Current Trends in Biotechnology and Pharmacy
Vol. 19(4) 2676-2683, October 2025, ISSN 0973-8916 (Print), 2230-7303 (Online)
DOI: 10.5530/ctbp.2025.4.46



# Development and Standardization of a New Ayurvedic Formulation from *Barringtonia acutangula* leaf

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## Abstract

*Barringtonia acutangula* (L) Gaertn. (Lecythidaceae) commonly known as freshwater mangrove and mango-pine in English, *Hijal* in Bengali, is a large evergreen mangrove tree grown in the coastal wetlands of Northern Australia and South-East Asian countries including India. This plant has traditionally been used in India for several important medicinal purposes. In the present study, the dried and powdered leaf of *B. acutangula* was extracted by hot water and a new oral liquid Ayurvedic formulation was developed from that decoction (*kwatha*) and preserved for subsequent standardization. Its organoleptic properties like description, colour, odour, taste were noted. Then different physico-chemical parameters of this product like specific gravity, pH, viscosity, solubility, total solids content, ash values, preliminary phytochemical analysis, thin layer chromatographic (TLC) profile, microbial and heavy metal contaminants were determined as per the protocols prescribed in the Ayurvedic Pharmacopoeia of India. These salient pharmacopoeial quality standardization parameters may be useful for ascertaining the quality, purity and stability of this newly developed Ayurvedic oral formulation from *B. acutangula* leaf for further compliance.

**Keywords:** *Barringtonia acutangula,* standardization, heavy metal, microbial, leaf.

## Introduction

Medicinal and aromatic plants and their constituents (phytochemicals) have immensely contributed to the development of contemporary or modern medicine. The prime merits of plant-based medicinal products seem to be their recognized efficacy, lower occurrence of critical adverse events and lower cost. Thus, medicinal plants and the traditional medicinal products thereof become more affordable to the majority of global population for meeting their basic healthcare requirements. The undiscovered wealth of the plant kingdom can still be an interesting viable source for the development of effective therapeutic products (1,2).

The ancient Indian medical system known as Ayurveda, which translates to 'science/knowledge of life' in Sanskrit, has traditionally been used for more than 5,000 years. Many academics believe that Ayurveda is the oldest medical science still being practised widely. In India and Nepal, where up to 80% of people report using Ayurvedic medicine, it is the most widely practiced along with the rest of the globe. Ayurvedic medicinal sources chiefly include plants, animals and minerals which may be used in raw form or most commonly, in the form of different pharmaceutical formulations which have traditionally been used for centuries in a small scale. Nowadays, Ayurvedic products are commercially manufactured and

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marketed worldwide. In modern days, however, the Ayurvedic formulations should meet the official standardization criteria to ascertain quality, safety and efficacy and also for pertinent regulatory compliance (3).

Medicinal plants constitute the main source of Ayurvedic medicines. *Barringtonia acutangula* (L) Gaertn. (Lecythidaceae) commonly known as Indian oak, freshwater mangrove and mango-pine in English, *Hijal* in Bengali, *Nichula* or *Hijjala* in Sanskrit, is a large evergreen mangrove tree grown in the coastal wetlands of Northern Australia and the South-East Asian countries including India (Fig. 1). All parts of this plant has traditionally been used in the Indian subcontinent for several important medicinal purposes.Its leaf has specifically been used as tonic and in the treatment of diarrhoea, dysentery and stomach disorders (4-8). Previous researchers have reported pharmacognostic parameters and different pharmacological effects like antidiabetic, hepatoprotective, immunomodulator, antimicrobial, anti-inflammatory and anti-arthritic properties of its leaf (9-14). Despite the traditional uses and extant scientific pharmacological attributes of its leaf, there is no mention of its formulation in current literature. In Chakradatta Sangraha, a classical Ayurvedic text of the 11$^{th}$ century AD; *B. acutangula* leaf juice is recommended to be taken with honey for the treatment of *Amaitsara* i.e., amoebic dysentery (8). Inspired from this excerpt, in the present study, a new oral liquid Ayurvedic formulation has been developed from the leaf of *B.acutangula* and it was subjected to pharmacopoial standardization.

Figure 1: *Barringtonia acutangula*

## Materials and Methods

### *Collection and authentication*

The mature leaves of *Barringtonia acutangula* (L) Gaertn. (Lecythidaceae) was collected on November 2023 from Kalyani, Nadia, West Bengal. The species was authenticated at the Central National Herbarium, Botanical Survey of India, Shibpur, Howrah, West Bengal, India and a voucher specimen (ref. no. BIPS/DC-01) was preserved at our laboratory for future reference purpose. The collected leaves were shade-dried, crushed into a coarse powder and kept in air-tight container for subsequent formulation development.

### *Reagents and chemicals*

All the reagents and chemicals were of premium analytical grade (EMSURE) procured commercially. Honey was procured of Dabur India Ltd. Thin layer chromatography was performed on silica gel 60$F_{254}$ pre-coated aluminium plates 2.5 × 7.5 cm (Merck). Heavy mental contents were estimated by atomic absorption spectrophotometer (AAS) equipped with graphite tube atomizer (GTA) of Agilent 280-FS AA with GTA-120. The glassware and plasticware were from Borosil and Tarsons respectively. Doubled-distilled water from all-glass still was employed throughout the studies except heavy metal contamination study, where Milli-Q water (Merck-Millipore) was used.

### *Preparation of formulation*

The dry powdered leaf of *B. acutangula* (250 g) was mixed with 1.5 litre of distilled water and kept for 48 h with occasional shaking. Then the mixture was heated below 60°C and thus reduced. The mixture was then strained, miscella was collected and further reduced it to 250 ml below 60°C. Then 0.5% of sodium benzoate, 0.01% of sodium methyl paraben and 0.0375% of sodium propyl paraben were added to it while at 40°C. After cooling to room temperature, 18.75 ml of honey (Dabur) was added and mixed it carefully. It was preserved in an amber coloured air tight glass bottle to be used in the subsequent studies. The formulation method is schematically demonstrated in Fig. 2.

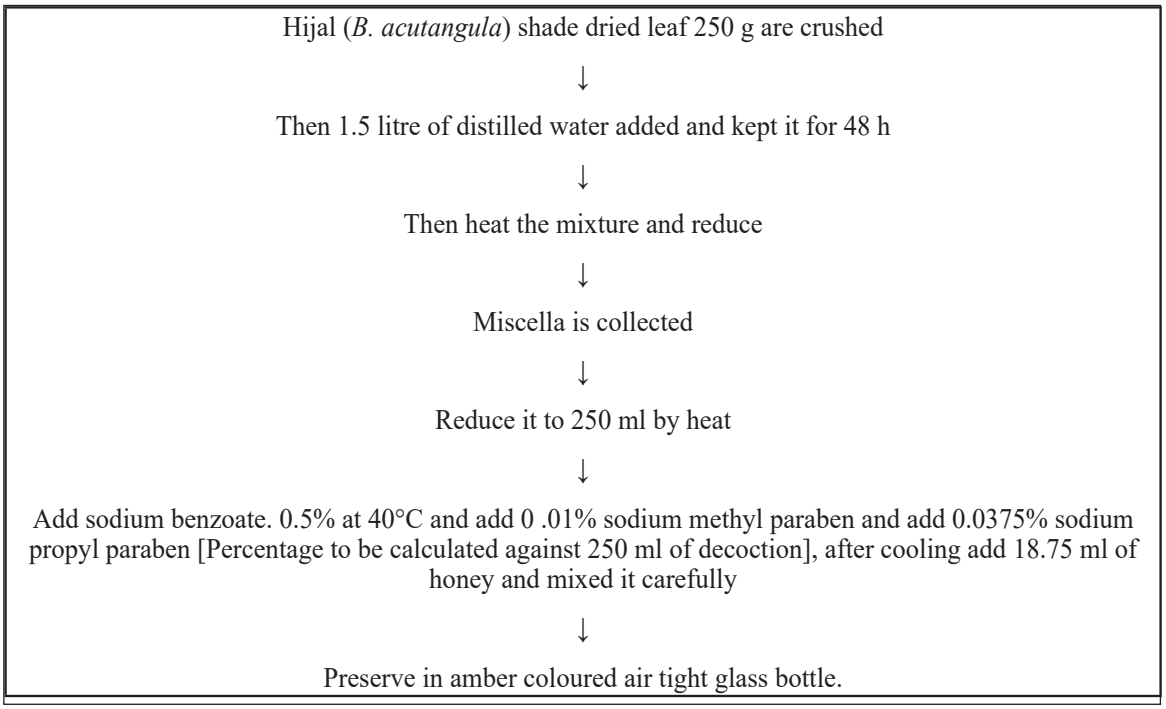


Figure 2: Formulation scheme.

***Organoleptic evaluation***

The sensory features like description, colour, odour, taste of the finished liquid formulation product thus prepared were noted.

***Physico-chemical and chromatographic parameters***

Different pharmacopieal physico-chemical parameters of the foregoing finished product like specific gravity, miscibility/solubility, pH, viscosity, solubility, preliminary phytochemical analysis, total solids content, ash values, extractive values of the finished liquid formulation product were determined as per the protocols prescribed in the Ayurvedic Pharmacopoeia of India. Thin layer chromatography (TLC) of the formulation was carried out on the plates pre-coated with silica gel $60F_{254}$ by using the mobile phase ethyl acetate: methanol = 5: 5 and the chromatogram was recorded.

***Microbial contamination***

The pharmacopoeia-prescribed microbial contamination tests namely total microbial plate count (TPC), total yeast and mould; and the presence of pathogens viz. *Staphylococcus aureus, Pseudomonas aeuroginosa, Escherichia coli* and *Salmonella sp*. were performed as per the protocols prescribed in the Ayurvedic Pharmacopoeia of India, just after preparation of the product and repeated after 6 months i.e., on the 181th day of its preparation, while stored in amber coloured air tight glass container at ambient temperature (15).

***Heavy metal content determination***

The formulation was processed in Teflon tubes by microwave assisted wet digestion and analyzed for arsenic (As), lead (Pb), cadmium (Cd) and mercury (Hg) by using atomic absorption spectrophotometer (AAS) equipped with graphite tube atomizer (GTA) as per the Ayurvedic Pharmacopoeia of India and according to the instrument's manual (Agilent 280-FS AA with GTA 120) as reported by the previous workers (15,16).

## Results and Discussion

### *Organoleptic evaluation*

Description: Free flowing syrupy liquid. Colour: Dark brown to blackish. Odour: Characteristic. Taste: *Kashay* to *Madhur* i.e., Astringent to Sweet.

### *Physico-chemical parameters*

### *Miscibility/solubility*

n-Hexane: Immiscible; Petroleum ether (60-80°C): Immiscible; Chloroform: Immiscible; Acetone: Miscible; Methanol: Miscible; Ethyl acetate: Immiscible; Water: Miscible.

***Specific gravity:*** 1.80 at 25°C.

***pH:*** 5.5

***Viscosity:*** 2.1 $KgM^{-1}S^{-1}$ at 25°C

***Ash values:*** total ash: 4.83 w/w, acid insoluble ash: 0.05 w/w.

***Total solids content:*** 88.83 %

### *Preliminary phytochemical screening*

The prepared Ayurvedic formulation demonstrated the presence of phytochemicals namely alkaloids, steroids, phenolic compounds, condensed tannins, saponins, carbohydrates and reducing sugars.

### *TLC: Rf values*

Plate observed at normal daylight: No visible spots., Plate observed in UV viewer chamber at the wavelengths 254 and 366 nm: $Rf_1$ = 0.68, $Rf_2$ = 0.21, $Rf_3$ = 0.07, plate observed at iodine chamber: $Rf_1$ = 0.78, $Rf_2$ = 0.68, $Rf_3$ = 0.21.

### *Microbial contamination*

The total microbial plate count (TPC) and total yeast and mould content of the prepared formulation were found to be below the permissible pharmacopoeial limits with the absence of pathogens viz. *Staphylococcus aureus, Pseudomonas aeuroginosa, Escherichia coli* and *Salmonella sp*. in it. The observed results with permissible official limits thereof have been presented in the Table 1. The repeat study of the foregoing official microbial parameters on it conducted after 6 months (180 days) did not show any appreciable deviation from the initial results (Table 1).

Table 1: Permissible limits and observed microbial loads of formulation prepared from *B. acutangula* leaf.

| Sl. no. | Parameters | Permissible limits as per API* | Results/g |
|---|---|---|---|
| 1 | Total microbial plate count | $10^5$/g | 92205 |
| 2 | Total yeast and mould | $10^3$/g | 889 |
| 3 | Staphylococcus aureus/g | Absent | Absent |
| 4 | Salmonella sp./g | Absent | Absent |
| 5 | Pseudomonas aeuroginosa/g | Absent | Absent |
| 6 | Escherichia coli/g | Absent | Absent |

* API: Ayurvedic Pharmacopoeia of India.

### *Heavy metal contents*

The toxic heavy metals namely lead and arsenic contents of the formulated product were found to be well below the prescribed official limits, whereas cadmium and mercury were not detected in it. The observed results with permissible limits thereof have been summarized in the Table 2:

Table 2: Official permissible limits and observed heavy metal contents of formulation prepared from *B. acutangula* leaf.

| Sl. no. | Heavy metals | Permissible limits (ppm) as per API[#] | Results (ppm) |
|---|---|---|---|
| 1 | Lead | 10 | 5.34 |
| 2 | Arsenic | 3 | 0.83 |
| 3 | Cadmium | 0.3 | < 0.05* |
| 4 | Mercury | 1 | < 0.05* |

*Below the limit of quantitation i.e., 0.05 ppm. [#]API: Ayurvedic Pharmacopoeia of India.

## Discussion

Along with contemporary or modern system of medicine, still now, traditional medicinal systems are well prevalent and official as well. Gastrointestinal diseases and related complications are the thrust area where the traditional medical practitioners or healers enjoy success and play a pragmatic role in their mitigation (17,18). As the leaf of *B. acutangula* has traditionally been used in the treatment of stomach related complications especially diarrhoea and dysentery and there is no such work on its formulation, the present work attempted development and standardization of a new Ayurvedic oral liquid formulation from *B. acutangula* leaf intended to be used for amoebic dysentery, motivated from Chakradatta Sangraha, the recognized classical Ayurvedic text of the 11$^{th}$ century AD, authored by Chakrapani Datta (see introduction).

Most of the Ayurvedic medicinal products are polyherbal/herbo-mineral formulations requiring considerable formulation skills. This newly developed Ayurvedic formulation (Fig. 2), on the other hand, is a simple monoherbal one which may be comparatively easier to formulate and standardize as well. The prepared formulation is an oral liquid formulation which is historically regarded as the most common and convenient type of traditional as well as contemporary pharmaceutical formulation with satisfactory patient compliance.

The most ancient and still widely practised traditional Indian medical system is Ayurveda. It is practised and its formulations are produced and used globally. When tested using contemporary criteria, the Ayurvedic formulations place a significant emphasis on establishing their clinical efficacy. However, occasionally it is discovered that these may be harmful or insufficiently effective in comparison to contemporary medicine. The absence of quality assurance and standardization is the primary cause of this. State of the art standardization is a crucial instrument for determining the level of purity and quality assurance of Ayurvedic and other herbal medicinal products.

Nowadays, the traditional or Ayurvedic medicinal formulations also require due standardizations for ascertaining their quality and stability as per the relevant official protocols/regulatory bodies for relevant compliance which may be mandatory for their marketing (19). In the present study, the shade-dried and powdered leaf of *B. acutangula* was extracted by hot water (decoction) and a new oral liquid Ayurvedic formulation was developed from that decoction (*kwatha*) with honey and subjected to subsequent standardizations. Its organoleptic properties like description, colour, odour, taste were noted. Then different pharmacopoial physico-chemical parameters of this finished product like specific gravity, pH, viscosity, solubility/miscibility, total solids content, ash values, preliminary phytochemical analysis, thin layer chromatographic (TLC) profile, microbial and heavy metal contaminants were determined as per the official protocols prescribed in the Ayurvedic Pharmacopoeia of India.

The organoleptic, physico-chemical, phytochemical and chromatographic studies

yield useful information pertaining to the quality and especially stability of the finished traditional pharmaceutical product. Microbial content of traditional Ayurvedic formulations taken internally, always plays an important role for the issues of its safety and efficacy (15,19). Therefore, the total bacterial count and total fungal count along with the tests for specific pathogens have been performed as per the pharmacopoeial protocols. The results indicated that, the presently formulated product is microbiologically safe to be consumed (Table 1). The same microbial contamination study repeated after 6 months (180 days) of closed storage at the room temperature also did not show any appreciable deviation from the initial results indicating recommended microbiological stability thereof for its continued usage period.

Heavy metal/metalloid contents in dietary/medicinal plants and herbal/Ayurvedic formulations thereof is a serious global concern nowadays (20). The most common heavy metals implicated in human/mammal toxicity include arsenic, lead, cadmium and mercury although nickel and chromium may also precipitate toxicity (21,22). These have no known physiological role yet may be of toxiciological interest (23). The Ayurvedic Pharmacopoeia of India recommends Ayurvedic formulations, should be checked for the presence of the first four above said heavy metals especially and prescribes limits for them (15). In the present study, the developed product was subjected to quantitative heavy metal analysis by AAS. The results indicated that the levels of all tested heavy metals were found to be well below the permissible pharmacopoeial limits (Table 2) indicating its legitimate oral administration as intended. In this way, the currently prepared formulation is at par with the other similar extant Ayurvedic formulations.

## Conclusion

To the best of present authors' knowledge, this is the first report of any pharmaceutical product/formulation development from *B. acutangula* to exploit its known therapeutic benefits. The salient pharmacopoeial quality standardization parameters reported in the present investigation may be useful for ascertaining the quality, purity and stability of this newly developed Ayurvedic oral liquid formulation from *B. acutangula* leaf for further compliance. The implication of the present findings may be taken into consideration whilst dealing with the development of Ayurvedic formulations for human or animal consumption. The present results may be of indicative reference value for future formulation development and quality assurance/control studies on this or other Ayurvedic or herbal formulation prepared by using *B. acutangula* leaf.

## Acknowledgement

The authors are thankful to the Department of Pharmaceutical Technology, Jadavpur University, Kolkata, West Bengal, India for necessary supports related to the present study.

## Funding

No funding source was applicable.

## Conflict of Interest

The authors declare that there is no conflict of interest.

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