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Research Article

Quality Evaluation and Standardization of An Ayurvedic Formulation *Bilvadileha*: A Bioanalytical Approach

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ABSTRACT

Bilvadileha; an Ayurvedic semi-solid formulation, is prescribed for the treatment of digestive impairment, tastelessness, emesis and excessive salivation. Though the formula composition and therapeutic claims of *Bilvadileha* are part of the Ayurvedic Formulary of India, the scientific methods for its quality and safety evaluation are yet to be documented. Hence, in the current work an attempt has been made to evaluate the quality parameters to be used for its preparation and processing. Standard operating procedure for the preparation of *Bilvadileha* was developed by the amalgamation of traditional methods and scientific tools. Preliminary phytochemicals in *Bilvadileha* were qualitatively estimated along with determination of crude fibre and reducing sugar content. Chromatographic standardization of *Bilvadileha* was carried out using HPTLC which included the determination of piperine content in *Bilvadileha* along with the evaluation of its stability samples stored for different storage periods. Safety of the formulation was affirmed in mice by carrying out acute toxicity study. The quality control parameters resulted after scientific evaluation of *Bilvadileha* can be used as reference standards by quality control/assurance unit of a pharmaceutical firm in order to have a proper quality check over its preparation and processing.

Keywords: Ayurvedic formulation, *Bilvadileha*, HPTLC, piperine, safety, standardization

1. INTRODUCTION

Bilvadileha; an Ayurvedic polyherbal semi-solid formulation, is used traditionally as a remedy for *Agnimandya* (digestive impairment), *Aruci* (tastelessness), *Chardi* (emesis) and *Praseka* (excessive salivation). The formula composition and therapeutic claims of *Bilvadileha* are documented in the Ayurvedic Formulary of India [1]. Due to the lack of standard operating procedure for formulating this medicine, its preparation by various manufacturers with the raw materials from different sources, poses a serious challenge to its consistency, quality and efficacy.

Quality evaluation and standardization of various Ayurvedic preparations of different matrices such as *Churna* [2], *Taila* [3], *Vati* [4], *Avaleha* [5] etc has been recently reported. Standardization of a Unani semisolid formulation, *Jawarish-e-Bisbasa* has also been documented [6]. But, quality evaluation of *Bilvadileha* remains an unexplored issue irrespective of increase in its popularity.

In the present work, raw materials of pharmacopoeial quality were used to prepare *Bilvadileha*. The standard operating procedure (SOP) was developed and *Bilvadileha* was prepared in-house by incorporating scientific tools in traditional methods. *Bilvadileha* was subjected to preliminary phytochemical evaluation and physicochemical evaluation which included the determination of ash content, loss on drying, reducing sugar and crude fibre content. Chromatographic characterization of *Bilvadileha* was done on the basis of piperine (Fig. 1) using HPTLC method. Effect of different storage periods on the formulation was studied using HPTLC. Safety of the formulation was evaluated in mice by conducting acute toxicity study.

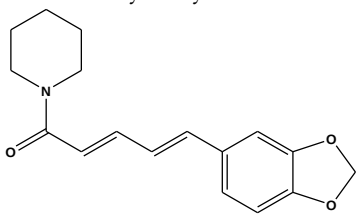

Figure 1: Structure of piperine

2. MATERIAL AND METHODS

2.1. Plant materials

Raw materials used in the current work were procured from local market, authenticated by Department of Botany, Ramnarain Ruia College, Mumbai and representative voucher specimens were deposited for future reference. Materials were dried in an incubator at 45°C, powdered, sieved through an 85-mesh (BSS) and stored in air tight containers.

2.2. Standard and reagents

The organic solvents and chemicals of analytical grade were procured from Merck Specialities Pvt. Ltd. (Mumbai). Piperine (≥ 99 % purity) was purchased from Sigma Aldrich (Germany).

2.3. Quality evaluation of raw materials

To assess the quality of plant raw materials used in the preparation of *Bilvadileha*, various parameters like ash values (total ash, acid insoluble ash and water soluble ash), loss on drying and foreign matter were determined using pharmacopoeial methods [7] and compared with the limits mentioned in the documented reports [8-11]. Quality of *Guda* was evaluated as per the published reports [12].

2.4. Preparation of *Bilvadileha*

Raw materials complying the pharmacopoeial quality and quantity were subjected to the preparation of *Bilvadileha* as per the composition (Table 1) mentioned in the classical reference [1]. The SOP for the preparation of the formulation involved following four steps:

2.4.1. Preparation of Bilva Kasaya

To the coarsely powdered *Bilva* root, water was added (1:8, w/v). The mixture was heated indirectly on a mild flame with continuous stirring till the water reduced to 1/4th of its original volume. The mixture was filtered through muslin cloth and the filtrate obtained was considered as *Bilva Kasaya*.

2.4.2. Removal of impurities in Guda

Guda was dissolved in sufficient amount of water and filtered through a muslin cloth to remove all the foreign matter and particulate impurities present in it.

2.4.3. Preparation of Paka

Bilva Kasaya and purified *Guda* solution were mixed and heated together in a vessel on a mild flame with continuous stirring till the desired thickness and consistency of one tar (thread) was attained.

2.4.4. Preparation of Bilvadileha

Finally, the mixture of plant powders was added to the *Paka*, and mixed thoroughly to obtain a semisolid medicated formulation - *Bilvadileha*.

Table 1: Ingredients for the preparation of Bilvadileha

Ingredients		Quantity
Sanskrit name	**Description**	
Bilva Kasaya	Decoction from root of *Aegle marmelos* L.	256.0 mL
Ghana	Rhizome of *Cyperus rotundus* L.	1.0 g
Dhanyaka	Fruit of *Coriandrum sativum* L.	1.0 g
Jiraka	Fruit of *Cuminum cyminum* L.	1.0 g
Truti	Seed of *Elettaria cardamomum* Maton.	1.0 g
Nagakesara	Stamen of *Mesua ferra* L.	1.0 g
Sunthi	Rhizome of *Zingiber officinale* Rosc.	1.0 g
Maricha	Fruit of *Piper nigrum* L.	1.0 g
Pippali	Fruit of *Piper longum* L.	1.0 g
Tvak	Stem bark of *Cinnamomum Zeylanicum* J.S. Presl	1.0 g
Guda	Jaggery	64.0 g

2.5. Chemical characterization of *Bilvadileha*

Phytochemical constituents in *Bilvadileha* were evaluated using preliminary phytochemical tests [13]. It was subjected to physicochemical evaluation in order to determine the crude fibre [14] and reducing sugar [15] content. The content of ash (total, water soluble and acid insoluble) and loss on drying for *Bilvadileha* was also determined.

2.6. Optimized extraction conditions for *Bilvadileha*

Extraction of phytochemical constituents from the complex matrix of *Bilvadileha* was achieved in methanol (1: 10, w/v). The mixture was vortexed for 1 min, kept standing overnight. It was filtered using Whatman filter paper (no. 1) and the filtrate was used for HPTLC analysis.

2.7. Chromatographic analysis

Chromatographic separation was achieved on TLC plates precoated with silica gel 60 F_{254} (E. Merck) of 0.2 mm thickness with aluminum sheet support. Samples were spotted using CAMAG Linomat IV (Switzerland) equipped with Hamilton syringe (100.0 µL). Plates were developed in a glass twin trough chamber (CAMAG) pre-saturated with mobile phase. Scanning device used was CAMAG TLC Scanner II equipped with winCATS software. The experimental condition was maintained at 20 ± 2°C. Detection of piperine in samples was carried out after derivatizing the plate with anisaldehyde sulphuric acid reagent. Photo-documentation was done with

CAMAG Reprostar 3 at 550 nm. Toluene: ethyl acetate: glacial acetic acid (8: 2: 0.1, v/v/v) was used as a solvent system.

2.8. Stability study

In order to study the impact of storage on the content of piperine, *Bilvadileha* was prepared on different days and stored. The content of piperine in *Bilvadileha* samples stored for 90, 75, 60, 45 and 30 days was determined using HPTLC and compared with the content of piperine in freshly prepared sample (stored for 0 day).

2.9. Safety evaluation

2.9.1. Animals

Albino Swiss mice of either sex weighing 18-22 g were procured from Haffkine Institute, Mumbai. All animals were housed in polypropylene cages under standard experimental conditions with 26 ± 2°C temperature and 12 h light-dark cycle. The animals were fed standard pellet diet (Amrut laboratory animal feed, India) and were provided water *ad libitum*.

2.9.2. Acute toxicity study

In order to evaluate safety of *Bilvadileha*, acute toxicity study (fixed dose procedure, OECD guide lines No. 420) [16] was conducted in healthy mice. Mice of either sex (three females and three males) received aqueous slurry of *Bilvadileha* (2.0 g/kg body weight) orally by gavage. A separate group (control) of six mice (three male and three female) received water. The animals were observed for toxic symptoms continuously for the first 4 h after dosing. Finally, the number of survivors was noted after 24 h. These animals were then maintained for further 13 days (total 14 days) with observations made daily for change in body weight, food and water intake. This study was approved by the Institutional Animal Ethics committee (CPSEA/315).

2.10. Statistical evaluation

Microsoft Excel - 2007 was used to determine mean and standard deviation during the analysis.

3. RESULTS AND DISCUSSION

3.1. Standardized operating procedure for the preparation of *Bilvadileha*

Herbal raw materials and *Guda* used for the preparation of *Bilvadileha* were of pharmacopoeial quality. A thorough check over the quantity of raw materials taken and the time required for processing them into finished product resulted in the development of standard operating procedure for the preparation of *Bilvadileha*. The average time required for preparation of *Bilva Kasaya* and *Paka* was 3 hours and 2 hours respectively. The organoleptic characters of *Bilvadileha* were as follows:

Colour: brown, **Odour:** characteristic, **Taste:** pungent and **Consistency:** semisolid.

3.2. Physicochemical standards for *Bilvadileha*

In the complex polyherbal matrix of *Bilvadileha*; flavonoids, glycosides, alkaloids, tannins and resins were found to be present as per their chemical tests. Ash content (total ash, acid insoluble and water soluble ash), loss on drying, crude fibre and reducing sugar content of *Bilvadileha* are summarized in Table 2.

Table 2: Physicochemical standards for Bilvadileha

Parameters	Results (Mean ± SD, n=3)
Total Ash (%)	2.13 ± 0.02
Acid insoluble ash (%)	0.21 ± 0.01
Water soluble ash (%)	0.86 ± 0.02
Loss on drying (%)	18.03 ± 0.08
Crude fibre (%)	1.018 ± 0.01
Reducing sugar (%)	0.12 ± 0.001

Figure 2: Piperine in methanolic extract of Bilvadileha at 550 nm using HPTLC. Track details 1: Bilvadileha, 2: Piperine

3.3. HPTLC analysis of *Bilvadileha*

Methanolic extract of *Bilvadileha* showed only piperine as a phytochemical constituent at $R_f = 0.43$ (Fig. 2, 3). The identity of piperine resolved from the matrix of *Bilvadileha* on TLC plate was confirmed by comparing its colour and R_f value with

that of the standard piperine. The content of piperine in freshly prepared *Bilvadileha* sample was 0.72 ± 0.004 mg/g. Till 3 months of storage, no remarkable variation in the content of piperine in *Bilvadileha* was observed (Table 3). Thus, the results of stability studies in the current work are strongly supported by the references in classical Ayurvedic text regarding the use of *Avaleha* [17].

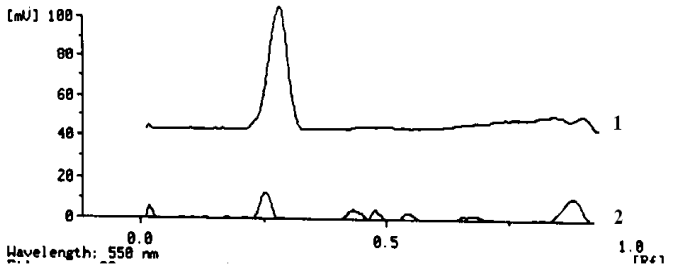

Figure 3: HPTLC densitometric profile as an overlay of Bilvadileha (methanolic extract) with piperine. 1) Piperine 2) Bilvadileha

Table 3: Stability study of Bilvadileha in terms of piperine content using HPTLC

Storage period of *Bilvadileha* (day)	Amount of piperine in mg/g (Mean ± SD, n=3)
0	0.72 ± 0.004
30	0.71 ± 0.003
45	0.72 ± 0.001
60	0.72 ± 0.002
75	0.72 ± 0.001
90	0.72 ± 0.002

Table 4: Cage side observations for animals during acute toxicity study

Parameters	Observation (for animals of each group including control)
Subcutaneous slug and abdominal distension	Nil
Dullness, opacity and ptosis of eyes	Nil
Discharge from the eyes	Nil
Breathing abnormalities	Nil
Condition of the fur and skin	Normal
Pupil diameter	Normal
Colour and consistency of faeces	Normal
Condition of teeth	Normal
Gait	Normal

3.4. Acute oral toxicity *Bilvadileha*

In acute toxicity studies, no significant change in body weight, food intake and water intake of the animals was observed compared to the animals of control group and also no mortality was recorded after the oral administration of *Bilvadileha* (Table 4). Safety of the formulation was established in terms of acute toxicity study which revealed that the formulation in the form of aqueous slurry can be considered with a wide margin of safety for oral use. Such reproducible modern techniques can increase the rate of acceptance of traditional Ayurvedic medicines in the local and global market.

4. CONCLUSION

Findings of the present study can be used to characterize the *Bilvadileha* sample in industry in terms of its consistency and uniformity. The standard operating procedure for the preparation of *Bilvadileha* can be followed by the manufactures to reduce the variation and discrepancies in the formulation. Since the current work focuses only on standardization of *Bilvadileha*, it is required to validate the therapeutic potential of the formulation through preclinical and clinical studies.

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