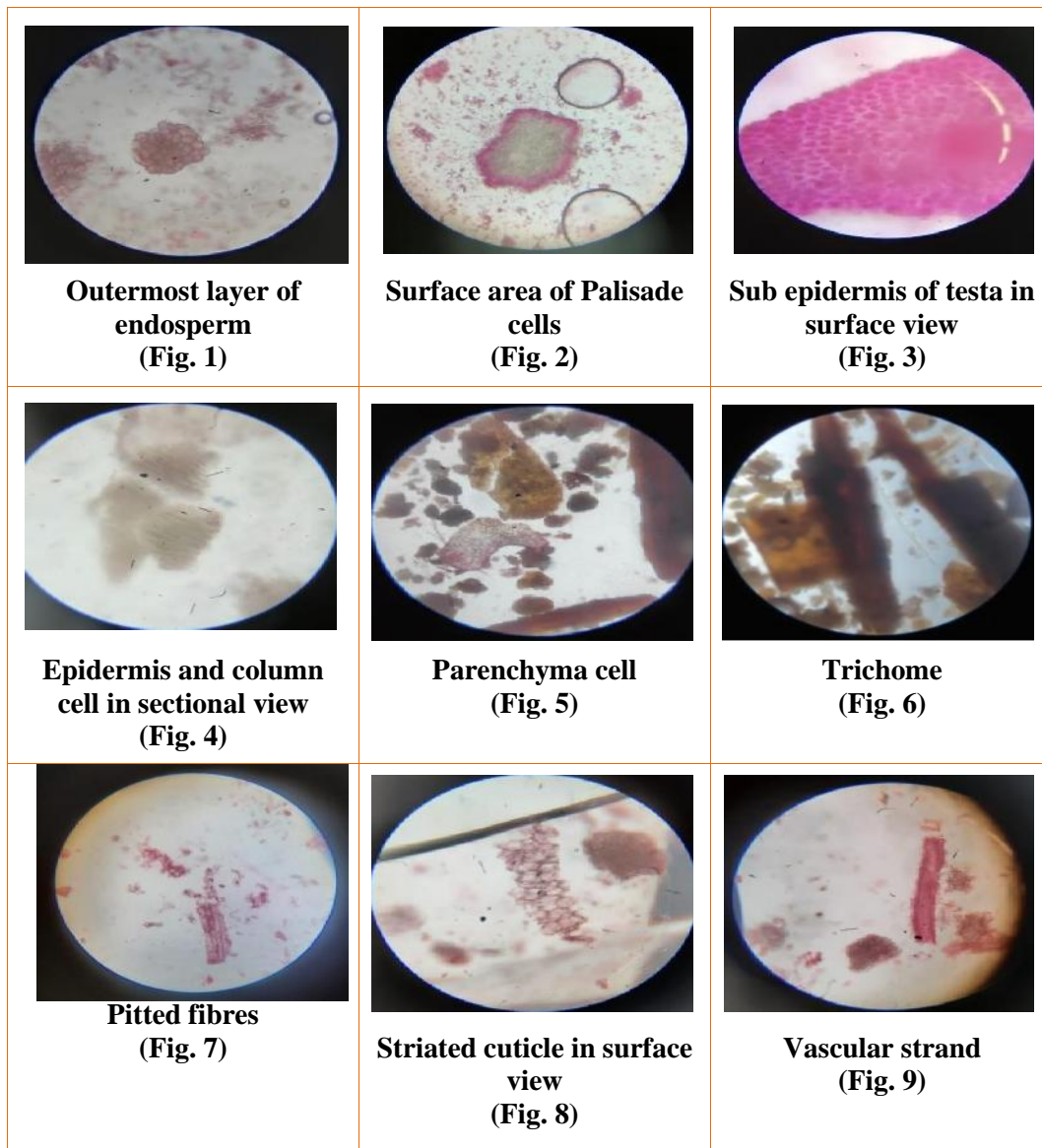
Sr no	Tests	TA	TF	CA	ZO	FF	F
1	Tannins	+	+	+	+	-	+
2	Glycosides	+	-	+	+	-	+
3	Terpenoids	+	+	+	-	-	+
4	Alkaloid	+	+	+	+	-	+
5	Flavonoid	-	+	-	-	-	-
6	Steroid	+	+	+	+	+	+
7	Saponin	+	-	-	+	+	-
8	Phenolic compound	-	-	-	-	-	-
9	Sterol	+	+	+	-	+	+
10	Anthocyanin	-	-	-	-	-	-
11	Carbohydrate	-	-	-	+	+	-
12	Starch	-	-	-	-	-	-

Key: (+) Present (-) Absent TG- *Trigonella foenum-graecum*, TA- *Trachyspermum ammi*

CA-*Centratherum*

anthelminticum, ZO- *Zingiber officinale*, FF- *Ferula foetida*.

Microscopic Evaluation of *Methi Ajwain Churna*



**Table 6: Physiochemical tests of *Methi Ajwain Churna***

Sr.no	Tests	Result
1	Loss on drying	7.38%
2	Total ash value	8.94%
3	Water soluble extract	29.59%
4	Alcohol soluble extract	18.16%
5	Bulk Density	0.430 g/ml
6	Tap Density	0.602 g/ml
7	Hausner ratio	1.4

**Table 7: Heavy metal testing**

Sr.no	Tests	Result
1	Lead	-
2	Copper	-
3	Zinc	-
4	Cadmium	-
5	Nickel	-
6	Cobalt	-
7	Chromium	-
8	Bismuth	-

**Table 8: Total Viable Count of Methi Ajwain Churna**

Media	Organism	Dilution	Cfu/0.1ml	Cfu/1ml	Average
Nutrient agar	Aerobic	10-3	$8 \times 10^3$	$0.8 \times 10^5$	$5.3 \times 10^5$
		10-4	$5 \times 10^4$	$5 \times 10^5$	
		10-5	$1 \times 10^5$	$10 \times 10^5$	
Nutrient agar	Anaerobic	10-3	$32 \times 10^3$	$3.2 \times 10^5$	$59 \times 10^5$
		10-4	$23 \times 10^4$	$23 \times 10^5$	
		10-5	$15 \times 10^5$	$150 \times 10^5$	
Sabourauds agar	Fungi	10-3	$4 \times 10^3$	$0.4 \times 10^5$	$0.4 \times 10^5$
		10-4	-	-	
		10-5	-	-	

**Table 9: UV Spectra**

Absorbance	Wavelength
200	1.041
250	2.439
300	2.308
350	2.227
400	0.796
450	0.518
500	0.490
550	0.427
600	0.374
650	0.386
700	0.360

Fig. 10 (200-700nm)

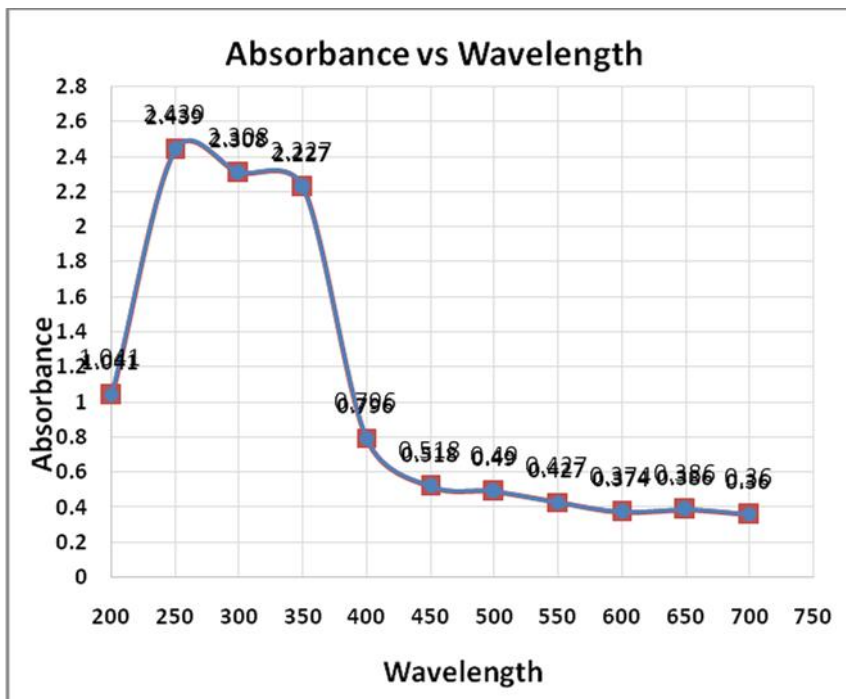
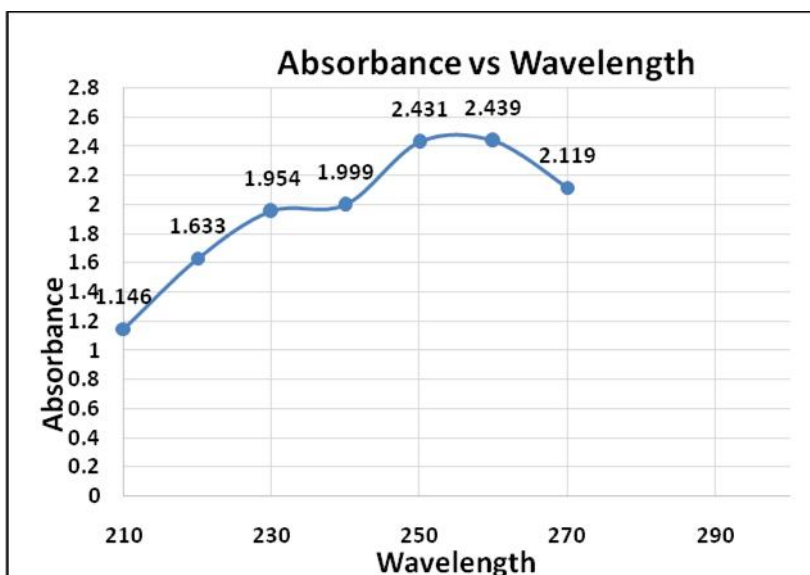


Table 10 UV spectra

Absorbance	Wavelength
210	1.146
220	1.633
230	1.954
240	1.999
250	2.431
260	2.439
270	2.119

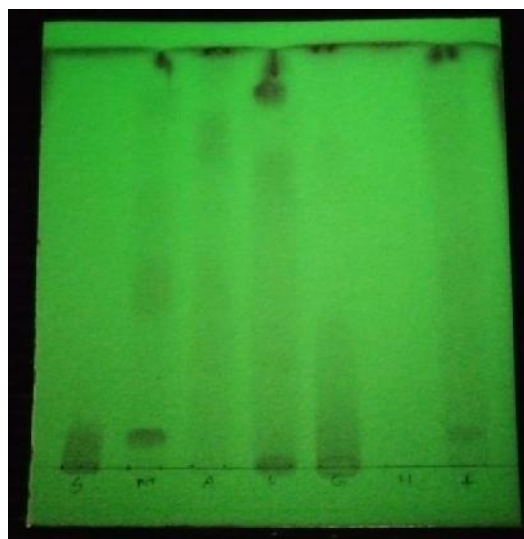
Fig. 11 (210-270nm)





### HPTLC Fingerprinting of Methi Ajwain Churna

Track 1	S -Standard (Tannic Acid)
Track 2	M - Methi
Track 3	A - Ajwain
Track 4	K - Kalijiri
Track 5	G - Ginger
Track 6	H - Hing
Track 7	F – Formulation



### Conclusion

The Methi Ajwain Churna was prepared as per the general method described in Ayurvedic formulary of India. This Ayurvedic formulation was prepared and examined at laboratory scale in order to check its various Phytoconstituents, Phytochemical content, heavy metals, antimicrobial testing. In the present study total viable count was within the limit in formulation. It is as per WHO norms. So it proves that Methi Ajwain Churna is free from microbial contamination. The findings of this study also highlighted the safety of the Methi Ajwain Churna. The tannic acid was present in both raw materials as well as formulation which was confirmed by HPTLC fingerprinting. It is concluded that, the formulation prepared satisfies all the limits and criteria set by AYUSH. The generated information of the present study will provide data which is helpful in the correct identification and authentication of this churna and may help in preventing its adulteration.

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