



Research Article

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Adraka Maricha Khandavaleha: Preparation and Analysis

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ABSTRACT

Preparation and Standardization of Ayurvedic compound is vital to assure therapeutic efficacy and safety. *Adraka Khandavaleha* is a drug of choice in Allergic skin diseases, dermatitis, chronic bronchitis, asthma, emaciation, anorexia, bloating, edema and inflammation. This formulation is having spicy test. The present study was aimed that to Prepare and Standardize the Traditional Polyherbal Avaleha formulation with respect to phytochemical screening, physicochemical analysis and High-performance thin layer chromatography (HPTLC) finger printing and stability studies. It is one of the common dosages from appreciated by all age group for their palatability and easy method of administration and feasibility. The formulation *Adraka Maricha Khandavaleha* was prepared by adopting reference of Bhaishajya Ratnavali. The tests results obtained like HPTLC fingerprint profile would serve as parameters for the identity of this poly herbal formulation. The results obtained may be considered as tools for assistance to the regulatory authorities, scientific organizations and manufacturers for developing standard formulation of great efficacy.

Keywords: *Adraka Maricha Khandavaleha*, Polyherbal ayurvedic formulation, Standardization, Phytochemical, High-performance thin layer chromatography (HPTLC).

INTRODUCTION

Formulations which contain two or more herbal drugs with multiple pharmacological action therapeutic effect are called as poly herbal formulation. Herbal formulations have reached widespread acceptability as therapeutic agents for many diseases and disorders [1]. It is necessary to maintain quality and purity of crude natural drug in commercial market because of their great and reliable potential. Hence there is need for standardization and development of reliable quality protocols of Ayurvedic formulations using modern techniques of analysis.

Ayurveda uses various dosage forms of formulations such as pills, powders, liquids (Ghrithas, kwathas, arishthas etc.) and semisolids like Avaleha. Assimilation of Avalehas starts from the buccal cavity. These can be defined as a food or a part of food that provides medical or health benefit along with the prevention and treatment of diseases. Chyawanaprashaavaleha, Kushmanda Avaleha are some of examples. *Adraka Avaleha* is a herbo-mineral formulation which is explained in Yoga Ratnakar in the context of Kasa Chikitsa Adhyaya [2]. *Adraka* (Ginger) can be compared with the confectionaries, nutraceutical and consumed worldwide as a spice and flavouring agent and is attributed to have many medicinal properties[5]. In traditional Indian medicine, ginger has been used in treating of wide variety of ailments including stomach aches, diarrhoea, nausea, asthma, respiratory disorders etc. Hence the present study was planned for the preparation and analysis of *Adraka Maricha Khandavaleha*.

MATERIALS AND METHODS

Raw Materials, Chemicals and Reagents

Plant Raw materials used for the preparation of *Adraka maricha khandavaleha* were procured from Ayurvedic Proprietary Medicines Shop (Mumbai) with the knowledge of Ayurvedic physician. The materials were dried in an oven preset at 45°C, powdered, sieved through an 85-mesh (BSS) sieve and stored in air tight containers.

Preparation of *Adraka Maricha Khandavaleha*

Raw materials complying the pharmacopoeial quality as per the composition [Table 1] . All the prepared powders Ghana, Pipali, Aruna, Karkatashringi were mixed thoroughly as per the standard protocol and stored in air tight container.

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Table 1: Formulation composition

S. No.	Ayurvedic name	Botanical /English name	Part used	Quantity (gm)
1	Pippali	<i>Piper longum</i>	Fruit	25
2	Pippalimoola	<i>Piper longum</i>	Root	25
3	Maricha	<i>Piper nigrum</i>	Fruit	25
4	Shunti	<i>Zingiber officinale</i>	Rhizome	25
5	Chitraka	<i>Plumbago zeylanica</i>	Root	25
6	Vidanga	<i>Emblica ribes</i>	Flower	25
7	Musta	<i>Cyperus rotundus</i>	Root	25
8	Nagakeshar	<i>Mesua ferrea</i>	Fruit	25
9	Twak	<i>Cinnamomum verum</i>	Leaf	25
10	Ela	<i>Elettaria cardamomum</i>	Fruit	25
11	Tamalapatra	<i>Cinnamomum tamala</i>	Bark	25
12	Kachura	<i>Curcuma zedoaria</i>	Flower	25
13	Adraka	Ginger rhizome paste	Fruit	384
14	Goghrita	Cow ghee	Root	192
15	Goksheera	Cow milk	Fruit	750
16	Sharkara	Sugar	Rhizome	384

Quality Evaluation

Organoleptic evaluation

The formulation was studied for its preliminary characters like colour, texture, odour and taste.

Microscopic evaluation

The formulation was evaluated microscopically to check the presence of different anatomical markers.

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. These extracts were used for all the tests.

Preliminary Phytochemical and Biochemical Evaluation

Phytochemical screening of some major secondary metabolites (Flavonoids, Tannins, Alkaloids, Glycosides, Terpenoids, Steroids, starch, Phenolic Compounds and Saponins) by performing preliminary colour based tests.

Physicochemical Evaluation

The prepared formulation was subjected for physicochemical studies like pH, Loss On Drying, Alcohol soluble extractive, Water soluble extractive and Ash value. The procedure for all these tests was followed as per ayurvedic pharmacopoeia.

Heavy Metal Determination

It is necessary to check the presence of heavy metals in ayurvedic preparations as heavy metals naturally come with the raw materials used to make the formulations[4]. Heavy metals may be used in some quantity because of their reputed therapeutic properties but at the same time they can pose a particular health risk when they accumulate in vital organs[3]. However, improper manufacturing processes may

result in dangerously high levels of heavy metals remaining in the final product. Heavy metals were assessed qualitatively using chemical tests.

Microbiological Assay

Total viable count was most widely accepted technique recommended by WHO for total count of microorganisms in plant materials and herbal formulations[6]. Total aerobic and anaerobic bacteria count is done by spread plate technique and then incubate at 30-35°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 °C for 24 hours. High count of fungi are risk because of the possibility to produce mycotoxin such as aflatoxin which are carcinogenic.

High Performance Thin Layer Chromatography (HPTLC)

10 µl of the filtered solution of raw materials, formulation extract and standard was applied on the HPTLC plate as per conditions mentioned in table 1a. for authentication of presence for raw material in formulation without any presence of adulterations. The same technique is also used for stability test to find out the evidence on how the quality of a drug substance or drug product varies with time under the influence of a variety of environmental factors such as temperature, humidity and light. For assessing stability, 10 µl of filtered solution of formulation extracts taken at 0 day, 15 days and 30 days after the day of preparation and marker compound piperine was applied on plate.

Table 1a: Chromatographic Conditions for HPTLC

Stationary Phase	HPTLC plates silica gel 60 F 254
Plate size	10.0x10.0 cm
Mobile Phase	Hexane : Ethyl Acetate : Glacial Acetic Acid (3:1:0.1)
Saturation Time	20 min.
Spot Volume	10 µl
Band Length	8.0mm
Solvent Front	80mm
Wavelength and Lamp	366nm & Mercury lamp
Sample Applicator	CAMAG Linomat 5
Sample Detection	CAMAG Visualizer : 200480
Number of Tracks	14 for fingerprinting and 4 for stability testing

RESULTS AND DISCUSSION

Adraka Maricha Khandavaleha (AMK) is an Ayurvedic formulations which involves complex mechanism, where triacylglycerols from ghrita interacts with other phytoconstituents of drug paste and undergoes hydrolysis resulting in the formation of fatty acid and glycerol. This was prepared at a laboratory scale as per standard literature under the guidance of ayurvedic practitioner. The observed results for various analytical parameters clearly indicates good quality of product. The organoleptic characters (table 2) indicates the perfect appearance, texture and consistency. Microscopic analysis under 45X showed various key anatomical markers that are Lignified fibres; xylem vessels; Brownish colouring matter; Acicular as well as Prismatic crystals; Sclerenchyma along with Epidermis were observed (table 3).

Physicochemical constants (table 4) helps to understand the quality of the formulation according to chemical nature. Loss on drying at 105°C

indicates the presence of moisture content. If moisture content is more than the permissible limit then the formulation is more likely to get infected by fungal growth. Less LOD value indicates the good preparation of khanda and longer stability of the formulation. Ash values indicates the unwanted inorganic matter in final product which was also found to be as per the standard acceptable limit. The extractive values, namely water soluble and alcohol soluble, indicate the amount of active constituent in a given amount of plant material when extracted with respective solvents, All the values were found to be as per permissible limit standards set by WHO.

Qualitative determination of heavy metals was done to assess the toxicity of the formulation and absence of heavy metals was observed (table 5).

Phytochemical and biochemical evaluation is very important as it gives complete profiling of various therapeutically active constituents present in final product AMK and its preliminary quality [7]. It was found that alkaloids, steroides and starch were present (table 6). Microbiological analysis also showed the presence of micro organisms and fungal organisms in the acceptable limits (table 7).

HPTLC studies gave the authenticated data about presence of raw materials in final product and also helps to obtain fingerprint of the formulation with presence of marker compound (fig 1).

Stability studies was done till 30 days and it as found that fingerprint of the formulation was quite stable indicating the stable quality content of the formulaion (fig 2). This can be continued for carrying out shelf life studies.

Table 2: Organoleptic Characters

S. No.	Characters	Ardrakamarichakhandavaleha
1.	Colour	Dark Brown
2.	Taste	Sweet & mild pungent
3.	Texture	Rough
4.	Odour	Sweat
5.	Consistency	Semi solid

Table 3: Microscopical evaluation

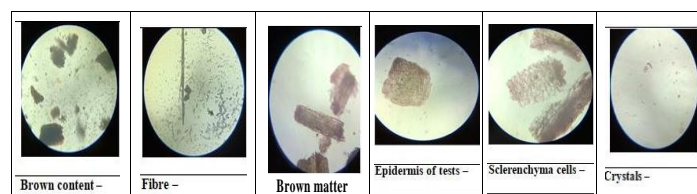


Table 4: Physicochemical evaluation

S. No.	Parameters	Ardraka Maricha Khandavaleha
1.	pH	6
2.	LOD	5.91%
3.	Alcohol soluble extractive	19.17%
4.	Water soluble extractive	18.60%
5.	Total Ash value	6.31 %
6.	Acid insoluble ash	3.35%

Table 5: Heavy Metal Determination

S. No.	Heavy Metals	Result
1	Lead	-
2	Chromium	-
3	Copper	-
4	Cadmium	-
5	Nickel	-
6	Zinc	-
7	Cobalt	-
8	Bismuth	-

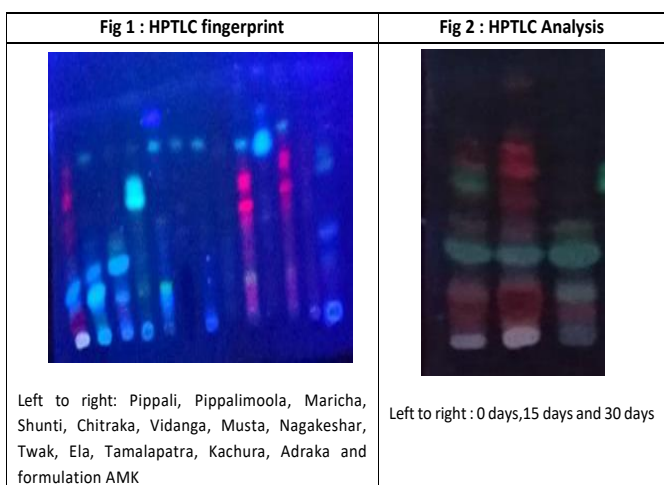
Table 6: Phytochemical Evaluation

S No.	Tests	Observation	Results
1.	Tannin: 1ml Aq. Extract + 0.1% FeCl ₃ dropwise	Brownish green or Blue black colour	-
2.	Alkaloids: 1ml Alc. Extract + 1ml conc. HCl + Hager's Reagent	Yellow ppt	+
3.	Glycosides: 1ml extract + 0.5ml Glacial Acetic acid + few drops of Dil. FeCl ₃ till colourless + 1ml Dil. H ₂ SO ₄	Brown Ring	-
4.	Flavonoids: 1ml extract+ 1ml Dil. ammonia solution + Conc. H ₂ SO ₄	Yellow colour disappear	-
5.	Steroids: 1ml extract + 1ml chloroform + Conc H ₂ SO ₄	Red colour after stand	+
6.	Phenolic Compounds: 1ml extract + dropwise FeCl ₃	Violet colourppt	-
7.	Saponin: 1ml extract + Few drops of olive oil+ Shake vigorously	Froth	-
8.	Terpenoids: 1ml extract +0.5ml CHCl ₃ + 1ml Conc. H ₂ SO ₄	Yellow colour	-
9.	Carbohydrate: 1ml extract + 1ml Fehling A + 1ml Fehling B	Blue Colour	-
10.	Proteins: 1ml extract + 1ml 4% NaOH + few drops 1% CuSO ₄	Violet or pink colour	-
11.	Starch: 1ml extract + iodine	Blue colour	+

Table 7: Microbiological Assay (Total Viable)

Media	Organism	Dilution	CFU/0.1ml	CFU/1ml	Average
Nutrient Agar	Aerobic	10-3	12 x 10 ⁻³	12 x 10 ⁻⁴	1.22 x 10 ⁶
		10-4	11 x 10 ⁻⁴	11 x 10 ⁻⁵	
		10-5	8 x 10 ⁻⁵	8 x 10 ⁻⁶	
Nutrient Agar	Anaerobic	10-3	15 x 10 ⁻³	15 x 10 ⁻⁴	4.50 x 10 ⁶
		10-4	9 x 10 ⁻⁴	9 x 10 ⁻⁵	
		10-5	7 x 10 ⁻⁵	7 x 10 ⁻⁶	

Sabourauds Agar	Fungi	10-3	54×10^{-3}	54×10^{-4}	1.45×10^6
		10-4	7×10^{-4}	7×10^{-5}	
		10-5	3×10^{-5}	3×10^{-6}	



CONCLUSION

The present analytical parameters can be used for routine quality control of the formulations. Ayurvedic medicine Adraka Maricha Khandavaleha AMK has been prepared by traditional method and standardized with the help of scientific quality control measures using modern analytical tools. Preliminary microscopical, phytochemical and physicochemical studies, qualitative heavy metal analysis, microbiological analysis and HPTLC studies gave complete quality control profile of the formulation. The analytical data generated here is initial step for setting standard limits for quality control parameters and study can be elaborated for getting stability studies data.

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