Preparation and Evaluation of Polyherbal Formulation Ayurvedic Churna for the treatment of Atopic Dermatitis

Darshana Sharma* and Dishant Gupta
Faculty of Pharmacy, Oriental University, Indore, (M.P.) – India
***Corresponding Author**
E.mail: darshana.sharma18@gmail.com

Abstract

People are usually unconcerned about their skin, yet skin illnesses can exacerbate an already bad situation. Nothing is more irritating and nasty than skin irritation and itching. Atopic dermatitis is one of the most common skin illnesses, characterised by itching, redness, dry areas, and elevated lumps on the skin. These disorders are especially frequent in youngsters, but they can afflict adults of any age. It is an illness that can be caused by germs, irritants, or allergies. Herbs are commonly used in the treatment of atopic dermatitis (AD) in India, and several herbs are thought to contain anti-inflammatory characteristics that can aid with AD. In te present paper ayuvedic churna was prepared as per mentioned in ayurveda using various herbs. The prepared herbal formulation in the form of churna was evaluated and the results were reported. The evaluation parameters of churna was found within the limit when checked with Ayurvedic Pharmacopoeia.

Key-words: Ayurvedic Churna, Herbs, Preparations
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Introduction

According to WHO, Herbal Medicine (HM) can be defined as a final medicinal product, well labelled and consisting of an active principle, which may include any part of the plant or the combination of different parts of a single or different plant. HM is widely used across the globe by the population of all genders, races and social status, due to its natural status, low costs, good efficacy and low toxicity. There are evidences of the use of HM 60,000 years ago, but more recently, the discovery of a Sumerian clay slab which was dated 5000 years ago was found, which was utilized for the manufacturing of drugs in ancient time. [1-2]

Ayurveda is one of the ancient medicinal systems which comprises of a many types of dosage forms (M.R. *et al.*, 2021). Some examples of ayurvedic dosage forms are *Vati* (pills), *Kalka* (paste), *Kwatha* (decoction), *Swarasa* (Expressed juice), *Churna* (Powder), *Asava* (Alcoholic preparation), *Rasa* (Mercurial Preparation), etc. Out of these dosage forms, *Churna* is the simplest dosage form. According to the Ayurvedic Formulary of India, *Churna* can be defined as fine powder of drugs or multiple drugs (Anonymous, n.d.). *Churna* is prepared by pulverization of any substance to make it into a fine powder. Recent advancement in research methodologies and technologies has assisted in the investigation and development of novel ayurvedic formulations. [3-5]

Atopic dermatitis (AD) is a skin condition connected with hyperreactivity of cutaneous which is usually triggered by the environmental factors. It results in chronic inflammation and

often leads to allergic rhinitis and asthma. AD is also known as 'atopic eczema'. The prevalence rate of atopic dermatitis (AD) during the 21st century varied across regions, with rates ranging from 1.2% to 17.1% in adults and from 0.96% to 22.6% in children in Asia. Understanding the prevalence and impact of AD is crucial for effective management and treatment strategies. [6-7]

Material and Methods

Selection, Collection and Authentication of Plant Material

The plants material i.e., Amla (*Phyllanthus emblica*), Haritaki (*Terminalia chebula*), Bahera (*Terminalia bellerica*), Vacha (*Acorus calamus*), Neem (*Azadirachita indica*), Manjistha (*Rubia cordifolia*), Katuki (*Picrorhiza kurroa*), Giloy (*Tinospora cordifolia*) and Daru Haldi (*Berberis aristata*) were used in the treatment of atopic dermatitis were selected for the present study based on literature review. All the raw materials used in formulation were purchased from local market of Indore (M.P.) 452010, India and identified morphologically and compared with standard Pharmacopoeial Monograph. The sample of crude drug was also identified & authenticated by Dr. S.N. Dwivedi, Retd. Professor & Visiting Professor, APS University, Rewa (M.P.). Voucher Specimen no was allotted. J/Bot/NCP-30-38.

Preparation of Polyherbal Formulation (Ayurvedic Churna)

All the plant parts were dried under shade and were made to fine powder and were mixed in ratio as mentioned in table 1. Three batches (AC-I, AC-II & AC-III) were prepared in laboratory as per procedure mentioned in Ayurvedic Pharmacopoeia. [8]

Table 1: Composition of Polyherbal Formulation (Ayurvedi Churna)

S/No.	Ingredients	Local Name	Part Used	AC-I	AC-II	AC-III
1.	*Phyllanthus emblica*	Amla	Fruits	25	25	50
2.	*Terminalia chebula*	Haritaki	Fruits	25	25	50
3.	*Terminalia bellerica*	Bahera	Fruits	25	25	50
4.	*Acorus calamus*	Vacha	Rhizomes	25	50	100
5.	*Azadirachita indica*	Neem	Leaves	25	50	100
6.	*Rubia cordifolia*	Manjistha	Whole plant	25	50	100
7.	*Picrorhiza kurroa*	Katuki	Stem	25	100	200
8.	*Tinospora cordifolia*	Giloy	Stem	25	100	200
9.	*Berberis aristata*	Daru Haldi	Rhizomes	25	100	200
10.	Black Salt	Kala Namak	-	qs	qs	qs

Evaluation of Polyherbal Formulation (Ayurvedic Churna) [9-13]

Organoleptic evaluation

The color, odor, taste of the three laboratory batches (AC-I, AC-II & AC-III) of polyherbal formulation were analyzed manually.

Determination of Flow properties

The flow properties of the three laboratory batches (AC-I, AC-II & AC-III) of polyherbal formulation were determined as per procedure mentioned above.

Determination of pH

The pH of 1% solution of formulated polyherbal formulation churna was **d**etermined using pH meter (Elico pH meter).

Determination of Heavy Metal Contamination

Heavy metal contamination in three laboratory batches (AC-I, AC-II & AC-III) of polyherbal formulation determined as per procedure mentioned below.

Preparation of Sample Solution (Lead, Cadmium and Iron)

Heavy metals analysis was done according to AOAC guidelines (2016) for non-volatile heavy metal. Take 5 g of the sample in a pre-weighed silica dish and keep it in a muffle furnace with initial temperature not higher than 100oC. Increase the temperature slowly to a maximum

of 450oC. Allow the dish to stand at least 8 hrs. or overnight. Wet ash with 1-3 ml water and evaporate on hot plate or water bath. Place the crucible in muffle furnace at not more than 200oC and raise the temperature to 450oC. Proceed with ashing at 450oC for 1-2 hrs. or longer. Repeat procedure until the sample is completely ashed. Add 5 ml of 6 M Hydrochloric acid to crucible ensuring that all ash comes into contact with acid. Evaporate acid on water bath or hot plate. Dissolve residue in 10-30 ml of 0.1 M Nitric acid. Cover with watch glass and let stand for 1-2hrs. Then stir solution in dish thoroughly with glass rod and filter the solution in a 100 ml volumetric flask and make up the solution upto the mark with deionised water. Analysis was done using atomic absorption spectrophotometer (GBC Avanta). The standard reference material of all the metals (E. Merck) was used for calibration and quality assurance for each analytical batch. Three replicates were analysed to assess precision of the analytical techniques, and results were averaged.

Preparation of Sample Solution (Mercury)

5-10 g of the plant material was taken in a 100 ml Round bottom flask of the bethge apparatus add 3 to 4 glass beads, 10-12 ml of concentrated Nitric acid and 2-5 ml of concentrated Sulphuric acid connect the flask to the condensate receiver and reflux condenser and Put the flask in the cold condition for about 1.5 hrs. Once, remove cold condition heat the flask and collect the nitric acid in the condensate receiver continue heating till the sulphuric acid starts. Fuming and chars the sample. Remove the burner, wait for a few minute and carefully allow the nitric acid to drain into the flask. Repeat this operation to all the sample solution becomes just pale yellow colour. Cool and then remove it from the condensate receiver. Collect all the condensate in 50 ml standard flask and make the solution with deionised water. Analysis was done by using Mercury analyser MA 5840. Three replicates were analysed to assess precision of the analytical techniques, and results were averaged.

Arsenic Content

Preparation of Standard Solution (10PPM)

0.33gms of arsenic trioxide was dissolved in 5ml of 2M Sodium hydroxide solution and then diluted to 250ml with water. One volume of this was the diluted to 100 volume with water.

Preparation of Sample

Preparation of Churna solution

The churna solution was prepared by means of diluting 1gm of churna to 100ml using distilled water. This is used to carryout limit test for iron and lead and also to perform qualitative test for mercury. 10ml of churna solution was pipetted out into a flask and about 10ml of concentrated nitric acid was added and evaporated to dryness on a waterbath. The residue was then dried at 130° C for 30minutes then about 10ml of hydrazine molybdate reagent was added and refluxed for 20minutes. The solution was then cooled and absorbance of both standard and test solution was measured at 800 nm usingn Perkin Elmer UV spectrophotometer.

Determination of Microbial Content

Total viable aerobic count

Preparation of sample: Dissolved 10g *churna* being examined in buffered sodium chloride peptone solution pH 7.0 and adjust volume 100ml with same medium.

Examination of sample: Total viable aerobic count in the sample was examined by using the plate count method by Digital colony counter by Chemiline.

C. For bacteria: Petri dishes of 10cm diameter were used; 1ml pretreated mixture was added to each dish and 15ml liquefied casein soyabean digest agar at a temperature not more than 45˚C. Two Petri dishes for each sample were prepared using the same dilution and incubated at 30- 40˚C for 3days. The number of colonies form was calculated using the digital colony counter. Results were calculated using plate with the greatest number of colonies but taking 300 colonies per plate as the maximum consistent with good evaluation.

Total yeast and mould count: Procedure was used as described in the test for bacteria using Chloramphenicol yeast glucose agar (CYGA) in place of casein soyabean digest agar and incubate the plates at 20-25˚C for 5days, unless

a more reliable count was obtained in shorter time. Results were calculated using the plates with not more than 100 colonies by using colony counter.

Test for *Escherichia coli*

Dissolved 10g *churna* in 90ml fluid casein digest-soya lecithin-Polysorbate 20 medium to get 1:10 dilution. 10ml mixture was added in 50ml nutrient broth in sterile screw-capped container, shake, allowed to stand for 1hr and shake again and incubated at 35-37˚C for 24hrs. After incubation, 1ml was added to 5ml of Mac-Conkey broth and incubated at 36-38˚C for 48hrs. If the contents of the tube show acid and gas indicated the possible presence of *E. coli.* Alternative test of an inoculating loop, Streak a portion from the enrichment culture (obtained in the previous test) on the surface of Mac-Conkey agar medium. Covered and invert the dishes and incubated at 45˚C for 24hrs. Growth of red, generally non-mucoid colonies of Gram negative rods, sometimes surrounded by a reddish zone of precipitation, indicated the presence of *E. coli.*

Test for *Salmonella* species Dissolved 10g *churna* in 90ml fluid casein digest-soya lecithin-Polysorbate 20 medium (1:10 Dilution). 10ml of mixture was added in 100ml nutrient broth in sterile screw-capped container, shake, allowed to stand for 4hrs and shake again and incubated at 35-37˚C for 24hrs. After incubation, 1ml enrichment culture to each of the two tubes containing (A) 10ml selenite F broth and (B) tetrathionate bile brilliant green broth and incubated at 36-38˚C for 48hrs. From each, streak looped on Bismuth sulphate agar and Xylose lysine deoxycholate agar media and incubated the plates at 36˚-38˚C for 24hrs. Well developed, black or green in Bismuth sulphate agar and red with or without black centers colonies in Xylose lysine deoxycholate agar indicated the presence of *Salmonella species.*

Physicochemical Evaluation

Standard procedures were used as per method described earlier.

Phytochemical Evaluation

The polyherbal formulation (Churna) was tested for presence or absence of phytoconstituents as per standard method.

Results and Conclusion

Three laboratory batches (AC-I, AC-II & AC-III) of Ayurvedic Churna (AC) were prepared in laboratory as per procedure mentioned in Ayurvedic Pharmacopoeia. Three laboratory batches of Ayurvedic Churna were prepared using standard procedure and were named as AC-I, AC-II & AC-III. One marketed formulations named MF-AC was purchased from local pharmacy store of Indore. These samples were stored at optimized conditions of temperature, light and moisture. The organoleptic characteristics, flow properties and pH of Ayurvedic churna i.e., AC-I, AC-II & AC-III) and MF-AC were evaluated results were mentioned in table 2.

Table 2: Physical Evaluation of Aurvedic Churna

S/No.	Parameters	Observations			
		AC-I	**AC-II**	**AC-III**	**MF-AC**
1.	Color	Light Brown	Brown	Dark Brown	Brown
2.	Odor	Characteristic	Characteristic	Characteristic	Characteristic
3.	Taste	Bitter	Bitter	Bitter	Bitter
4.	pH	5.15	5.58	5.92	5.50
5.	Tap density	0.587	0.578	0.583	0.591
6.	Bulk density	0.667	0.644	0.649	0.669
7.	Angle of repose	24.01	23.59	23.62	23.91
8.	Hausner ratio	0.891	0.889	0.898	0.899
9.	Carr's index	11.62	11.46	11.32	11.92

Analysis of heavy metal in three laboratory batches (AC-I, AC-II & AC-III) like Lead (Pb), Cadmium (Cd), Mercury (Hg), Iron (Fe) and Arsenic content were carried out. Results are shown in Table 3. Heavy metals after entering in the human body can't be removed easily. On long usage they accumulated in the body ultimately produce toxicity by hindering the normal physiological activity. Hence WHO and various pharmacopoeias have prescribed the limit for heavy metals. Results showed that concentration of lead and cadmium in AC-I, AC-II & AC-III was found within the limit. The presence of heavy metal in AC-I, AC-II & AC-III was less than the limit prescribed in The Ayurvedic Pharmacopoeia of India.

Table 3: Heavy Metal concentration in Ayurvedic Churna

Formulation Code	Heavy Metals (mg/kg)				
	Lead (Pb)	Cadmium (Cd)	Mercury (Hg)	Iron (Fe)	Arsenic Content (PPM)
AC- I	3.65	0.15	0.03	1.65	2.10
AC-II	3.62	0.15	0.03	1.66	2.15
AC-III	3.68	0.16	0.04	1.68	2.20
MF-AC	3.25	0.14	0.03	1.55	2.05

Microbial analysis of three laboratory batches of Ayurvedic Churna i.e., (AC-I, AC-II & AC-III) included total viable aerobic count, total yeast and mould, *E. coli, S. Typhi* count. The results are given in Table 4. Pathogenic bacteria in vegetative and non-vegetative forms are harmful to human body because they produce diseases. Hence WHO and pharmacopoeia of the advance countries have prescribed the limit for total aerobic viable count, total yeast and mould, *E. coli* and *Salmonella* species. Results indicated that Total aerobic viable count was within the limit and Total yeast and mould, *E. coli and Salmonella* species were absent in *Ayurvedic churna*. This indicated that total aerobic viable count, total yeast and mould, *E. coli* and *Salmonella* Species in *Ayurvedic churna* were lower than the limit prescribed in The Ayurvedic Pharmacopoeia of India.

Table 4: Microbial analysis of Ayurvedic Churna

S/No.	Microbial Analysis	Observations			
		AC-I	AC-II	AC-III	MF-AC
1.	Total aerobic viable count	210 CFU/gm	250 CFU/gm	270 CFU/gm	150 CFU/gm
2.	Total yeast and mould	Absent	Absent	Absent	Absent
3.	*E.coli*	Absent	Absent	Absent	Absent
4.	*Salmonella Sp*	Absent	Absent	Absent	Absent

The physicochemical evaluation of the three laboratory batches of Ayurvedic Churna i.e., (AC-I, AC-II & AC-III) and MF-AC was done and reported in table 5.

Table 5: Physicochemical Evaluation of Ayurvedic Churna

S. No.	Formulation Code	LOD	FOM	TA	AIA	ESEV	WSEV	SI	FI
1.	AC- I	1.15	0.65	8.83	1.18	14.89	20.84	2.22	2.10
2.	AC-II	1.11	0.98	10.42	3.10	13.50	18.10	3.14	4.22
3.	AC-III	1.18	1.02	11.49	2.89	15.10	17.82	2.17	3.56
4.	MF-AC	1.10	0. 05	7.48	1.10	14.11	17.28	2.10	2.15

Note: All reading are Mean, n=3

The polyherbal formulation Ayurvedic Churna i.e., (AC-I, AC-II & AC-III) and MF-AC was tested for presence or absence of phytoconstituents as per method described earlier. Various phytochemical present were revealed and are mentioned in table 6.

Table 6 Phytochemical screening of extract of raw material

Formulation Code	Alkaloids	Carbohydrates	Tannins and phenols	Glycosides	Gums and mucilage's	Fixed oils and fats	Saponins	Protein and free amino acid	Volatile oil
AC- I	+	+	+	-	-	-	+	+	-
AC-II	+	+	+	-	-	-	+	+	-
AC-III	+	+	+	-	-	-	+	+	-
MF-AC	+	+	+	-	-	-	+	+	-

Abbr.: + = Present; - = Absent

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