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# PREPARATION AND QUALITY EVALUATION OF A POLYHERBAL FORMULATION: *KARKATI BEEJ CHURNA*

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**ABSTRACT**

Herbal medicines, made up of different plants materials are used worldwide to treat various disorders and are widely accepted by large number of population. But due to climatic changes and lack of knowledge *w.r.t.* identification and preparation of the drugs, it is essential that quality evaluation of herbal medicines must be carried out to manifest the drug as potent and safe. *Karkati beej churna* is an ayurvedic herbal formulation which is used in the treatment of urinary disorders. The present study focusses on the quality evaluation of *Karkati beej churna w.r.t.* parameters like organoleptic chracteristics, physicochemical parameters, microscopic evaluation, phytochemical screening and chromatographic profiling. A Thin layer chromatographic profile was obtained for saponins as saponins are considered as one of the plant diuretics. The results thus obtained for all the above mentioned parameters can be used for future references.

**Keywords:** *Karkatibeejchurna*, Chromatography, Quality control, Pharmacopoeia, HPTLC, Polyherbal formulation.

## 1. INTRODUCTION

Ayurveda is the oldest system of medicine used in India and a large number of population is dependent on this traditional system of medicine [1]. Due to the effectiveness of the ayurvedic medicines and establishment of guidelines for quality control, population worldwide is now interested in traditional systems of medicines [2]. Yet, due to higher demand for natural products, supply of good quality herbal plant materials is a challenge due to adulteration and misinterpretation of the herbal plant material [3]. Also, parameters like environmental conditions, harvesting time, geographical variations, storage conditions, predators and genetic factors play an important role that affects the quality and quantity of phytochemicals [1, 2, 4]. Hence standardization and quality evaluation of herbal drugs (single drugs and formulations) is an essential criterion to render the drug as safe and effective [1]. It includes authentication of the herbal plant material, quality control parameters like proximate analysis, microscopic and macroscopic characteristics, chromatographic profiling, shelf life studies etc.; for which the guidelines are prescribed by WHO [1,2,5].

There are different ancient books which comprise of verses i.e. Sanskrit shloka that gives information about the preparation and dosage form of ayurvedic drugs [4, 6]. The monographs prescribed by Indian Council of Medical Research (ICMR) quality standards and Ayurvedic Pharmacopoeia of India (API) contains information on different ayurvedic drugs and also contains standardized protocols which aid in standardization of herbs and formulations [1, 7]. Hence for the acceptability of the herbal drugs, to render them safe and to have consistency in their production, developing and standardizing methods for quality assessment of herbal drugs is of utmost importance [1,8].

## 2. MATERIAL AND METHODS

### 2.1. Collection of raw materials

Raw materials like *Emblica officinalis* (Amla fruit), *Terminalia chebula* (Haritaki fruit), *Terminalia Bellirica* (Bibhitaki fruit), *Cucumis sativus* seeds (Cucumber seeds)

and crystals of rock salt were procured as a whole (dry) from local market. They were crushed in a mixer grinder and sieved individually using sieve no. 85 and stored in an air tight bottle [9]. All the raw materials used were authenticated from Agharkar Research Institute, Pune and authentication certificates were obtained for the same.

### 2.2. Reagents, chemicals and instruments

All the reagents and chemicals like Chloroform, Glacial acetic acid, methanol, ethanol, hydrochloric acid and anisaldehyde used in this study were of analytical grade.

### 2.3. Preparation of the formulation

The preparation of the formulation of *Karkati beejchurna* was done as per the reference mentioned in the ancient text "*Brihad Nighantu Ratnakara*; part-5. As per the shloka, cucumber seeds powder, *triphala* (mixture of fruits of amla, *haritaki* and *bibhitaki* fruit powders) and *saindhavnamak* (pink rock salt) was mixed in equal proportions [6]. The prepared churna was stored in an air tight container for further analysis [6].

### 2.4. Physicochemical Analysis

#### *2.4.1. Determination of Organoleptic parameters*

*Karkati beejchurna* was evaluated on the basis of colour, texture, odour and taste [7, 8].

#### *2.4.2. Determination of moisture content*

Two gms of the *churna* sample was taken in a tarred petri plate (pre-conditioned), and dried at 10°C for 5 hours. The final weight was noted [7, 8].

#### *2.4.3. Determination of ash content*

Two gms of the *churna* sample was taken in a tarred crucible (pre-conditioned) and incinerated at a temperature not more than 450°C until the ash was carbonless. After cooling, the final weight was noted [7,8].

#### *2.4.4. Determination of acid insoluble ash*

The ash obtained above (in 2.4.3) was boiled with 25 ml hydrochloric acid for 5 minutes, filtered using whatmann 41 (ashless) and was washed with water; further igniting to constant weight. Crucible was cooled and final weight was taken [7, 8].

#### *2.4.5. Determination of water soluble ash*

The ash obtained above (in 2.4.3) was boiled with 25 ml water for 5 minutes, filtered using whatmann 41(ashless),washed with water; further igniting to a temperature not more than 450°C. Crucible was cooled and final weight was taken [7, 8].

#### *2.4.6. Determination of pH*

Five gms of sample was weighed, added to a beaker containing 100 ml water and kept on standby for 24 hours. It was filtered the next day and pH of the filtrate was recorded on a calibrated pH meter [7, 8].

#### *2.4.7. Determination of extractive values*

*2.4.7.1. Alcohol soluble extractive*

Five gms of the sample was weighed (W1) and macerated in 100 ml alcohol, shaken frequently for first 6 hours and later kept on standby for 24 hours. It was filtered the next day and 25ml of the filtrate was evaporated in a tarred evaporating dish to dryness. It was further dried at 105°C and weighed (W2). Alcohol soluble extractive value was calculated with reference to the original weight of the sample [7, 8].

*2.4.7.2. Water soluble extractive*

Five gms of the sample was weighed (W1) and macerated in 100 ml water, shaken frequently for first 6 hours and later kept on standby for 24 hours. It was filtered the next day and 25ml of the filtrate was evaporated in a tarred evaporating dish to dryness. It was further dried at 105°C and weighed (W2). Water soluble extractive value was calculated with reference to the original weight of the sample [7, 8].

### 2.5. Determination of powder flow property

Physical parameters like Bulk and tap density Carr's index (Compressibility index), Housner's ratio and Angle of repose were calculated for the *churna* [8, 10, 11].

### 2.6. Microscopic evaluation

Microscopy is one of the best aid to identify the drug and check for the adulterations which lead to less effective and less potency of the drug [5, 7]. Hence *Karkati beej churna* was subjected to microscopic evaluation and the powder characteristics are recorded for the same [12, 13].

### 2.7. Phytochemical testing

Phytochemical testing was performed on three different extracts to determine the presence of secondary metabolites like tannins, phenols, alkaloids etc. [10].

### 2.8. High Performance Thin Layer Chromatography

High Performance Thin Layer Chromatography (HPTLC) technique was used to generate a chromatographic profile of saponins present in the formulation as saponins are considered to be one of the diuretics that aids in regulation of urinary disorders [14]. Different extraction techniques like sonication, soxhlet and rotary extractions were used using solvents like water and methanol [7]. 2gm of sample was extracted using water and methanol in rotary (18 hours), sonication (30 minutes) and soxhlet (6 hours), filtered and used for HPTLC analysis. 20cmx20 cm plate was used to apply water and methanol extracts along with saponins standard using CAMAG Linomat V sample applicator, and the plate was developed in the mobile phase with slight modification *i.e.* Chloroform: acetic acid: methanol (6.4:1.6:1.2) V/V/V with a saturation time of 20 minutes[15]. Densitometric scanning was performed using CAMAG scanner III using the software vision CATS by CAMAG. Derivatization was done using Anisaldehyde sulphuric acid reagent; heated at 105°C and the plate was rescanned at 540nm and 366nm. Saponin standard was used to compare the bands obtained in water and methanol extracts.

## 3. RESULTS AND DISCUSSION

### 3.1. Organoleptic characteristics

The organoleptic characteristics of *Karkati beej churna* are represented in table 1.

**Table 1: Results for organoleptic evaluation**

| Parameters | Results |
|---|---|
| Appearance | Powder |
| Colour | Yellowish brown |
| Odour | Characteristic |
| Taste | Sour and salty |

### 3.2. Physicochemical parameters

The Physicochemical parameters of *Karkatibeej churna* are listed in the table 2.

**Table 2: Results for physicochemical parameters**

| PARAMETERS | RESULTS |
|---|---|
| Moisture content | 3.78% ±0.001054 % |
| Total ash | 36.42% ±0.000265% |
| Acid insoluble ash | 0.585% |
| Water soluble ash | 26.62% |
| pH(5%aqueous) | 4.033333 ±0.057735 |
| Alcohol soluble Extractive | 24.32% |
| Water soluble Extractive | 74.48% |

### 3.3. Powder flow property

The powder flow property of *Karkati beej churna* are as indicated in table 3. The flow property indicates the quality of the powder. As per the results obtained for Carr's index and Housner's ratio, the flow property for *churna* is fair while that the results obtained for angle of repose, the flow property for *churna* is poor [10].

**Table 3: Results for powder flow property**

| Parameters | Results |
|---|---|
| Bulk density | 0.45 g/ml |
| Tap density | 0.56 g/ml |
| Carr's index (Compressibility index) | 19.64286 |
| Housner's ratio | 1.244444 |
| Angle of repose | 48.41° |

### 3.4. Microscopic evaluation

The powder shows presence of group of xylem vessels with reticulate pitting 1(a), trichome 1(b), starch grains 1(c & d), macro-sclereid 1(e & f) and osteo-sclereid 1(g) under 45X.

### 3.5. Phytochemical testing

The *churna* formulation showed the presence of many phytochemicals and the data is represented in table 4. The formulation *Karkati beej churna* contains many different classes of phytochemicals like tannins, phenols, carbohydrates, saponins, flavonoids etc. Mostly, the presence of these phytochemicals were detected in water extracts as compared to methanol and n-hexane extracts. This phytochemicals act synergistically making the drug more effective and potent against the diseases.

**Table 4: Results for phytochemical testing**

| TESTS | RESULTS | | |
|---|---|---|---|
| | Water extract | Methanol Extract | n-hexane extract |
| Alkaloids | - | - | - |
| Carbohydrates | + | + | - |
| Reducing sugars | + | + | + |
| Flavonoids | + | + | - |
| Saponins | + | - | - |
| Tannins | + | + | - |
| Steroids | - | + | - |
| Proteins | - | - | - |
| Phenols | + | + | - |
| Amino acids | - | - | - |
| Terpenoids | - | - | - |
| Phytosterols | - | + | + |
| Anthocyanin | - | - | - |
| Volatile oils | - | - | + |
| Glycosides | - | - | - |

*Present (+)* *Absent (-)*

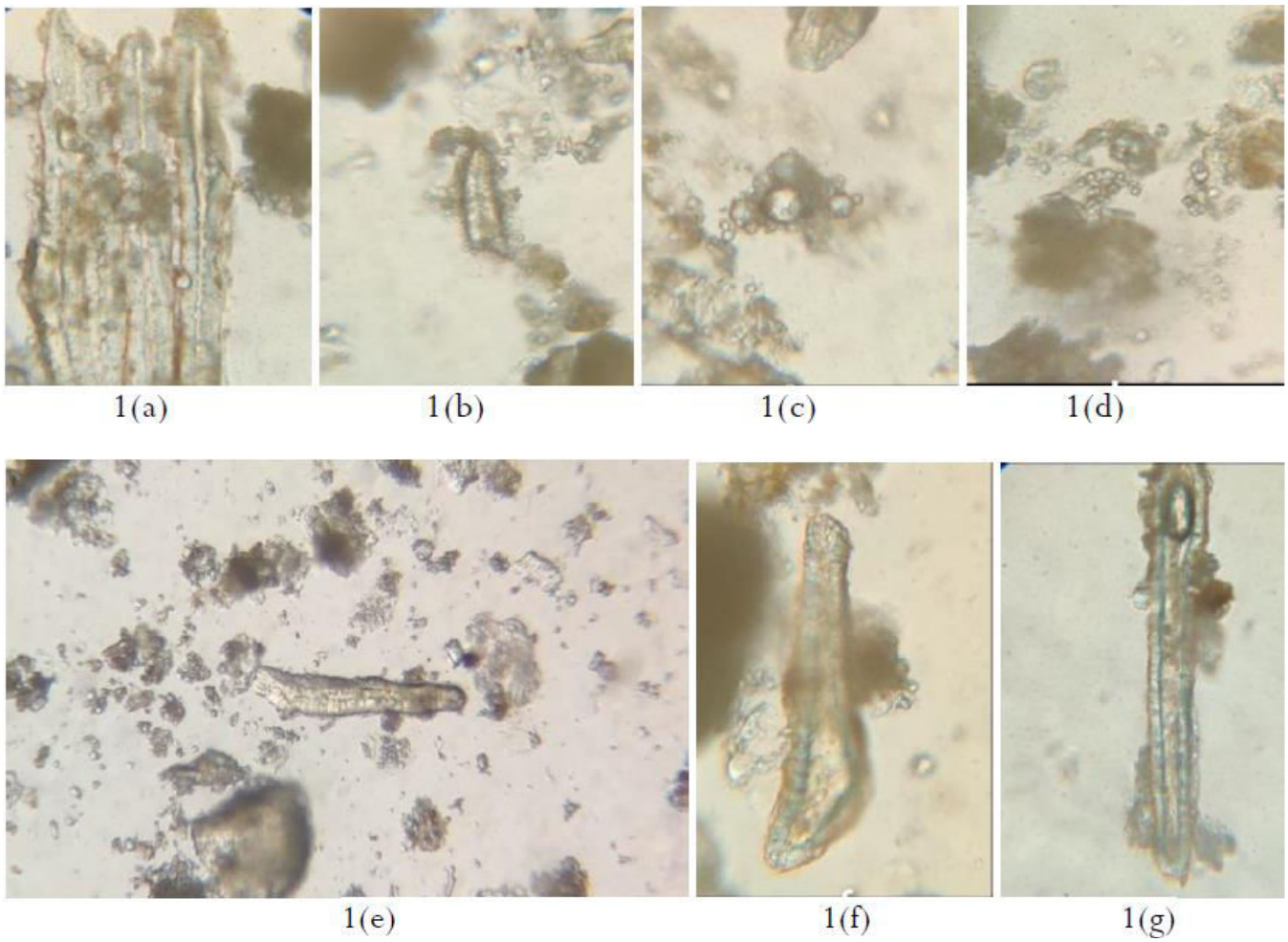


**Fig. 1: Results for microscopic evaluation**

### 3.6. Chromatographic profiling

The chromatogram obtained showed the presence of blue, blue-violet and yellow-brown coloured bands after derivatization in white light confirming the presence of saponins; while the bands showed better resolution under 366nm after derivatization as it showed blue, blue-violet and green fluorescent bands[15].

Track details for the same are as follows:
Track 1: Water rotary extract 2µl,
Track 2: Water rotary extract 5µl
Track 3: Saponin standard in water 30 µl
Track 4: Saponin standard in water 15 µl
Track 5: Water sonicator extract 2 µl
Track 6: Water sonicator extract 5µl
Track 7: Methanol rotary extract 2 µl
Track 8: Methanol rotary extract 5 µl
Track 9: Saponin standard in methanol 30 µl
Track 10: Methanol sonicator extract 30 µl
Track 11: Methanol sonicator extract 30 µl
Track 12: Saponin standard in methanol 15µl
Track 13: Methanol soxhlet extract 30 µl
Track 14: Methanol soxhlet extract 30 µl

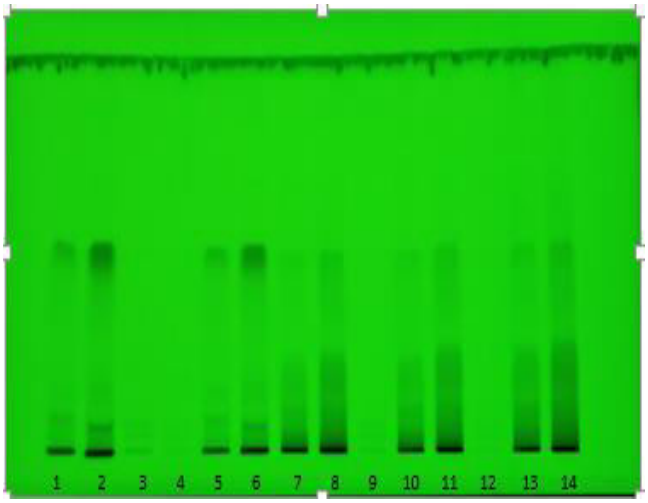


**Fig. 2: Bands at 254nm**

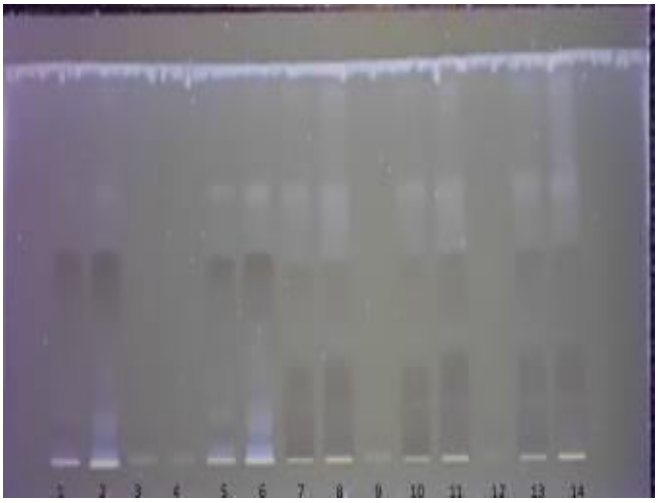

**Fig. 3: Bands at 366nm**

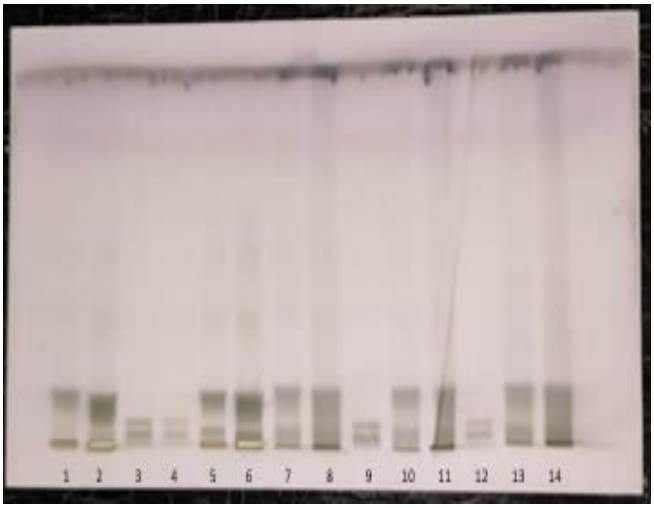


**Fig. 4: Bands in white light (After derivatization)**

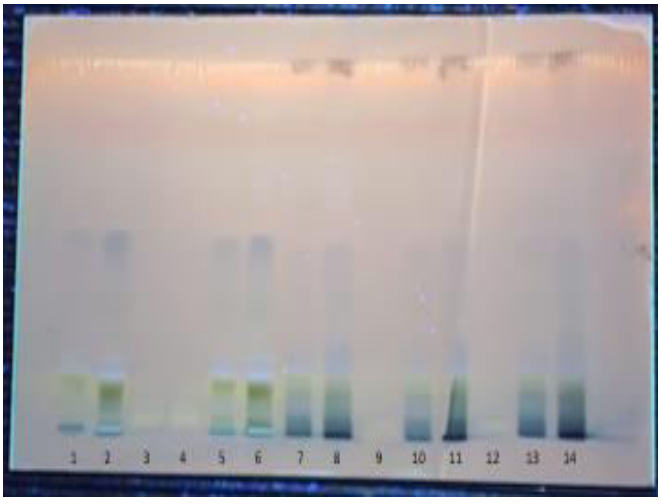


**Fig. 5: Bands at 366nm (After derivatization)**

## 4. CONCLUSION

As per the pharmacopoeial standards, various parameters like physicochemical, phytochemical, powder flow property, microscopical and chromatographic profiling were employed for the quality evaluation of *Karkati beej churna*. The preparation of *Karkati beej churna* was done according to the ancient text, *Brihad Nighantu Ratnakar*a under the guidance of an ayurvedic practitioner. Quality evaluation and standardizing the methods for herbal formulations aids in setting up limits which would help in batch to batch consistency for producing an effective, potent and safe quality of herbal medicines.

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***Conflict of interest***

None declared

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None declared

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