

## **A New Process of Standardization for Promising Antioxidant Herbal Formulation of *Momordica charantia Linn.* (Family- Cucurbitaceae ) by using several parameters and Analytical techniques.**

\*Subhajit Ghosh<sup>(a)</sup>, Dr.Hanumanthachar Joshi<sup>(a)</sup>, Dr.P.N.Murthy<sup>(b)</sup>, Dhirendra Kumar Tarai<sup>(c)</sup>

(a) Sarada Vilas College Of Pharmacy, Mysore, Karnataka, India.

(b) Royal College of Pharmacy and Health Sciences, Berhampur, Odisha, India.

(c) K.V. Virani Institute of Pharmacy and Research Centre, Badhada, Gujarat, India.

**Keywords:-** Herbal Drug, Herbal Raw Material, *Momordica charantia Linn*, Herbal Formulation, Standardization, Chromatographic Fingerprint.

**\*Author for Correspondence :-**

**Permanent address:-** Dr. S. P. Ghosh Road, Bagbazar, Chandannagar, Dist:- Hooghly, West Bengal, Pin-712136, India.

**Present address:-** Sarada Vilas College Of Pharmacy, Krisnamurthy Puram, Mysore-570004, Karnataka, India.

**Mob no:** +919459690122.

**E-mail:** [subhajitm pharm07@gmail.com](mailto:subhajitm pharm07@gmail.com),

**Institutional E-mail address:** - [mysoresvcp@gmail.com](mailto:mysoresvcp@gmail.com)

**Institutional Phone no:-** +918212488612

**ABSTRACT:-**

Since the age of ancient times to till date herbal drugs have been used for the treatment of a wide range of diseases. In the recent time more number of people throughout the world is very much concentrating to use of herbal products in the health care system for that herbal products have played a key role in the world health system. Now the people of the world wide need some alternative medicine has a resulted in the growth of the markets of herbal products and interest in our traditional system of medicine. In modern system of Herbal Drug Technology is used for converting raw herbal materials or plant products or food products in to a specific formulation which is use as a medicine, where for this processing, standardization and quality control is necessary with the proper integration of modern technology with the help of Scientific and Traditional knowledge is important. In order to prove constant and promising composition of herbal preparation for here Antioxidant activity, adequate parameters and Analytical methods that have to be applied such as Thin layer Chromatography (TLC), High Performance Liquid Chromatography (HPLC) etc.

**Abbreviations:-**

MC- *Momordica charantia Linn.*

## **1. Introduction.**

Somehow having a relationship between food and health has become a topic for study with a great interest. Some substantial evidence having of the beneficial effects of foods that are rich in fruits and vegetables. Asia or some parts of Asia and including India also has a great biological diversity that's why from the ancient time to till date peoples are using Ayurvedic or herbal formulations and so many Ayurvedic formularatory or herbal industries are here, starting of Ayurveda from India everybody knows about it, that can be explored to yield extracts for therapeutic application to control and/or prevent some chronic diseases. Polyphenols from fruits and vegetables (1). Can be divided into several classes some examples are hydroxyl benzoic acids or benzoic acid derivatives containing hydroxyl groups, hydroxyl cinnamic acid and its derivatives, Anthocyanins, Proanthocyanidines, Flavonols, Flavones, Flavanols, Flavanones, Isoflavones, Stilbenes and Lignans etc. They contribute substantially to the antioxidant effects of many fruit species, vegetables and having positive potential to health.

*Momordica charantia Linn.* of the Cucurbitaceae family, traditionally the fruits, seeds and leaves are used as an anti-diabetic agent. In India where the plant is better known as "Karela," it is used as a tonic, emetic and laxative (2). Traditional Chinese medicine includes uses for the fruits, leaves and seeds in gastroenteritis, diabetes, tumors and some viral infections (3). This paper will try to focus on the standardization process of herbal formulation (Churna) prepared by MC dried fruits, by using some different parameters for identifying the chemical constituents present in MC and try to correlate these constituents with the pharmacologic effects observed.

Briefly, MC is a monoecious herbaceous vine with bright green, lobed leaves and numerous, small fragrant flowers. Mature fruits of the wild balsam pear (MC) are 2-7cm in length and 1.4-2cm in width. They are ovoid to oblong, pointed at both ends, and are covered with small warts and have a 8-10 irregular longitudinal ridges marked by prominent triangular tubercles. The green soft pericarp turns orange and eventually red with age. At maturity, the three valves of the dehiscent fruit curl back to expose 5-20 seeds each surrounded by a scarlet, sticky, pulpy aril. Seeds are 5-9mm long and are slightly three-toothed at the apex and base; the sculptured faces are regular and pale brown with irregular black areas and corrugated black margins (4).

*Momordica charantia Linn.* (Ampalaya, Bitter Gourd, Karela, Bitter Melon) has been shown to have a variety of chemical constitutions. A petroleum ether extract (4) of the dried and powdered whole plant provided a highly volatile aromatic ethereal oil, fixed oil, free fatty acids, cholesterol and carotene. After exhaustion with petroleum ether, the dried material was macerated with ether. The ether extract provided the pigment cyanophyll and xanthophylls, resins, glycosides and an alkaloid. The alcoholic extract that followed the ether extraction provided further proof of the presence of a glycosides. In 1962 Lotlikar and Rao (5) isolated from the fruits of MC a non-nitrogenous substance identified as a "charantin." It is a whitish crystalline material melting at 266-268 °C with decomposition. Gamma-aminobutyric acid was also isolated by Durand et al. (6) from MC. Baldwa et al. (7) isolated an insulin-like compound from the fruits and tissue cultures of *M. charantia L.* this plant insulin has 17 amino acids in two chains bound together with sulfide bonds. The infra-red spectrum was super imposable of that on standard zinc crystalline bovine insulin. This plant insulin is stable at 4°C and is denatured by heat. Two storage proteins have been isolated and purified from seeds of MC. (8) these proteins contain a very high concentration of glutamic acid plus glutamine and arginine. The ripe fruit and leaves provide a guanylat cyclase inhibitor with an estimated molecular weight of 5,000 to 50,000 estimated by gel filtration and is acid stable and heat labile. In 1978, Lin et al. (9) reported the isolation of lectins; proteins that can agglutinate blood cells, from the seeds of MC. Molecular weights of 23,678 and 31,762 were determined by gel electrophoresis. The same lectins were isolated and purified by

Li (10). Charantin, isolated by Lotilkar and Rao (5) was later found by Sucrow (11) in 1965 to be a mixture of sitosteryl-3 $\beta$ -D-Glucoside and 5,25-stigmastadiene-3 $\beta$ -ol D-Glucoside. Additional compounds were isolated and identified by Ulubelen and Sankawa (12) in 1979 from the tissue cultures of the unripe fruits of MC. These are diosgenen  $\beta$ -sitosterol, 7-stigmasten-ol, 5-stigmasten-3 $\beta$ , 25 diol, 5,25-stigmastadiene-3 $\beta$ -ol and 7,25-stigmastadiene-3 $\beta$ -ol. The leaves yielded additional compounds such as n-octacosans ( $C_{28}H_{58}$ ), triacontanol ( $C_{30}H_{62}O$ ) and a new phytosphingosin (structure to be established)

Multiple mechanisms have been proposed as the cause of bitter melon's hypoglycemic property. Some research works support the insulin-like activity of MC. Others propose a decrease in hepatic gluconeogenesis, or increase hepatic glycogen synthesis or an increased peripheral glucose oxidation in erythrocytes and adipocytes, or an increase pancreatic insulin secretion through increased beta-cell production in the pancreas.

We found no study in the literature that evaluated the content of regarding potential Antioxidant compounds and their specific extraction process with optimize value, Standardization process etc , the activity should be maximized if controlling some parameters like selecting best method of extraction , solvent system, temperature and easiest way to standardization of the prepared formulations ( Churna)by using MC dried fruits(21,22,24,25).

The phenolic compounds in fruit and vegetable-rich diets have attracted researchers' attention because of their health promoting effects such as lowering the risk of cardiovascular disease, cancer, or other conditions associated with aging. The biological mechanisms behind these health-promoting effects include protection against free radicals, free radical mediated cellular signaling, inflammation, allergies, platelet aggregation, ulcers, viruses, tumors and hepatotoxicity.(3,4,5,15,16,18,26).

However, there are few studies on the identification of phytochemical compounds and Standardization of herbal formulation where the main active ingredient is Ethanolic extract of MC and it having promising antioxidant activity, mainly Ethanolic extract of MC fruit. Furthermore, knowledge concerning the

antioxidant activity and content of bioactive compounds in MC may be useful for genetic improvement programs to select the varieties with higher nutritional values and specific way of herbal formulation including standardization. Thus, the objective of this study was to evaluate the correct as well as scientific and easiest way of standardization process of herbal formulation where MC is the active herbal ingredient, with maximum antioxidant potential by using different parameters(27).

## **2.Materials and Methods.**

### **2.1.Chemicals.**

1. Ethanol 2. Methanol 3. Phosphomolybdic acid 4. Chloroform were all purchased from Merck chemicals, India, all the reagents used were of analytical grade

### **2.2.Preparation Of Fruit Extracts.**

The crude drugs used in preparation of Churna were collected from Nadaun, Himachal Pradesh, India in the month of September 2014 and Authenticated by, University of Rajasthan, Jaipur, Herbarium,The Department of Botany, Voucher Number:-RUBL 211449, Candidate Number:-1949/Subhajit..One In - House formulation was prepared, as per the procedure mentioned in Ayurvedic text. An Antioxidant churna was prepared. This is a herbal formulation, consisting of Bitter Melon *Momordica charantia Linn* as ingredient (fruit ) with specific morphological parts of the plants (fruit of herb) used. Take the ingredient of pharmacopoeia quality, in a stainless steel pan at a low temperature(  $\leq 50^{\circ}\text{C}$  ) till it becomes free from moisture. Powder the ingredient take in a pulverizer and pass through sieve number 85. Weigh accurately ingredient, mix thoroughly and pass through sieve number 44 to obtain a homogeneous blend. Pack it in tightly closed containers to protect from light and moisture. Store in a Cool and Dry place.

5gms Powder of Sample was extracted with 300ml Ethanol by using reflux filtration method till complete extraction of Sample is attained (i.e. for at least 3 to 4 Hrs). The extract was filtered with a whatman no 1 filter paper then the filtrated was dried using a rotary vacuum evaporator at  $\leq 50^{\circ}\text{C}$ . to dissolve the filtrate with 25ml Methanol in a volumetric flask, this solution is further diluted to 10ml by Methanol; The obtained solutions were poured in to a brown bottles with screw caps and stored at  $-18^{\circ}\text{C}$  until further use. Triplicate determinations ( $n= 3$ ) were carried out during the study. this solution is used as test solution for evaluation and separation of constituents in sample. Then solidified for TLC,HPLC etc others study(28).



Fig:- After the preparation of Churna

#### Formulation Composition:-

**Table 1: Ingredients of An Antioxidant Churna:**

Serial	Sanskrit	English	Scientific name	Part Used	Quantity

No.	name	name			
1.	Karavella	Bitter melon	<i>Momordica charantia</i> Linn,Family- Cucurbitaceae	Fruit	1 Part

### 2.3. Morphology or Organoleptic evaluation.

The material was examined for probable adulterants and foreign matter adhering to the surface was removed. Organoleptic evaluation was used for identification of sensory characteristics like colour, odour, taste, shape, size, texture and fracture. In this methods, description, general condition of the drug size, shape outer surface inner surface are referred. A sensory or organoleptic character describes colour, odour taste, consistency.(2,4)

### 2.4. Microscopy evaluation

The inner pseudoparenchyma cells are oval or rounded, the contain fixed oil & protein the whole tissue is devoid of cellulose and lignin. various parameter includes in microscopy

A. Leaf content B. Trichome C. Stomata

#### A. Determination of leaf content

In this include parameter like stomatal number, stomatal index, vein islet number, vein termination number was determining by standard methods.

**B & C. Determination of stomatal index**

The stomatal index is the percentage of the number of stomata formed by the total number of epidermal cells including the stoma being counted as one cell. Place leaf fragment of about 5x5mm in size in a test tube containing about 5ml chloral hydrate.

$$\text{Stomatal index} = (S / (E + S)) \times 100$$

Where: S= Total number of stomata in a given area of leaf

E= Number of epidermal cells (including **Trichomes**) in the same area of leaf.

**2.5. Chemical evaluation.**

As per monogram given in classic text (13, 29).

**2.6. Physical evaluation.**

Physico chemical investigations of the formulations carried out including determination of extractive values, ash values and loss on drying by I R moisture balance.

**a) Loss on drying**

Loss on drying is the loss of mass expressed as percent w/w. About 10g of samples of formulation was accurately weighed in a dried and tared flat weighing bottle and dried at 105°C for 5hrs. Percentage was calculated with reference to initial weight.(2,19,28)

**b) Determination of total ash**

Ashing involves an oxidation of the components of the products. A high ash value is indicative of contamination, substitution, adulteration or carelessness in preparing the formulation. Total ash determination constitutes detecting the physiological ash (ash derived from plant tissue) and non physiological ash (ash from extraneous matter, especially sand and soil adhering to the surface of the drug). For its detection, 2g of powdered material was placed in a suitable tared crucible of silica previously ignited and weighed. The powdered drug was spread into an even layer and weighed accurately. The material was incinerated by gradually increasing the heat, not exceeding 450°C until free from carbon, cooled in a desiccators, weighed and percentage ash was calculated by taking in account the difference of empty weight of crucible & that of crucible with total ash (19).

**c) *Determination of Acid insoluble ash***

The ash obtained as above was boiled for 5min with 25ml of dilute hydrochloric acid; the insoluble matter was hot water and collected on an ashless filter paper, washed with ignited to constant weight. The percentage of acid-insoluble ash with reference to the air-dried drug was calculated (19).

***Determination of solvent Extractive values***

**d) *Alcohol soluble extractive value***

5g of coarsely powdered air-dried formulation was macerated with 100 ml of alcohol in a closed flask for twenty-four hours, shaking frequently during six hours and allowing standing for eighteen hours. It was then filtered rapidly; taking precautions against loss of solvent. 25ml of the filtrate was evaporated to dryness in a tared flat-bottomed shallow dish at 105°C to constant weight and weighed. The percentage of alcohol-soluble extractive was calculated with reference to the air dried drug and is represented as % value(13,19).

**e) *Water soluble extractive value***

5g of coarsely powdered air-dried formulation was macerated with 100 ml of chloroform water in a closed flask for twenty-four hours, shaking frequently during six hours and allowed to stand for eighteen hours. It was then filtered rapidly, taking precautions against loss of solvent. 25ml of the filtrate was evaporated to

dryness in a tared flat bottomed shallow dish at 105°C to constant weight and weighed. The percentage of water-soluble extractive was calculated with reference to the air-dried drug and is represented as % value (17,19)

***f) Determination of pH***

The pH of different batches of formulation were determined using pH paper (Range 3.5–6) and (6.5–14) with standard glass electrode(19).

**Physical Characteristics of Powder:**

Physical characteristics like bulk density, tap density, angle of repose, Hausner's ratio and Carr's index were determined for different formulations.

***g) Bulk density and Tape density***

The term bulk density refers to a measure used to describe a packing of particles or granules. The equation for determining bulk density (Db) is:  $Db = M/V_b$  Where M is the mass of the particles and V<sub>b</sub> is the total volume of the packing. The volume of the packing can be determined in an apparatus consisting of a graduated cylinder mounted on a mechanical tapping device (Jolting Volumeter) that has a specially cut rotating can. 100gm of weighed formulation powder was taken and carefully added to the cylinder with the aid of a funnel. Typically the initial volume was noted and the sample was then tapped until no further reduction in volume was noted. The initial volume gave the Bulk density value and after tapping the volume reduced, giving the value of tapped density(19).

***h) Angle of repose***

Angle of Repose has been used as an indirect method of quantifying powder flow ability; because of its relationship with interparticle cohesion. As a general guide, powders with angle of repose greater than 50 degree have unsatisfactory flow properties, whereas minimal angle close to 25 degrees correspond to very

good flow properties. The fixed funnel and the free standing cone method employs a funnel that is secured with its tip at a given height, which was taken 2.5 cm (H), above the graph paper that is place on flat horizontal surface. Powder or granulation was carefully poured through the funnel until the apex of the conical pile just touched the tip of the funnel.  $\tan \alpha = H/R$  or  $\alpha = \text{arc tan } H/R$

Where  $\alpha$  is the angle of repose,  $R$  being the radius of the conical pile (19).

#### i) **Hausner's ratio**

It is related to inter particle friction and as such can be used to predict the powder flow properties. Powders with low interparticle friction such as coarse spheres, have a ratio of approximately 1.2, whereas more cohesive, less flowable powders such as flakes have a Hausner's ratio greater than 1.6. The equation for measuring the Hausner's ratio is:

Hausner's ratio =  $D_f / D_o$ , where  $D_f$  = Tapped density and  $D_o$  = Bulk density(19)

#### j) **Carr's index**

Another indirect method of measuring the powder flow from bulk density is Carr's index. The equation for measuring Carr's index is: % compressibility =  $(D_f - D_o / D_o) \times 100$  where  $D_f$  = Tapped density and  $D_o$  = Bulk density(19).

#### k) **Determination of foreign matter**

Drugs should be free from moulds insects, animal, faecal matter and other contamination such as earth stones and extraneous matters(19).

**Percentage of foreign organic matter =  $n \times W \times 94,100 \times 100 / S \times M \times P$**

Where: n= number of chart particles in 25 fields.

S= number of spores in the same 25 field.

W= weight in mg of lycopodium taken.

M= weight in mg of the sample (calculation on the sample dried at 105°C.

P= number of characteristics particles per mg of the pure foreign matter.

94,000= number of spores per mg of lycopodium.(1-2)

### **I) Solubility**

The presence of adulterant in a drug could be indicated by solubility studies identify by various solvents.  
(10,19)

#### **i. Alcohol**

5 gm of powdered material along with 100 ml of alcohol are shaken well occasionally for the first 6 hours and kept undisturbed for 18 hours. The liquefied extract thus obtained was concentrated in an vacuum oven and the percentage was calculated with the weight of the drug powder taken.

#### **ii. Water**

The procedure adopted for solubility percentage of alcohol is used with chloroform water instead of alcohol to get the water solubility.

### **2.7. Analytical Evaluation & Standardization.**

#### **i) Thin Layer Chromatography**

Primarily TLC, chromatographic evaluation was done due to determination of the number of chemical constituent (s) present in that formulation going to run with that particular mobile phase then doing HPLC(17).

**ii) High performance Liquid chromatography (HPLC):- (Recent Advancement) HPLC fingerprinting of Ayurvedic formulation containing herbal ingredients:-**

Standardization of churna was carried out by organoleptic study, phytochemical analysis; qualitative organic, inorganic analysis, HPLC fingerprint study is one of the essential tool for standardization of Herbal Formulation, qualitative organic analysis the churna's showed the presence of alkaloids, phenols, tannins, glycosides, and flavonoids.(14,17,19,20)

### **2.8. Stasistical Analysis.**

Data were reported as the mean  $\pm$  the standard Error Mean (SEM) of the three replicates for each sampled species. The results were analyzed by one-way analysis of variance (ANOVA), followed by Tukey's test ( $p < 0.05$ ). The relationship between the antioxidant compounds (i.e., phenolics, ascorbic acid) and antioxidant activity was evaluated by Pearson's correlation. All analyses were performed using the statistical software SPSS (SPSS 16.0, SPSS Inc., Chicago, USA).

## **3. Results And Discussion.**

### **3.1. Morphological and organoleptic evaluation:-**

#### **Table :-02 Organoleptic Character of Prepared Churna Including Ingredient:**

S. No.	Parameters	<i>Momordica charantia</i>	Prepared Churna
1	Appearance	Powder	Powder
2	Colour	Light Brown	Light Brown
3	Odour	Astringent	Astringent
4	Taste	Bitter	Bitter

The physical comparison of different batches of prepared formulation is given in Table 5. The results obtained with the different batches in-house formulations were found to be comparable and variation was insignificant.

Identification of herb is based on macroscopical and microscopical features. Macroscopical feature involves odour, taste, color, size shape and special feature of plant and microscopically involves leaf content, trichome, stomata etc. Certain microscopical features and chemical test comes under evaluation and standardization of herbal drug. Evaluation of drugs means confirmation of its identity and determination of its quality and purity and detection of adulteration. (1,5)

Standardization expression is used to describe all measures which are taken during the manufacturing process and quality control leading to a reproducible quality. It's also involve the study from birth of plant to its clinical application. It's also include the herbal drugs preparation to a define content of a constituent or a group of substance with known therapeutic activity respectively by addition of excipients or by mixing herbal drugs preparation.(1,20) In other words it's ensuring that every packet of medicine has correct ingredient in correct amount and will induce intended therapeutic effect(23).

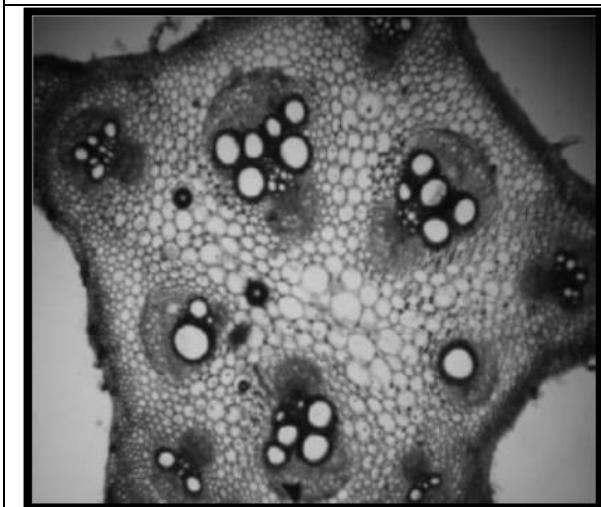
### 3.2. Miroscopic Evaluation:-



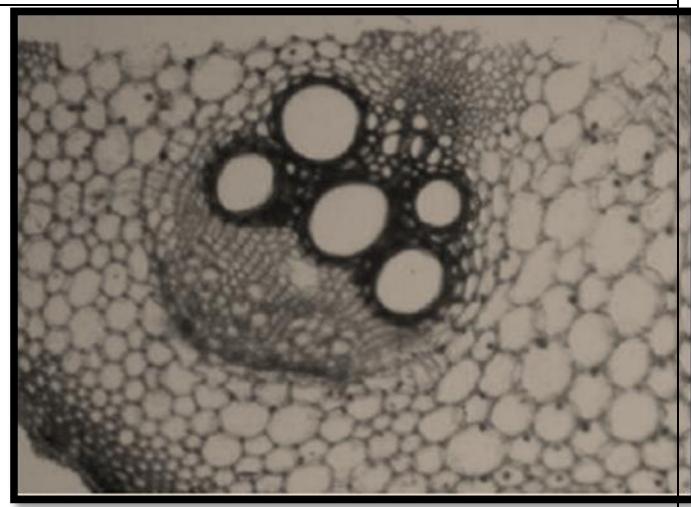
( A )



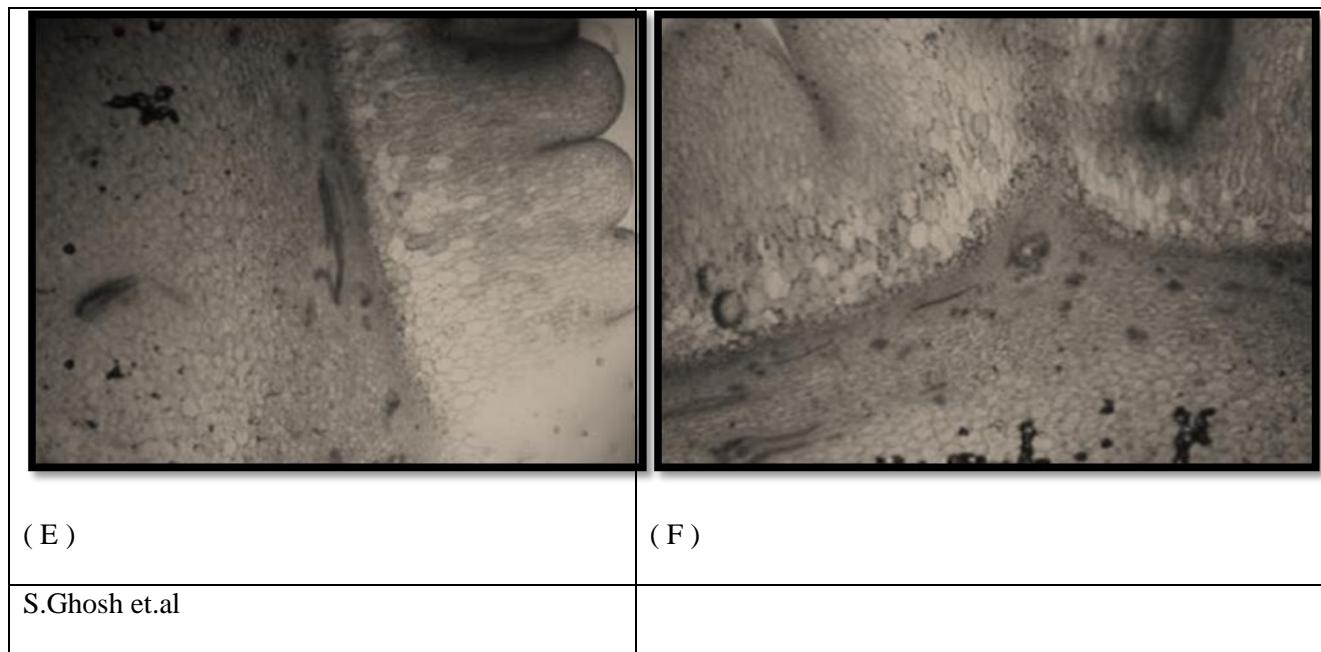
( B )



( C )

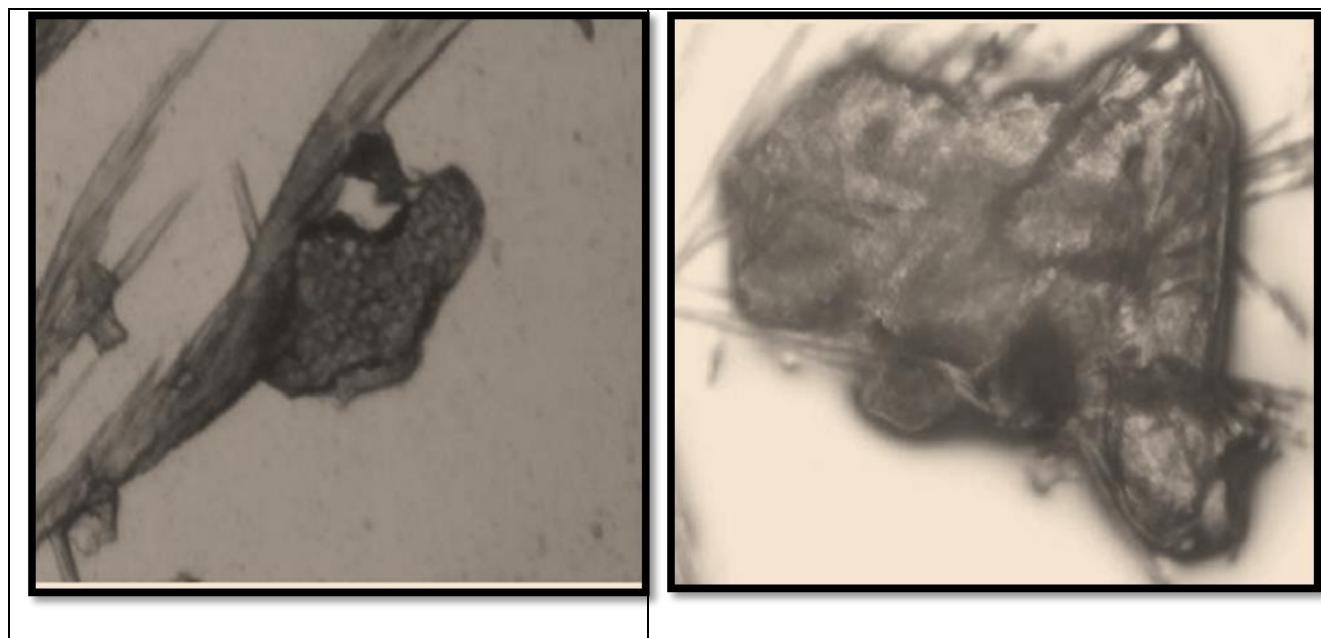


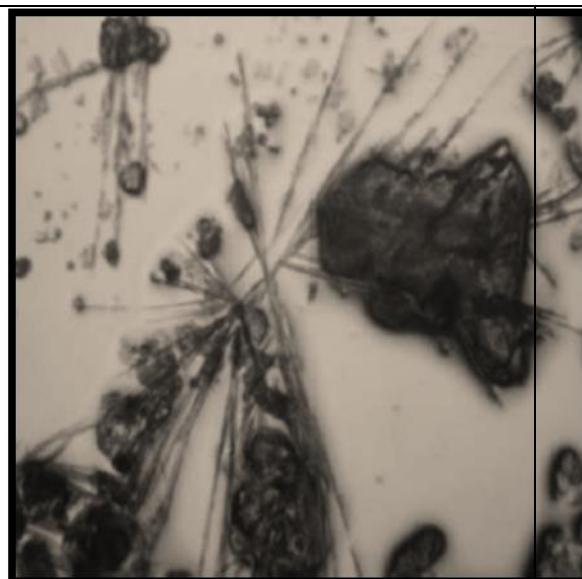
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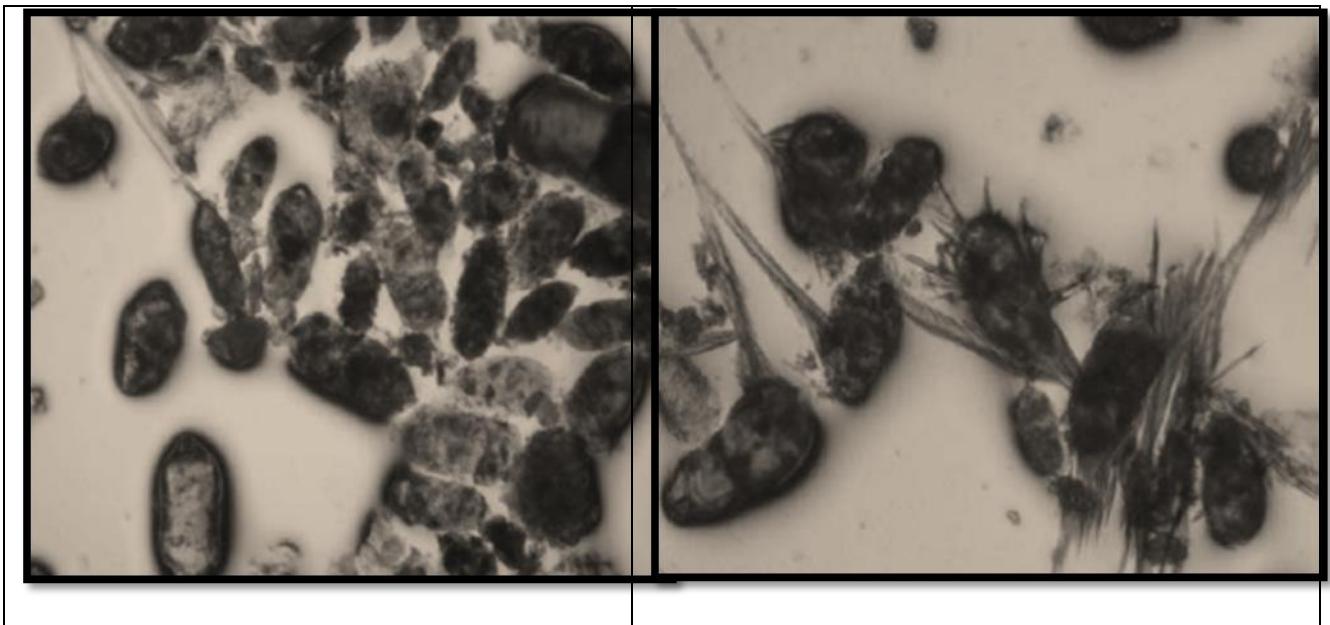
Microscopy of *Momordica charantia* Fruits

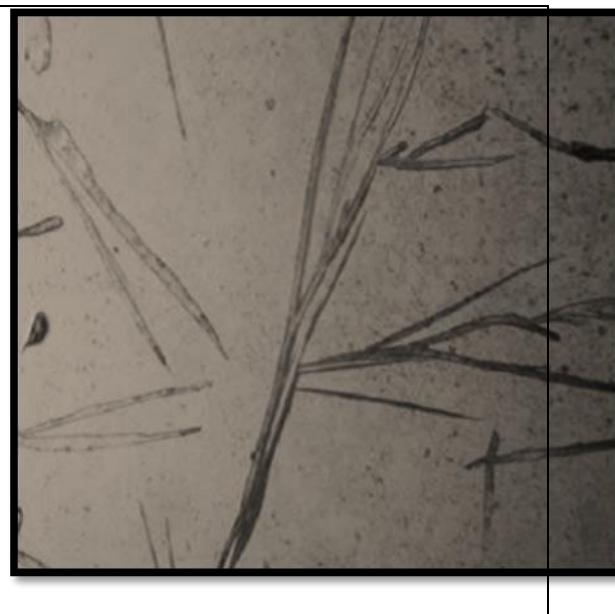
**Powder Character Of *Momordica charantia* Fruits:-**





Powder Microscopy Of *Momordica charantia* Fruits





### Microscopic Evaluation ( Table no-03)

S.No	Evaluation	Result
1.	Stomatal Density	800 mm <sup>-2</sup>

### 3.3. Chemical Evaluation.

#### Phytochemical screening or Chemical Evaluation of Prepared Churna:

In this include chemical test, assay, physical properties, purification and identification of active constituents are chemical methods of evaluation. It also includes phytochemical evaluation.( Table 4,5,6,7)

Table :-04

S. No.	Phytoconstituent	<i>Momordica charantia</i>

<b>1</b>	Glycoside	Positive
<b>2</b>	Alkaloids	Positive
<b>3</b>	Tannins	Positive
<b>4</b>	Flavonoids	Positive
<b>5</b>	Phenols	Positive

The Chromatographic Investigation of different batches of in-house formulation the  $R_f$  value given below.

### **3.4. Physical Evaluation.**

**Table :05 Physical Investigation of Prepared Churna:**

S. No.	Parameter	Prepared Churna (Different Batches)					Mean values + SEM
		Batch 1	Batch 2	Batch 3	Batch 4	Batch 5	
1	Loss on Drying 105°C	4.00	5.60	4.90	5.00	5.70	<b>5.04<math>\pm</math>1.521</b>
2	Water soluble Extractive Value (%)	60.40	66.70	71.25	66.50	68.70	<b>66.71<math>\pm</math>8.974</b>
3	Alcoholic Soluble Extractive Value (%)	41.20	45.20	47.80	45.80	50.80	<b>46.16<math>\pm</math>7.895</b>
4	Total Ash (%)						<b>13.6<math>\pm</math>0.894</b>

		14.30	13.40	13.30	13.50	13.50	
5	Acid Insoluble Ash (%)	0.70	0.70	0.80	0.80	0.80	<b>0.76±0.122</b>
6	p <sup>H</sup>	5.4	5.8	5.7	5.7	5.5	<b>5.62±0.367</b>

The physical characteristics of the in-house formulation (average values along with standard deviation) (n = 5) are shown in Table 7. The results of the In - House formulation indicate that flow ability of the formulation was found to be poor, which was further confirmed by high values of Hausner ratio & Carr's Index.

➤ **Table.:06- :** limiting value of more two essential parameter or Bitter melon *Momordica charantia Linn*, Family-Cucurbitaceae dry extract.

S.No	Parameters	Specification
1.	Heavy Metals	Not more than 25PPM
2.	Assay ( on dried basis)	Not less Than 35% W/W

**Table:-07 Physical Parameter study of Prepared Churna:**

S. No.	Parameters	Results

		CGS Units	S.I Units
1	Bulk Density	0.4436 ± 0.002 (gm/ml)	443.6 ± 0.184 (kg/m <sup>3</sup> )
2	Tapped Density	0.6240 ± 0.012 (gm/ml)	624±1.363 (kg/m <sup>3</sup> )
3	Carr's Index	40.66 %	
4	Hauner's Ratio	1.40 ± 0.4	
5	Angle of Repose	30.7 ± 0.2	

The result obtained for phytochemical screening reveals that phytoconstituents like glycosides, alkaloid and flavonoids, phenols were present in prepared In - House formulation (Table 2). Above study indicate that some constituent (s) carried by the herbal ingredient (*Momordica charantia*).

### 3.5. Analytical Evaluation & Standardization.

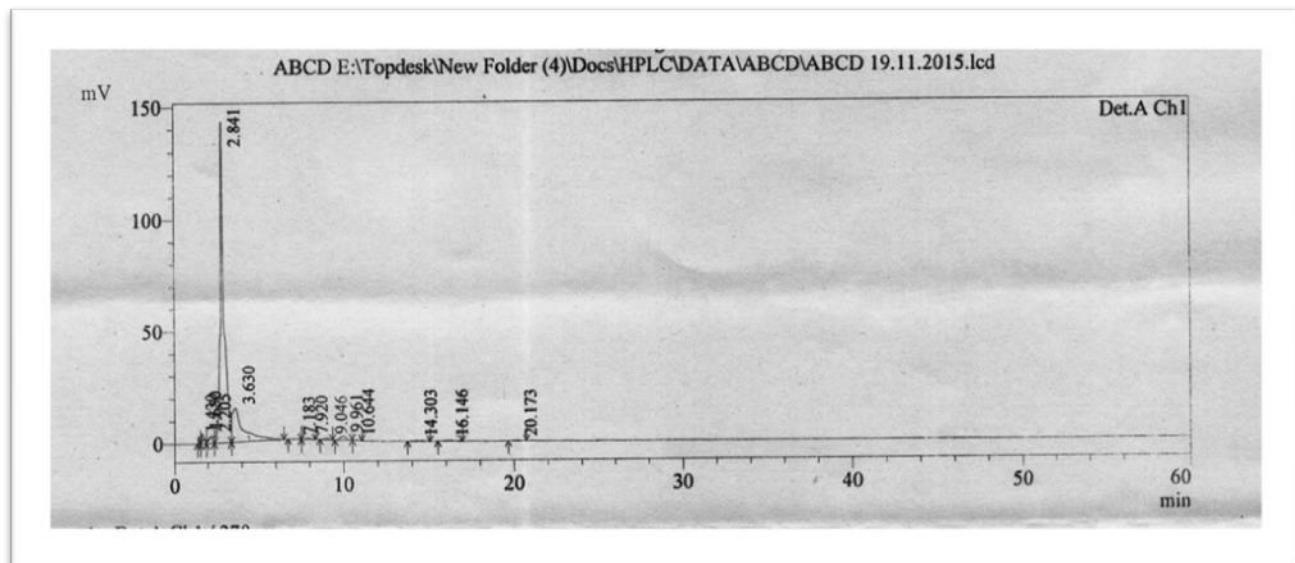
#### A) Investigation by TLC :-

Ethanolic extract of *churna* of the *Momordica charantia Linn* fruit, using the solvent system Chloroform: Methanol- 90:10 After development of the plate, allow it to dry in air and examine under U.V. (366 nm) four fluorescent zones at Rf. 0.24 (red), 0.62 (light sky blue), 0.97 (sky blue), 0.99 (red & sky blue). On exposure to Iodinen vapour four spots appear at Rf. 0.18, 0.47, 0.68 and 0.99 (all yellow). On spraying with 5%

Methanolic Phosphomolybdic acid reagent nine spots appear at Rf. 0.04, 0.17, 0.35, 0.44, 0.51, 0.60, 0.75, 0.81 and 0.99 (all blue), then we are going to study by HPLC with the solvent system of Methanol & Water, here changing the polarity than TLC for good separation according to polarity, HPLC is using like finger print for standardization of this herbal formulation.

### B) Investigation by HPLC:-

Each time 20 $\mu$ l of the test solution prefiltered through 0.45 membrane filter was injected into water's HPLC ( Australia ) with C-18 RP Bondapack column, setting its pressure by Auto-pressure between ( 0-4000 PSI ) and the flow rate was set at 1.5 ml/min. The solvent system used was **Methanol: Water-35:65** by Auto-selector and sample were run for 1 hour. By PDA detector peak were observed at 270 nm to have best results.



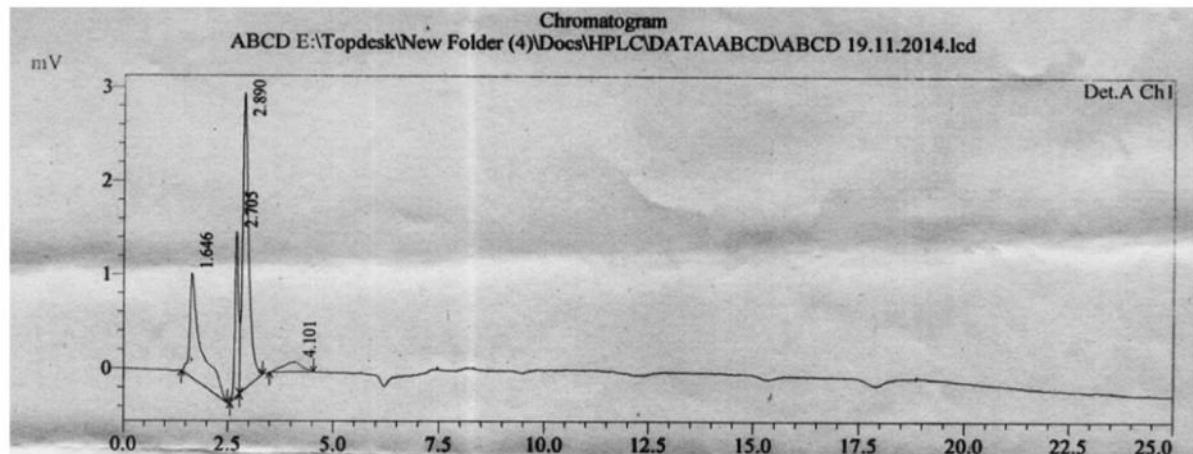
Detector A ch1 270nm

**Peak table**

Serial Number	Ret.Time	Area	Height	Theoretical Plate
1.	1.430	15534	3489	1863.736
2.	1.650	60681	4562	329.257
3.	2.205	74640	3332	73.099
4.	2.841	2211269	143126	2286.048
5.	3.630	703611	14916	120.544
6.	7.183	3434	140	1817.736
7.	7.920	8212	386	3332.005
8.	9.046	11166	630	7479.208
9.	9.961	38461	1640	4186.528
10.	10.644	2211	107	8312.977
11.	14.303	12294	338	3288.889
12.	16.146	12188	344	4294.148
13.	20.173	4666	146	7681.842
		3158366	173156	

Analyzed By:- Subhajit Ghosh

Date :-19/11/2014



**Det.A Ch1/270nm**

**Detector A Ch1 270 nm**

**Peak Table**

<b>S.No</b>	<b>Ret.Time</b>	<b>Area</b>	<b>Height</b>	<b>Theoretical Plate</b>
1.	1.646	20800	1110	464.396
2.	2.705	10388	1757	3365.950
3.	2.890	32141	3170	1960.620
4.	4.101	3284	107	391.257
5.		66613	6144	

Analyzed By:- Subhajit Ghosh

Date :-19/11/2014

#### **4.Conclusion**

The process of Standardization of Herbal drugs comprises total information about the formulation or herb and controls to essentially guarantee consistent composition of all herbal formulations including Analytical operations for identification , markers and Assay of active principles. There is no legal control model over

medicinal or herbal plants. Different countries define medicinal plants or formulations derived from them in different ways and have adopted different approaches to licensing, dispensing, manufacturing and trading to ensure their safety, quality and efficacy. Fingerprinting of herbal formulations is utilized for the authenticity and quality control of herbal formulation or herbal drugs, chemical fingerprints obtained by chromatographic techniques, in this article we are using some physical parameters ,TLC and HPLC, all the results are showing as a fingerprint of that particular herbal formulation which having promising antioxidant activity , as well as responsible for so many pharmacological activities. Instrumental analytical methods are the most potent tools for quality control of traditional herbal formulation. More over all herbal formulation manufactures must follow WHO guidelines for quality control.

Further, the Combination of fingerprinting and quality multi-component analysis is a novel and rational method to address the main issues of quality control of herbal formulation. The advancement of analytical techniques will serve as a rapid and specific tool in the herbal research, thereby, allowing the manufactures to set quality standards and specifications so as to seek marketing approval from regulatory authorities for therapeutic efficacy, safety and self life of herbal drugs. The applications of high- technology oriented advanced techniques will serve as a rapid and unambiguous tool in the herbal research, thereby benefiting the entire Pharmaceutical industry as well as herbal formulation users also.

### **Conflicts of interest**

None of the authors have any conflicts of interest to declare.

### **Acknowledgement**

The authors gratefully acknowledge to our,The Ayurvedic Formulary of India. Government of India, Ministry of Health and Family Welfare, New Delhi, The Ayurvedic Pharmacopoeia of India, Government of India, Ministry of Health and Family Welfare, New Delhi, India, all Ayurvedic practitioners who help us and

Special thanks to Mrs.Bithika Tarafder,Mr.Sambhu Nath Ghosh,Mrs.Kalayani Ghosh,Mrs.Indrani Ghosh For providing all facility to complete this work successfully .

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