PREPARATION AND STANDARDIZATION OF AGNIMUKH CHURNA: A POLY-HERBAL FORMULATION

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ABSTRACT

Herbal medicine is a plant derived preparation with therapeutic health benefits. It contains either raw or processed ingredients from one or more plants. Traditional system of medicine is effective but it lacks standardization. Standardization of herbal formulation is essential in order to assess the quality, purity, safety and efficacy of the drug. The present work is an attempt to standardize the Agnimukh churna an Ayurvedic polyherbal preparation which is used as appetizer and digestive stimulant medicine. In-house and two marketed preparations have been standardized as per Indian Pharmacopoeia and WHO guidelines on the following parameter viz. Organoleptic characters, Physical characters, Physio-chemical properties, Preliminary phyto chemical screening and HPTLC fingerprinting. These results will be useful towards establishing pharmacopoeial standards for the Agnimukh churna formulation.

KEYWORDS: Agnimukh Churna, Standardization, HPTLC Fingerprinting.

INTRODUCTION

Herbal medicines are complex chemical mixtures obtained from a plant which is widely used in health-care in both developed and developing countries [1] for the maintenance of health as well as in the prevention, improvement or treatment of physical and mental illness. In the present era, universal trend has been shifted from synthetic to herbal medicine i.e. Return to Nature.[2] Due to less side effects, more effectiveness, cheap in cost, well acceptance, not harmfulness. A Herbal medicine is effective but lacks standardization, due to the complex nature and inherent variability of the chemical constituents of the plant based drugs, it is difficult to establish quality control parameter. [3] The World Health Organization (WHO) has appreciated the importance of medicinal plants for public health care in developing nations and has evolved guidelines to support the member states in their efforts to formulate national policies on Traditional medicine and to study their potential usefulness including evaluation, safety and efficacy. [4]

Standardization is essential in order to assess the quality, purity, safety and efficacy of the drug. Therefore it is imperative to establishment of quality control parameters for traditional medicine which will be alignment with modern technology.[5] High Performance Thin Layer Chromatography (HPTLC) is a valuable quality assessment tool for the evaluation of botanical materials. It allows for the analysis of a broad number of compounds both efficiently and cost effectively. Additionally, numerous samples can be run in a single analysis thereby dramatically reducing analytical time. With HPTLC, the same analysis can be viewed collectively in different wavelengths of light thereby providing a more complete profile of the plant than is typically observed with more specific type of analysis. [6] High Performance Thin Layer Chromatography (HPTLC) can provide an electronic image of the chromatographic fingerprint and a densitogram to detect the presence of marker compounds in a plant sample.[7]

Agnimukh Churna is an appetizer and digestive stimulant. It reduces the flatulence, abdominal distention and intestinal gas. It improves the bowel movement and helps to get complete bowel evacuation. Agnimukh Churna was selected because it had no previous scientific works been reported. So to prepare and standardize procedures of the churna, [8,9] the present work was attempted.

MATERIALS AND METHODS

1. Plant material

The Agnimukh Churna comprises of fruits of *Cuminum cyminum* (cumin), rhizomes of *Zingiber officianalis* R (Saunthi), fruits of *Piper nigrum* L (Marica), leaves of *Menthe piperata* L (Peppermint), Sendha namak (rock salt), Kala namak (black salt) and Nimbu satva (Citric acid). All these ingredients were procured from local

market of Hyderabad, India and authenticated on basis of morphological and microscopically characters.

2. Preparation of Agnimukh churna

In house formulation of Agnimukh Churna was prepared accordingly first Roast cumin seeds and keep it aside for further use. Now, grind to make powder of each herb including roasted cumin seeds except citric acid and peppermint. Mix appropriate quantities of all the herbs (*Piper nigrum*-554mg, *Zingiber officinale*-554mg, *Cuminum cyminum*-1.1gm, Sendha namak-1.65gm, Kala namak-554mg) and make a mixture. Grind to make powder of citric acid crystals- 554mg and add it to the herbal mixture prepared in the second step. Then, pestle peppermint-13.9 mg in a stone mortar and mix it in the above made mixture. Pass the Churna through sieve number 80 # to prepare a homogeneous blend. Agnimukh Churna is ready to use now. Preserve it in an air tight glass container.

3. Marketed samples

The marketed samples of various brands Agnimukh Churna i.e. Baidyanath (formulation 1) and Sanjeevika (formulation 2) and the in-house preparation (formulation 3) were standardized based on their oganoleptic charecters, physical characteristics and physicochemical properties.

4. Evaluation of Agnimukh Churna
1. Organoleptic evaluation

Organoleptic evaluation refers to evaluation of formulation by color, odor, taste, texture etc. The organoleptic characters of the sample were carried out based on the method described by Siddique et. Al. [10]

2. Physicochemical Investigation A. Determination of Total ash

Ash values are used to determine quality and purity of crude drug. It indicates presence of various impurities like carbonate, oxalate and silicate. A high ash value is indicative of contamination, adulteration, substitution or carelessness in preparing the formulation. Total ash determination constitutes detecting the physiological ash (ash derived from plant tissue) and nonphysiological ash (ash from extrageneous matter, especially sand and soil adhering to the surface of the drug). For its detection, 2g of each formulation were placed separately in a suitable tarred crucible of silica pre viously ignited and weighed. The powdered drugs were spread into an even layer and weighed accurately. The materials were incinerated by gradually increasing the heat, not exceeding 450°C until free from carbon, cooled in a desiccator, weighed and percentage ash was calculated by taking in account the difference of empty weight of crucible & that of crucible with total ash. [11]

B. Acid insoluble ash

The acid insoluble ash consist mainly silica and indicate contamination with earthy material. The ash obtained as above was boiled for 5min with 25ml of dilute hydrochloric acid. The insoluble matter was collected on an asshless filter paper, washed with hot water and ignited to constant weight. The percentage of acid-insoluble ash with reference to the air-dried drug was calculated.

C. Water soluble ash

The water soluble ash is used to estimate the amount of inorganic compound present in drugs. The ash was boiled for 5 minutes with 25 ml of water; collected insoluble matter in an ash less filter paper, washed with hot water, and ignited for 15 minutes at temperature not exceeding 450°C. Subtract the weight of the insoluble matter from the weight of the ash. The difference in weight represents the water-soluble ash. The percentage of water-soluble ash with reference to the air-dried drug was calculated.[12]

D. Extractive value

The extractions of any crude drug with a particular solvent yield a solution containing different phytoconstituents. The compositions of these phytoconstituents depend upon the nature of the drug and the solvent used. It also gives an indication whether the crude drug is exhausted or not.[12]

E. Alcohol soluble extractive value

5g each formulation was macerated with 100ml of alcohol 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 tarred 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]

F. Water soluble extractive value

5g each formulation was macerated with 100ml 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 tarred 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. [14]

G. Loss on drying

Moisture content of drugs should be at minimal level to discourage the growth of bacteria, yeast or fungi during storage. Loss on dying is the loss of mass expressed as percent w/w. About 10g of dug samples of each formulation was accurately weighed in a dried and tarred flat weighing bottle and dried at 105°C for 5hrs. Percentage was calculated with reference to initial weight.[15]

3. Physical characteristics of Agnimukh Churna A. Bulk density and Tap density

The term bulk density refers to a measure used to describe a packing of particles or granules. Bulk density is the ratio of given mass of powder and its bulk volume.

Bulk density = W/V0 gm/ml

W= mass of the powder, V0= untapped volume The volume of the packing can be determined in an apparatus consisting of agraduated 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.[16,17]

Tapped density= W/Vf gm/ml

W= mass of the powder, Vf= tapped volume

B. Angle of repose

Angle of Repose has been used as an indirect method of quantifying powder flowability because of its relationship with interparticle cohesion. The internal angle between the surface of the pile of powder and the horizontal surface is known as the angle of repose.

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.[16,17]

Angle of repose = tan^{-1} (h/r)

h= height of the pile, r= radius of the pile.

C. Hausner ratio

Hausner ratio is related to interparticle 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 ratio greater than 1.6. [16,17]

Hausner ratio = Tapped density/ Bulk density
D. Carr's index

Carr's index is another indirect method of measuring the powder flow from bulk density.

% Compressibility index = [(tapped density-bulk density)]/ tapped density $\times$ 100

4. Preliminary phytochemical screening

Preliminary Phythochemical Analysis of In house and marketed formulation of Agnimukh Churna were carried out including Test for alkaloids, glycosides, flavonoids, terpenoids, tannins, steroids, carbohydrates, proteins and Saponins.[18]

5. Limit test for Iron
Preparation of Standard Solution (20 PPM)
One volume of 0.1726% w/v solution of ferric ammonium sulphate solution was diluted in 0.05 M sulphuric acid to ten volume using distilled water.

Procedure
Limit test was performed in Nessler's cylinder. 2ml of test and standard solutions were taken in separate cylinders and then 2ml of 20% solution of citric acid and 0.1 ml thioglycollic acid were added. The solution was then mixed and made alkaline with iron free ammonia, diluted to 50ml with distilled water. It was then allowed to stand for 5minutes and colour obtained in sample was compared with of standard colour. If the colour produced in test is more when compared to that of standard solution then the sample was said to fail the limit test and said to pass the test if vice versa occurs. [9]

6. HPTLC Finger printing
HPTLC study of methanolic extracts marketed formulation-1 (Baidhyanath) were carried out along with the different marker compounds corresponding to the active ingredients to ensure the presence of active ingredients in the formulations.

Sample Preparation
10 mg of sample powder was weighed and extracted with 25ml of methanol on boiling water bath for 25minutes consecutively three times using fresh potion of 25ml methanol, filtered and concentrated. 50 μl of this solution was applied on TLC plate.[19]

Standard Solution Preparation
10 mg of standard piperine was weighed and dissolved in 10 ml of methanol (1000 μg/ml). From this 1ml was diluted to 100 ml to get solution having concentration 100μg/ml. In similar manner standard solution of Zerumbone was prepared having concentration 100 μg/ml. 2 μl of each of this solution was applied on TLC plate.

Instrumentation and Chromatographic conditions
The samples were spotted in the form of bands of width of 6 mm with space between bands of 10 mm, with a 100 μL sample syringe (Hamilton, Bonaduz, Switzerland) on precoated silica gel aluminium plate 60 F 254 (10 cm ×10 cm) with 250 μm thickness (E. MERCK, Darmstadt, Germany) using a CAMAG Linomat 5 sample applicator (Switzerland). The slit dimensions 5 mm × 0.45 mm and scanning speed of 20 mm/sec was employed. The linear ascending development was carried out in 10 cm×10 cm twin trough glass chamber (CAMAG, Muttenz,

Switzerland) using n-Hexane: Ethyl Acetate: Acetic Acid (5 : 3.5 : 1.5 v/v) as mobile phase. The optimized chamber saturation time for mobile phase was 20 min. The length of chromatogram run was 8 cm and development time was approximately 20 min. TLC plates were dried in a current of air with the help of a hair drier. After development by WINCATS software version 1.4.2. densitometric scanning was performed on CAMAG thin layer chromatography scanner at 210 nm.[20]

RESULTS AND DISCUSSION
In house Agnimukh churna was prepared in accordance with the Ayurvedic formulary of India. Two Marketed Agnimukh churna formulations were procured from Baidyanath and Sanjeevika. As a part of standardization procedure, all the formulations were tested for Organoleptic characters, Physical characteristics, Physico-chemical properties, Heavy metal test and HPTLC fingerprinting.

Organoleptic evaluation reveals that Agnimukh churna appears like brown colour, fine powder bitter taste and characteristic odour [Table I]. The physiochemical properties of Agnimukh churna such as total ash, acid insoluble ash, water soluble ash, alcohol soluble extractive, water soluble extractive and loss on drying were calculated and results were shown in Table II. The results are expressed as mean (n=3) ±Standard deviation (SD). Variations were observed in most of the physicochemical parameters studied. The total ash value of formulation 1 was found to be lower than that for 2, 3. Acid insoluble ash value for formulation 1 was found to be 19.167 ± 1.6 and in case of formulation 2 and 3 this was found to be 22.333 ± 1.53 and 23.333 ± 1.53 respectively. The water soluble extractive values of formulations were found to be much higher than alcohol extractive values. Percentage weight loss on drying or Loss on drying at 105°c was minimal that indicates that less bacterial, fungal or yeast growth.

Table I Organoleptic Evaluation.

Parameter	Formulation 1	Formulation 2	Formulation 3
Appearance	Powder	Powder	Powder
Colour	Dark brown	Light brown	Dark brown
Odour	Characteristic	Characteristic	Characteristic
Taste	Bitter	Bitter	Bitter

Formulation 1- Baidyanath, Formulation 2- Sanjeevika, Formulation 3- In house

Table II Physico-chemical Parameters Evaluation.

S. no.	Physico-chemical parameter	Formulation 1	Formulation 2	Formulation 3
1	Total Ash Value	21.00 ± 1	26.33 ± 1.52	26.00 ± 1.3
2	Acid insoluble ash	19.167 ± 1.6	22.333 ± 1.53	23.333 ± 1.53
3	Water Soluble Extractive value (%w/w)	26.3333± 3.21	24.8666± 2.01	40.3333± 0.57
4	Alcohol soluble extractive value (%w/w)	30.5± 0.5	43.83 ± 0.76	40.5 ± 0.5
5	% Loss on drying (w/w)	0.09± 0.87	0.09 ± 0.78	0.12± 0.66

Table III Physical Parameters Evaluation.

S. no.	Physical parameters	Formulation 1	Formulation 2	Formulation 3
1	Bulk density (gm/ml)	0.33	0.4	0.38
2	Tapped density(gm/ml)	0.526	0.666	0.55
3	Hausners ratio	1.215	1.365	1.337
4	Carr's index	23.8%	19.52%	24.92%
5	Angle of repose	34±1.89	33± 1.97	34±1.66

Table- IV Determination of phytochemical constituents.

S.NO.	Phytochemical	Result
1.	Alkaloids	+
2.	Glycosides	-
3.	Flavonoids	-
4.	Terpenoids	+
5.	Tannins	+
6.	Steroids	+
7.	Carbohydrates	+
8.	Proteins	+
9.	Saponins	-

"**Fig.1**"Limit test for Iron Results.

1 2 3   4   5

"**Fig. 2**" HPTLC plate seen at visible light

Track 1: Blank (Methanol) - 2μl
Track 2: Piperine in Methanol (200ng/band) - 2μl
Track 3: Zerumbone in Methanol (200ng/band) - 2μl
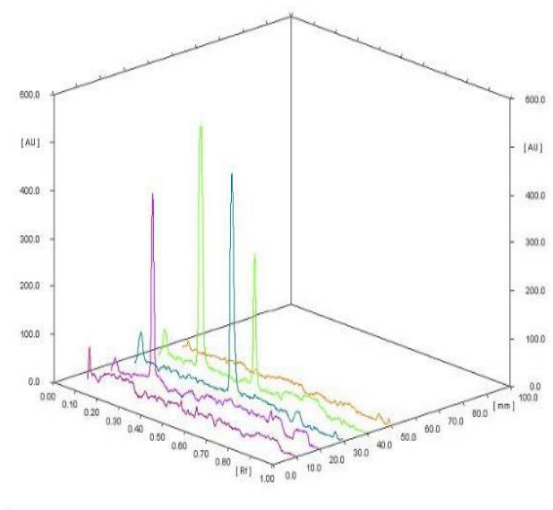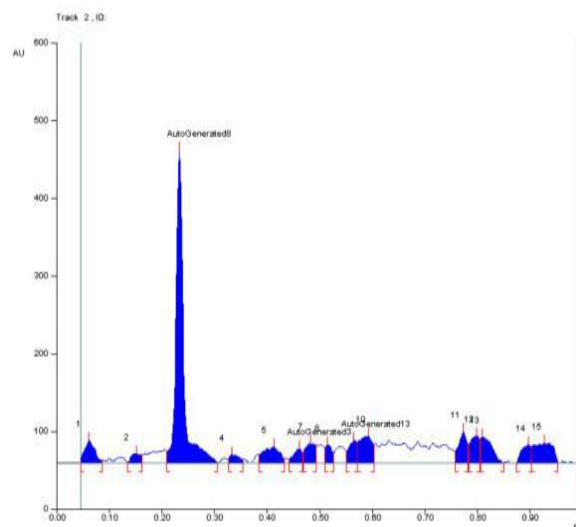Track 4: Agnimukh churna sample in Methanol - 2μl
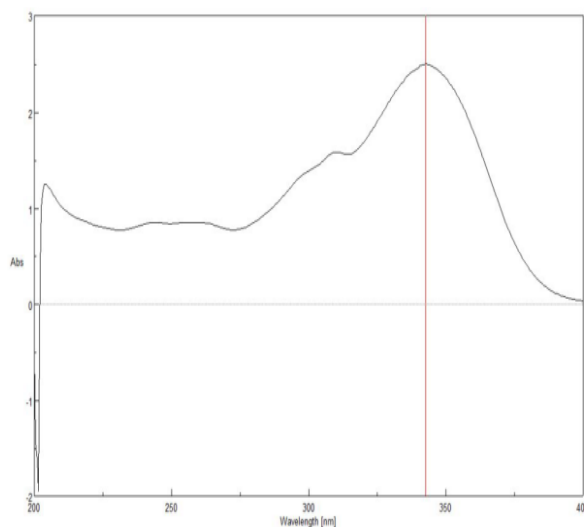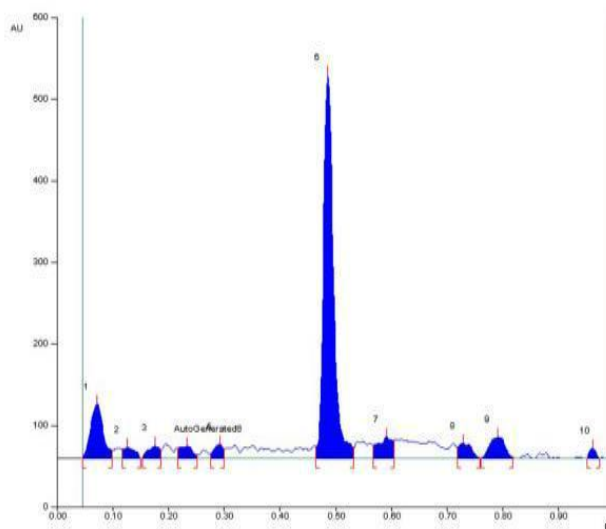Track 5: Blank (Methanol) - 2μl

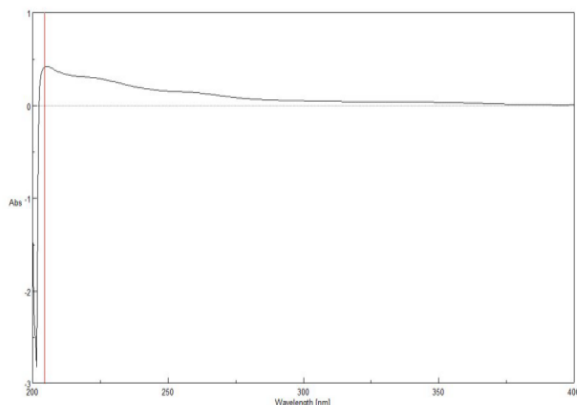Figure: 3 D Densitogram (All tracks scanned at 210 nm)

"**Fig. 4**" 2D Densitogram of Track 2 (Standard Piperine, 2000ng/band) [Rf- 0.23; Area-14240.34].
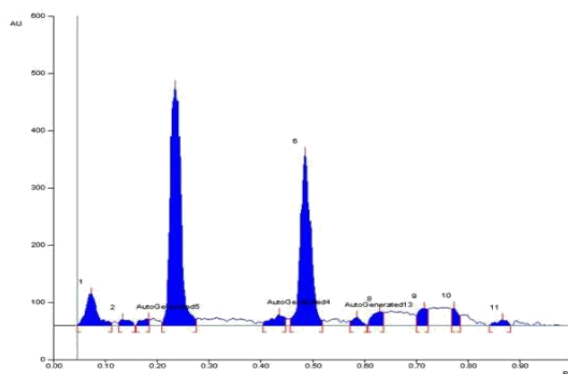

"**Fig. 5**" UV Spectrum of standard Piperine.

"**Fig. 6**" 2D Densitogram of Track 3 (Standard Zerumbone, 2000ng/band) [Rf- 0.49; Area-15578.34].

"Fig. 7" UV Spectrum of standard Zerumbone

"Fig. 8" 2D Densitogram of Track 4.

Table V: Quantitative values of Piperine and Zerumbone in Agnimukh churna.

PIPERINE		
Type of sample	Area	Concentration
Standard	14240.34	200
Sample	15840.56	222.474
ZERUMBONE		
Type of sample	Area	Concentration
Standard	15578.34	200
Sample	6984.12	89.6645

The physical properties of Agnimukh churna (In house and marketed formulations) are shown in Table III. The results of the marketed and in-house formulations were found to be comparable and indicate poor flow property. Preliminary phytochemical screening revealed the presence of Alkaloids, Terpenoids, Tannins, Steroids, Carbohydrates and Proteins Table IV. Heavy metal of iron was absent in marketed and in-house formulations (fig. 1).

HPTLC fingerprinting of Agnimukh churna (methanol extract) were developed in n-Hexane: Ethyl Acetate: Acetic Acid (5 : 3.5 : 1.5 v/v) solvent system (fig 2). The active principles of Agnimukh churna, Piperine and

zerumbone taken as marker compounds. The 3 D Densitogram of Agnimukh churna along with marker compounds piperine and zerumbone scanned at 210 nm (fig 3). Piperine resolved at Rf.- 0.23; Area- 14240.34 (fig 4). Sharp peaks of piperine (standard and sample) were obtained at wavelength 340nm (fig 5). Zerumbone resolved at Rf -0.49; Area 15578.34 (fig 6). Zerumbone (standard and sample) sharp peak were Obtained at 200nm (fig 7). The 2 D Densitogram of Agnimukh churna were found to contain both piperine (Rf.- 0.23) and zerumbone (Rf -0.49) (fig 8). Quantification of piperine and zerumbone in Agnimukh churna, were obtained automatically (Table V). Quantity of Piperine found in 50 µL of sample 222.474 ng. Therefore 01 ml of extract contains – 222.474 ng * 20 = 4449.489 ng = 4.449 µg of piperine. So 100 mg of sample contains - 4.449 mg of piperine i.e 4.449 % w/w of piperine. Quantity of zerumbone found in 50 µL of sample 89.6645 ng. Therefore 01 ml of extract contains – 89.6645 ng * 20 = 1793.29 ng = 1.7933µg of Zerumbone. So 100 mg of sample contains - 4.449 % w/w of piperine and 1.793 % w/w of Zerumbone.

CONCLUSION
The Agnimukh Churna was standardized by intervention of modern scientific quality control method (HPTLC Fingerprinting). The HPTLC densitometric method has been developed for quantification of piperine and zerumbone in Agnimukh Churna. The results obtained are used as tool for the quality control or quality assurance of Agnimukh churna.

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