

# Pharmacognostic Standardization of an Ethnomedicinal Aquatic Herb, *Monochoria hastata* (L.) Solms for its Antibacterial Potentiality

Debabrata Misra<sup>1</sup>, Manab Mandal<sup>1</sup>, Narendra Nath Ghosh<sup>2</sup>, Vivekananda Mandal<sup>1\*</sup>

Debabrata Misra<sup>1</sup>, Manab Mandal<sup>1</sup>, Narendra Nath Ghosh<sup>2</sup>, Vivekananda Mandal<sup>1\*</sup>

<sup>1</sup>Plant and Microbial Physiology and Biochemistry Laboratory, Department of Botany, University of Gour Banga, Malda, West Bengal, INDIA.

<sup>2</sup>Department of Chemistry, University of Gour Banga, Malda, West Bengal, INDIA.

## Correspondence

Vivekananda Mandal

Department of Botany, University of Gour Banga, Malda, West Bengal, INDIA.

Phone no: +91 9679008986

E-mail: mandalvivek@gmail.com

## History

- Submission Date: 18-12-2017;
- Review completed: 23-12-2017;
- Accepted Date: 05-02-2018

DOI : 10.5530/pj.2018.3.87

## Article Available online

<http://www.phcogj.com/v10/i3>

## Copyright

© 2018 Phcog.Net. This is an open-access article distributed under the terms of the Creative Commons Attribution 4.0 International license.



## ABSTRACT

**Objectives:** To evaluate antibacterial potentiality, pharmacognostic characteristics and quality control parameters including heavy metals, like lead (Pb) and arsenic (As) accumulation in the aerial parts of an aquatic herb, *Monochoria hastata* (L.) Solms. **Methods:** Antibacterial assay was done by agar well diffusion method. Pharmacognostic studies like morpho-anatomical and physicochemical analyses were carried out for organoleptic, microscopic and macroscopic evaluations of living aerial parts, and powder microscopy, fluorescence, proximate and elemental analysis of the dried powder sample. Elements and heavy metals contents were determined by carbon, hydrogen, nitrogen, sulphur / oxygen (CHNS/O) analyzer and inductively coupled plasma mass spectrometry (ICP-MS), respectively. **Results:** *M. hastata* (L.) Solms aerial parts showed antibacterial activity against gastrointestinal and topical pathogens. It exhibited an amphistomatic and hydromorphic anatomical characters. The distinguishing features were the presence of stomata in upper and lower epidermis, broad air chambers, cuticle, collateral vascular bundles, sclereid fibres, pitted tracheids, pitted vessels, calcium oxalate crystals and annular vessels in leaf. The powder sample contained very less amount of acid insoluble ash than water soluble ash and complete absence of foreign organic matter. Carbon, hydrogen, nitrogen and sulfur (CHNS) ratio was 33:6:5:1 and lead content was lesser than the recommended upper limit though the arsenic content was higher than the permissible upper limit. **Conclusions:** Though the plant has traditionally been used as a potent Ethno-medicinal herb to cure boils, gastritis, hepatopathy and as laxative, but no such evaluation of pharmacognostic identity and quality parameters have been done so far. This is the first report on its pharmacognostic characters and quality control issues like heavy metal accumulation and physicochemical parameters for future use as powder drug.

**Key words:** Fluorescence characteristics, Heavy metal accumulation, *M hastata* (L.) solms, Pharmacognostic standardization, Traditional medicinal plants.

## INTRODUCTION

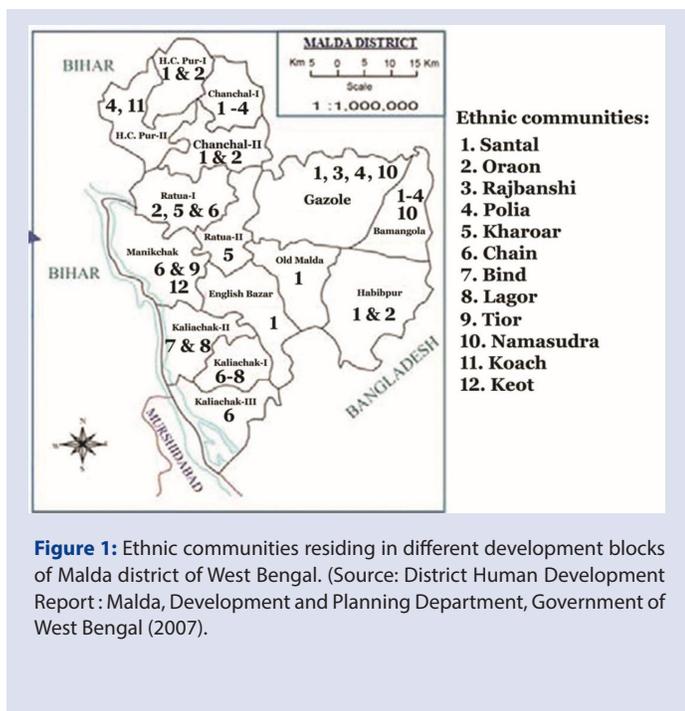
Indigenous people use medicinal plants either as original or as semi-synthetic herbal preparations in various ailments without side effects.<sup>1</sup> Medicinal plants are considered as safer and cost effective also.<sup>2</sup> 70% of Indian population use herbs as remedy based on their traditional and indigenous knowledge. Indian Systems of Medicine (ISM) including Ayurvedic, Unani, Siddha, Homoeopathy, Naturopathy and tribal medicines use about 400 plants. Herbs are the potent source of several novel modern drugs. Pharmaceutical sector in India use 280 medicinal plant species.<sup>3</sup> Hydrophytes or plants grown on aquatic, wet and marshy lands also have medicinal uses like the plants grown in terrestrial habitat.<sup>4</sup> India is a country having a vast ethnic diversity and is rich in different indigenous knowledge system. Many ethnic communities inhabit in Malda (24° 40' 20" N and 25° 32' 08" N latitude and 87° 45' 50" E and 88° 28' 10" E longitude), a district situated in West Bengal province of this nation where 'Kaviraaj',

'Vaidya', 'Ojha', 'Jan Guru' and aged knowledgeable persons of different ethnic communities living in Malda are the Ethno-medicine practitioners (Figure 1). They have information regarding the use of different medicinal plants curing several ailments.<sup>5</sup>

There are many pertinent literatures which support the Ethno-medicinal use of herbs in various diseases and ailments. Some of the published works done so far on the traditional uses of edible and medicinal plants in Malda district are mentioned chronologically in the Table 1.

*Monochoria hastata* (L.) Solms is one of the potent Ethno-medicinal herbs used in India. Root stock of this plant increases vitality.<sup>12</sup> Young shoots are used as green leafy vegetables and leaf juice is applied to cure boils by Bodo, Koch-Rajbongshi and Rangia tribes.<sup>13</sup> Root stock and leaves of *M. hastata* (L.) Solms are also used in gastropathy,

**Cite this article:** Misra D, Mandal M, Ghosh NN, Mandal V. Pharmacognostic Standardization of an Ethnomedicinal Aquatic Herb, *Monochoria hastata* (L.) Solms for its Antibacterial Potentiality. Pharmacogn J. 2018;10(3):533-40.



**Figure 1:** Ethnic communities residing in different development blocks of Malda district of West Bengal. (Source: District Human Development Report : Malda, Development and Planning Department, Government of West Bengal (2007).

hepatopathy and anti-lipoxygenase (LOX) activity.<sup>14</sup> The leaves of the herb are laxative and its paste is served to cattle with diarrhoea as a tonic.<sup>15</sup> Hence, this herb might have some anti-enteric efficacy against some gastrointestinal microflora. In West Bengal, this aquatic plant is used as medicinal herb and fodder.<sup>16</sup> In Malda, this plant species is dominant as emergent hydrophytes in the water bodies such as Piasbari nursery pond, Sagardighi, Asokpally pond, Rajgunge, pond near Ramkeli.<sup>17</sup>

The identity, quality, purity and safety of an Ethno-medicinal herb to be used as powder drugs, is an important concern due to conventionally used immediate natural sources may contain some hazardous contaminants including heavy metals, toxic substances and foreign materials. It can be achieved by a stepwise standardization process which includes pharmacognostic studies like organoleptic, macroscopic as well as microscopic and eco-physiological features and also the evaluation of physicochemical parameters, viz. moisture content, ash values, fluorescence analysis, as well as by toxicological investigation, viz. heavy metals and foreign matter contents etc.<sup>18</sup> Organoleptic or physical properties like colour, odour, size, shape, taste etc.; microscopic and macroscopic char-

acters are very important to identify a plant as a crude drug source.<sup>19</sup> Estimation of proximates such as ash values, loss on drying, presence of foreign organic matter etc. in herbal material helps us to justify its safety and efficacy and to ensure reproducible quality of it as crude powder drug.<sup>20</sup> Ash value gives an indication of its purity level in presence of various impurities like carbonates, oxalates and silicates.<sup>21</sup> Loss on drying can be estimated by quantifying the amount of moisture present in the plant sample. It is very much noteworthy regarding its storability as moisture gives suitable growth condition to microorganisms contamination and spoilage.<sup>22</sup> A medicinal plant material should be entirely free from any visible sign of contamination by any organic foreign materials such as moulds or insects, and other animal contamination, including animal excreta as they may produce toxic substances like aflatoxins.<sup>23</sup> Fluorescence analysis is also an important tool for the screening of some compounds which do not fluoresce themselves but when they are treated with some reagents and solvents are converted into fluorescent derivatives.<sup>24</sup> Medicinal properties of a plant directly or indirectly depend on the elemental concentrations of that plant. Medicinal plants contain major elements which are widely used as precursors of different metabolite biosynthesis pathways. Elemental concentrations of medicinal plants regulate the curative ability of certain diseases. Carbon and hydrogen are the major elements commonly present in all organic molecules while nitrogen and sulphur is present in some special organic compounds like alkaloids, glucosinolates, camalexin, defensins etc.<sup>25</sup> Hence, it is important to know the elemental concentration in medicinal plants which gives indications on what kind of organic metabolites are present in the plant sample.<sup>26</sup> Evaluation of CHNS ratio is also important as it may act as a purity index. This ratio is affected when there is any adulterant in the crude drug sample.

Water hyacinth (*Eichhornia crassipes*) and other aquatic plants have been reported to uptake arsenic of different concentrations and thereby reduce arsenic pollution.<sup>27</sup> Investigation of heavy metals accumulation in frequently utilized medicinal plants collected from environmentally diverse locations of north western India proved that consumption of raw medicinal herbs grown in toxic heavy metal sites caused serious consequences on human health.<sup>28</sup> Arsenic (As) contaminated drinking water beyond the permissible limit has deleterious effects on human health viz. cardiovascular problem, gastrointestinal disorders, haematological ailments, neurological effects, skin diseases etc. Higher levels of lead (Pb) are carcinogenic and effects on the central nervous system and memory (dyslexia).<sup>29</sup> Hence, checking the level of heavy metal contamination in herbal plant material, specially the aquatic one is an important concern before its medicinal use. International agencies like World Health Organization (WHO), Food and Agriculture Organization (FAO) has suggested the analysis of absorption, elimination and toxic profiles of

**Table 1:** Published works on the traditional uses of edible and medicinal plants of Malda district.

Title of the work	Name of the author (s) with year of publication
Inventory of some ethno-medicinal plants in wetlands areas in Maldah district of West Bengal	Chowdhury and Das (2009) <sup>6</sup>
Wild edible plants consumed by local communities of Maldah district of West Bengal, India	Chowdhury and Mukherjee (2012) <sup>7</sup>
Some less known plants from Malda district of West Bengal used for the treatment of arthritis, rheumatism and gout	Mitra and Mukherjee (2013) <sup>8</sup>
Indigenous knowledge of plants in local healthcare management practices by tribal people of Malda district, India	Saha et al. (2014) <sup>9</sup>
Ethnobotany of Chanchal Block of Malda District of West Bengal (India): plants used in local healthcare	Saha et al. (2014) <sup>10</sup>
Ethnobotany, traditional knowledge and socioeconomic importance of native drink among the Oraon tribe of Malda district in India	Saha et al. (2015) <sup>11</sup>

heavy metals in the herbal medicines and also put forwarded the other relevant necessary chemical, biological, and environmental analysis as a critical issue in their guidelines.<sup>30</sup> Though very little is known regarding the potential influences of heavy metals on pharmacological activities of plant derived natural drugs. Yet, the heavy metal content analysis is necessary as a safety measure.

The present investigation was dealt with the study of antibacterial bioassay guided organoleptic and morpho-anatomical studies, analysis of fluorescence, estimation of proximate contents, determination of CHNS ratio, levels of arsenic and lead heavy metals present in the leaf of the aquatic medicinal herb, *M. hastata* (L.) Solms. This kind of study helps to channelize indigenous knowledge of herbal use and contributes to improve health care delivery system by alternative and complementary medicines in today's pluralistic society.

## MATERIALS AND METHODS

### Microorganisms and Chemicals

Gram '+' bacterial strains of *Staphylococcus aureus* MTCC 96 (*S. aureus*) and *Streptococcus mutans* MTCC 497 (*S. mutans*), and Gram '-' strain of *Escherichia coli* MTCC 571 (*E. coli*) were purchased from the Microbial Type Culture Collection and Gene Bank (MTCC), Chandigarh, India. It is an affiliate member of the World Federation for Culture Collections (WFCC) and is registered with the World Data Centre for Microorganisms (WDCM). All the chemicals including n-Hexane, ethyl acetate, methanol, formalin, absolute alcohol, safranin, fast green, acetic acid, canada balsam, H<sub>2</sub>SO<sub>4</sub>, NaOH, KOH, H<sub>2</sub>SO<sub>4</sub>, HNO<sub>3</sub>, HCl, Na<sub>2</sub>HPO<sub>4</sub>, FeCl<sub>3</sub>, distilled water, aniline, silver nitrate, hydrogen peroxide, acetic acid, silica gel, light green, safranin etc. were of analytical grade and were procured either from Merck Limited India or from Sisco Research Laboratories, Mumbai, India.

### Collection and Authentication of Plant Materials

Fresh leaves of *M. hastata* (L.) Solms were collected from Jalalpur wetland of Kaliachak-I Development Block of Malda district in West Bengal and was identified by Dr. R. Gogoi, Scientist-D, Central National Herbarium of Botanical Survey of India, Howrah-711 103, West Bengal (India) with the specimen voucher no. UGB/DM/01.

### Processing of Plant Sample, Extraction and Partial Purification

Collected plant materials were washed thoroughly under running tap water, rinsed in distilled water and dried in hot air chamber at 50°C for one week. Dried leaves were uniformly grinded using a mixer-grinder machine and the leaf powder was stored at 4°C in an air tight container. Fifty g of leaf powder soaked in 500 ml absolute ethyl acetate and 50% methanol in water were separately extracted using a soxhlet apparatus at 40°C for 48 hrs. The extracts were filtered through Whatman No. 1 filter papers and made pigment-free by passing through the activated charcoal columns. The de-pigmented filtrates were collected and concentrated using a rotary vacuum evaporator (Superfit, R-150, Mumbai, India). The concentrated extract was stored at 4 °C until use for antibacterial activity test. The ethyl acetate extract was further purified using silica gel (100-200 mesh) column as the stationary phase, and n-Hexane and ethyl acetate at a ratio of 1:1 (v/v) as the mobile phase. The purified fraction was air dried and kept in an Eppendorf tube for further studies.

### Culturing of Microorganisms and Evaluation of Antibacterial activity

The bacterial strains were maintained on agar slant at 4°C and activated at 37°C for 24 h on nutrient agar (HiMedia, Mumbai, India) before any

antibacterial assay. Overnight culture of each bacterial strain grown in broth was diluted to 0.5 McFarland turbidity standard for inoculation and seeded on Mueller Hinton agar (MHA) (HiMedia M173-500G) plates by using sterilized swabs. The medium was allowed to dry under laminar air flow and wells were cut by using sterilized corn borer (7 mm diameter). 20 µl each of the concentrated extracts and fraction was placed into the agar wells. 3µl Ciprofloxacin antibiotic (50µg/ml) was used as positive control and respective solvents as negative control. Plates were incubated at 37°C for 24 h and diameter of growth inhibition zones were determined.<sup>31</sup>

### Organoleptic Evaluation

Various sensory parameters like color, odor, appearance, texture and taste of the plant material had been studied as per standard procedure.<sup>32</sup>

### Macroscopic and Microscopic Evaluation

Different characteristic features of leaf lamina and petiole of *M. hastata* (L.) Solms like size, shape, margin, surface, venation pattern etc. including the type of stem and nature of inflorescence were morphologically studied.<sup>33</sup> All the slides prepared for the leaf and powder microscopy were visualized under a compound microscope (Magnus MLX) in 40X objective and photographs were taken using the camera, Magnus (MagCam DC10).

### Leaf and Powder Microscopy

In this study, fresh leaf was dipped in distilled water and transverse sections (T.S.) were cut manually. Thin sections were mounted on glass slide with glycerine without any staining reagent. Various histological and eco-physiological characters, such as cell composition, adaptive structures and histochemical features were qualitatively studied under microscope and photomicrographs were taken. For powder microscopy, shade dried leaves were finely powdered in a mixer-grinder machine and studied under the microscope. A pinch of the powder was placed in the water taken in a watch glass and remained undisturbed for 2 h. 1 drop of it was taken on a glass slide and stained with one drop of 1.0 % w/v safranin in 70% ethanol. After 5 min it was washed two times with distilled water and stained with 0.1% w/v light green in 90% ethanol for 5 min. After washing two times with distilled water the slide was covered with cover slip and examined under microscope. Different cell components i.e. vessels, fibres, cortex cells, calcium oxalate crystals were studied.<sup>34</sup>

### Biochemical test and Microscopy for Calcium Oxalate Crystals

The presence of calcium oxalate crystals in the leaf tissues was confirmed by the chemical method as described by.<sup>35</sup> The leaf powder sample was treated with 2N acetic acid for 15 min to remove phosphate and carbonate. These were then treated with 1% silver nitrate in 15% hydrogen peroxide (1:1, v/v) for 15 min at 22°C. Then the treated sample was washed in distilled water and counter stained with 2% safranin for 3 min and observed under compound microscope.

### Determination of Extractive values

The powder sample was taken in conical flasks and soaked with various solvents like absolute n-hexane, diethyl ether, chloroform, dichloromethane, ethyl acetate, methanol, 90% methanol in water, 50% methanol in water and water separately and capped. The capped flasks were then agitated in an orbital shaker at 100 rpm for 24 h. After obtaining the filtered extract, it was then transferred into weighed Petri plates and concentrated to dryness by keeping filtrates for complete evaporation of solvent. The extractive value in percentage was calculated by using following formula:

$$\text{Extractive value(\%)} = \frac{W_e}{W_p} \times 100$$

Where,  $W_e$  = Weight of dried extract and  $W_p$  = Weight of plant powder sample.

### Qualitative Assessment of Phytochemical Constituents

Phytochemical compounds present in the 50% methanol extract were assessed through the phytochemical screening tests following specific protocols with some minor modification.<sup>36</sup>

### Fluorescence Analysis

Fluorescence analysis of leaf powder was carried out by standard protocol.<sup>37</sup> In this analysis the plant sample was treated with various basic and acidic solvents such as aqueous and alcoholic sodium hydroxide (NaOH), hydrochloric acid (HCl), sulphuric acid (H<sub>2</sub>SO<sub>4</sub>), nitric acid (HNO<sub>3</sub>), ferric chloride (FeCl<sub>3</sub>) and dibasic sodium bi phosphate (Na<sub>2</sub>HPO<sub>4</sub>) and then observed in UV/ visible chamber under visible, short UV-A wavelength (366 nm) and long UV-B wavelength (254 nm) of light simultaneously.

### Preparation of Ash and Estimation of Various Ash values

3gm of leaf powder was incinerated in a Silica crucible over the heater. The charred mass was heated in muffle furnace at 600-650°C until the ash became white and free from carbon. It was cooled and weighed on the ash less filter paper. The water soluble ash, acid insoluble ash, sulphated ash and total ash were determined following the standard protocol.<sup>38</sup>

### Estimation of Moisture Content

The loss on drying was determined by estimating the moisture content evaporated on drying at a temperature not exceeding 115°C as per the procedure put forwarded by The Indian Pharmacopoeia.<sup>39</sup> The amount of moisture was calculated using the following formula:

$$\% \text{ moisture content (Wb)} = \frac{Ww - Wd}{Ww} \times 100$$

Where,  $Ww$  = Wet weight of sample and  $Wd$  = Dry weight of sample.

### Determination of Foreign Organic Matter

100g of leaf powder sample was weighed and spread on a tray in a thin layer. The sample was inspected with the unaided eye and also with the use of a 6x lens, and the foreign organic matter was separated manually. Separated foreign matter was weighed and the percentage value was determined as per Indian Pharmacopoeia.<sup>40</sup>

### CHNS and Heavy Metals Contents Analysis

Total carbon, hydrogen, nitrogen and sulphur contents were determined using a CHNS analyser (Make: Thermo Fisher Scientific Instruments; Model: FLASH 2000 CHNS/O Analyzers; Country: USA), and lead and arsenic contents were determined by ICP-MS according to the method described by Mandal *et al.* (2017).<sup>41</sup>

### Statistical Analysis

All the experiments except elements and heavy metals analysis have been done in triplicate and the results accepted as Arithmetic Mean  $\pm$  Standard Error at 5% level.<sup>42</sup>

## RESULTS

### Antibacterial Evaluation

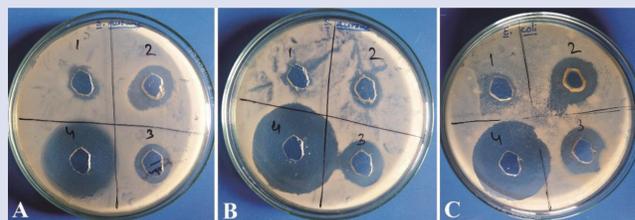
The different solvent extracts and purified fraction of *M. hastata* (L.) Solms leaf exhibited antibacterial efficacy by showing bacterial zone inhibition as shown in the Figure 2 (A-C).

### Organoleptic Evaluation

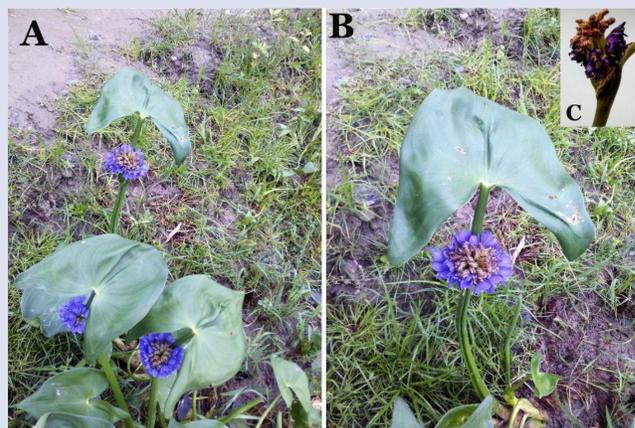
The leaves showed green leathery appearance from both side without any trichome. The leaf powder was green in colour, rough in texture, mildly aromatic in odour and insipid in taste. The stem covered with the remains of old leaf sheaths at the base was white, prostrate to erect, soft, spongy and herbaceous in nature.

### Macroscopic Evaluation

Morphological study indicated that leaves were simple, arrow shaped or hastate with smooth margin, petiole was hollow, up to 3-4 ft long. The lamina had parallel venation. Surface was smooth. The size of the leaf varied from 45 to 60 cm in width and length, respectively. Root was adventitious, laterally grown in tufts from the tip of the rhizomatous stem. Stem was short rhizomatous, simple covered with leaf sheath. Inflorescence was 6-9 cm long shortly stalked spike with 25-60 densely arranged 13-16 mm long brilliant purple blue sub-umbellate raceme flowers (Figure 3 A-C).



**Figure 2:** Antibacterial evaluation of *M. hastata* (L.) Solms leaf. (A) against *S. mutans*; (B) against *S. aureus*; (C) against *E. coli*. Here, '1' indicates bacterial zone inhibition by 50% methanol extract, '2' indicates bacterial zone inhibition by purified ethyl acetate fraction, '3' indicates bacterial zone inhibition by absolute ethyl acetate extract and '4' indicates bacterial zone inhibition by antibiotic drug.



**Figure 3:** Macroscopy of *M. hastata* (L.) Solms. (A) Whole plant; (B) Leaf with inflorescence; (C) Inflorescence.

## Leaf and Powder Microscopy

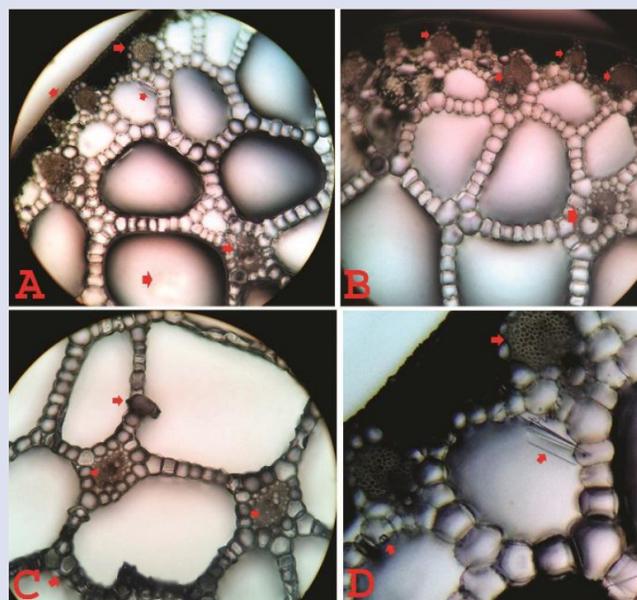
T.S. of lamina showed that the upper and lower both the epidermis contained stoma. Wide air spaces were present in the median part. Epidermal cells were covered with a very thin cuticle. Collateral vascular bundle showed wide meta-xylem and narrow proto-xylem elements enclosed in a parenchymatous sheath. Vascular bundle of mid-rib showed wide circular thin walled meta-xylem with circular mass of phloem (Figure 4 A-C). T.S. of petiole showed thick cuticle and ground tissue including palisade layer followed by 3 layers of air-chambers divided by thin uniseriate partition filaments. Collateral vascular bundles were located in the intercepts of the partition filaments. A central air canal was surrounded by a thin membranous plate of circular cells, called diaphragm. Needle shaped raphides with pointed ends were also observed (Figure 5 A-D). Powder microscopy of *M. hastata* (L.) Solms leaves indicated the presence of sclereidal fibres, pitted tracheids, pitted vessels, calcium oxalate crystals and annular vessels (Figure 6 A-D).

## Determination of Extractive values

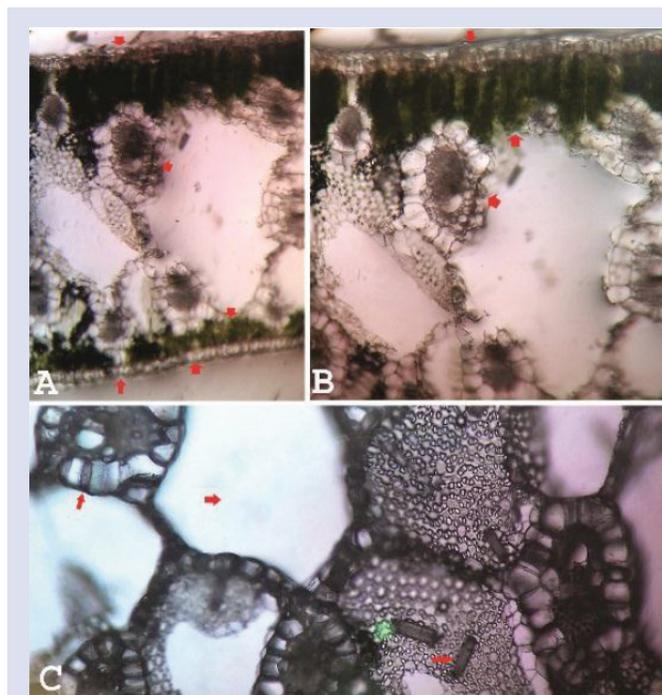
The extractive values calculated from the yields in different solvents are shown in the Figure 7. The extractive values in different solvents indicated that water and 50% methanol extract showed the highest extractive values while n-hexane and diethyl ether extract showed the lowest extractive values compared to other solvents. Chloroform, dichloromethane and ethyl acetate had more or less same extractive value which is lesser than water and methanol but greater than n-hexane and diethyl ether.

## Phytochemical, Proximate and Fluorescence analysis

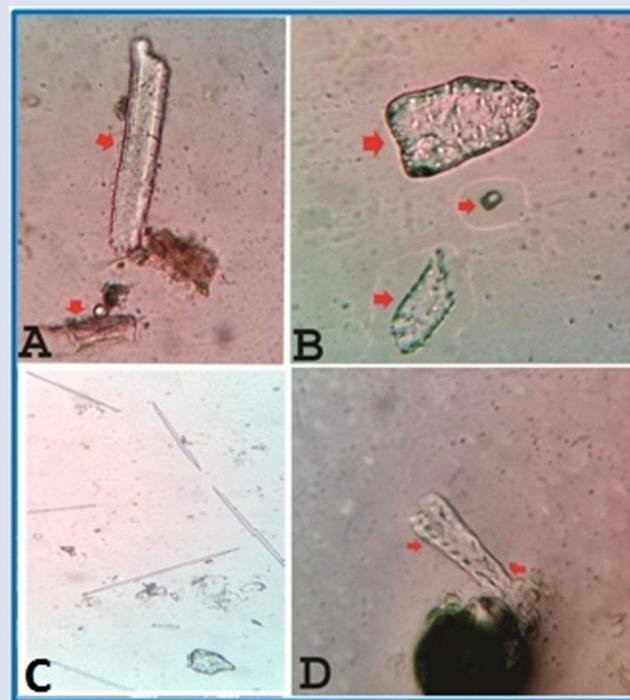
Phytochemical constituents present in the 50% methanol extract were phenols, flavonoids, saponins, steroids, carbohydrates, glycosides and alkaloids. The results of proximates and fluorescence analysis are presented in Table 2 and Table 3, respectively.



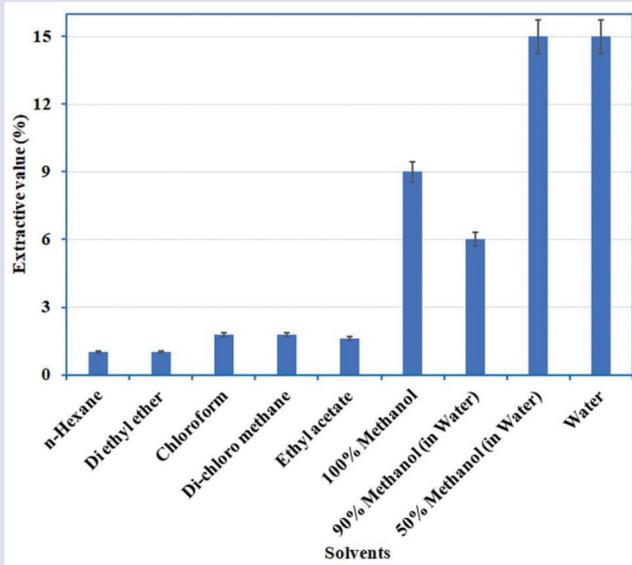
**Figure 5:** Microscopy of *M. hastata* (L.) Solms leaf petiole. (A) T.S. of petiole showing cuticle, epidermis and other visceral parts; (B) T.S. of petiole showing vascular bundles and air-chambers; (C) T.S. of petiole showing central air canal with diaphragm; (D) Presence of raphides.



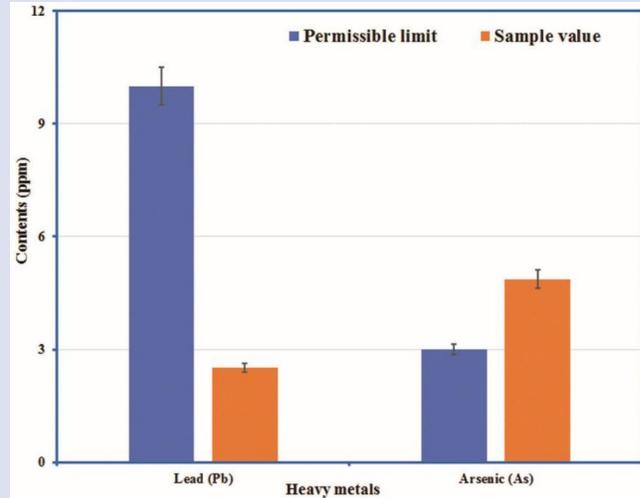
**Figure 4:** Microscopy of *M. hastata* (L.) Solms leaf. (A) Transverse section (T.S.) of lamina; (B) T.S. of lamina showing epidermis, chlorenchyma tissue and vascular bundles; (C) T.S. of midrib.



**Figure 6:** Powder microscopy of *M. hastata* (L.) Solms leaf. (A) Sclereidal fibre; (B) Pitted tracheid, pitted vessel and raphide; C. Needle shaped calcium oxalate crystals; (D) Annular vessel.



**Figure 7:** Extractive values of *M. hastata* (L.) Solms leaf powder in different solvents.



**Figure 8:** Lead (Pb) and Arsenic (As) contents of *M. hastata* (L.) Solms leaf powder.

**Table 2:** Proximate analysis of *M. hastata* (L.) Solms powder sample.

Proximates	Contents
Total ash	28%
Acid insoluble ash	2%
Water soluble ash	15%
Sulphated ash	8%
Moisture content	8.65%
Foreign organic matter	0%

**Table 3:** Fluorescence analysis of powder of *M. hastata* (L.) Solms leaf.

Experiments	Visible/Day light	UV Light	
		254 nm	365nm
Powder only	Green	Brown	Black
Powder + 1N NaOH (aqueous)	Yellow	Green	Black
Powder + 1N NaOH (alcohol)	Yellow	Brown	Black
Powder + 1 N HCl	Yellow	Brown	Black
Powder + 50% H <sub>2</sub> SO <sub>4</sub>	Light Brown	Dark brown	Black
Powder + Nitric acid	Orange	Brown	Black
Powder + Ferric chloride	Yellow	Green	Black
Powder + Na <sub>2</sub> HPO <sub>4</sub>	Brown	Dark brown	Black

### Elements and Heavy Metals Analysis

Carbon, hydrogen, nitrogen and sulphur (CHNS) contents of the leaf powder sample were 27.85 %, 5.25 %, 4.31 % and 0.85 %, respectively. Lead and arsenic contents in the powder sample are shown in the Figure 8.

### DISCUSSION

The crude extracts and the purified fraction have potential antibacterial efficacy against both Gram-positive and Gram-negative bacteria. The amphistomatic and hydromorphic characters, features like presence of cuticle, collateral vascular bundles, sclereidal fibres, pitted tracheids, pitted vessels, calcium oxalate crystals, annular vessels are the major identifying characters of *M. hastata* (L.) Solms as the crude drug source.<sup>43</sup> Sclereidal fibres confirm the presence of woody material in the crude drug component, and also indicates its purity. Low moisture content suggests better stability of the powder sample against microbial degradation.<sup>44</sup> The unique fluorescence characteristic features are helpful to assess the purity level of the powder drug sample. Ash content analysis showed very significantly lesser amount of acid insoluble ash than that of water soluble ash which ensures the presence of negligible amount of contaminants like silica, carbonates etc. Carbon, hydrogen and nitrogen ratio (C: H: N: S) was 33: 6: 5: 1. *M. hastata* (L.) Solms contains a significant amount of nitrogen and sulphur which was not detected in another aquatic medicinal herb, *Eclipta prostrata*, yet it contains amine and nitrile compounds.<sup>45</sup> This is an indication to definitely have nitrogenous compounds in *M. hastata* (L.) Solms. The powder sample was completely free from any organic impurities. Lead content in the leaf powder sample was within the permissible limit while the arsenic content was higher than the upper permissible limit as recommended by the Department of Ayurveda, Yoga and Naturopathy, Unani, Siddha and Homeopathy (AYUSH) for medicinal plants of India.<sup>46</sup> It may be due to bioaccumulation of arsenic absorbed by this aquatic plant from the water body where it grew. In Malda, arsenic (As) concentration in ground water is higher (0.05 – 1.434 mg/Lt) than the permissible limit of BSI (0.05mg/Lt). Arsenic affected development blocks are English Bazar, Kaliachak-I, Kaliachak-II, Kaliachak-III, Manikchak, Ratua-I and Ratua-II.<sup>47</sup> Hence, this particular aquatic herb may be used in bioremediation and restoration of arsenic polluted wetlands and from the herbal use point of view the plant sample must be checked before use as the arsenic content is high.

### CONCLUSION

It can be concluded that *M. hastata* (L.) Solms leaf has potential antibacterial activity. The heavy metal contents analysis suggests that appropriate safety measures should be taken before use of this herb as medicine. This study will be helpful for authentication of *M. hastata* (L.) Solms as the

crude drug source. It will also help further researchers to maintain the standards of this plant for their research projects. This pharmacognostic standardization study will be used in pharmaceutical preparations if necessary to recognize of *M. hastata* (L.) Solms from its adulterants. It ensures the identity, quality and purity of the crude drug for the human welfare. This study may be a diagnostic tool for using *M. hastata* (L.) Solms as a phytoremediator of arsenic.

## ACKNOWLEDGEMENTS

We are thankful to Edward Food Research and Analysis Centre (EFRAC) for conducting the analysis of elements and heavy metals contents of the plant sample.

## CONFLICT OF INTEREST STATEMENT

We declare that we have no conflict of interest.

## ABBREVIATIONS USED

**AYUSH:** Ayurveda, Yoga and Naturopathy, Unani, Siddha and Homeopathy; **CHNS/O:** Carbon, hydrogen, nitrogen, sulphur / oxygen; **FAO:** Food and Agriculture Organization; **ICP-MS:** Inductively coupled plasma mass spectrometry; **ISM:** Indian Systems of Medicine; **LOX:** Anti-lipoxygenase; **MHA:** Mueller Hinton agar; **MTCC:** Microbial Type Culture Collection and Gene Bank; **WHO:** World Health Organization.

## SUMMARY

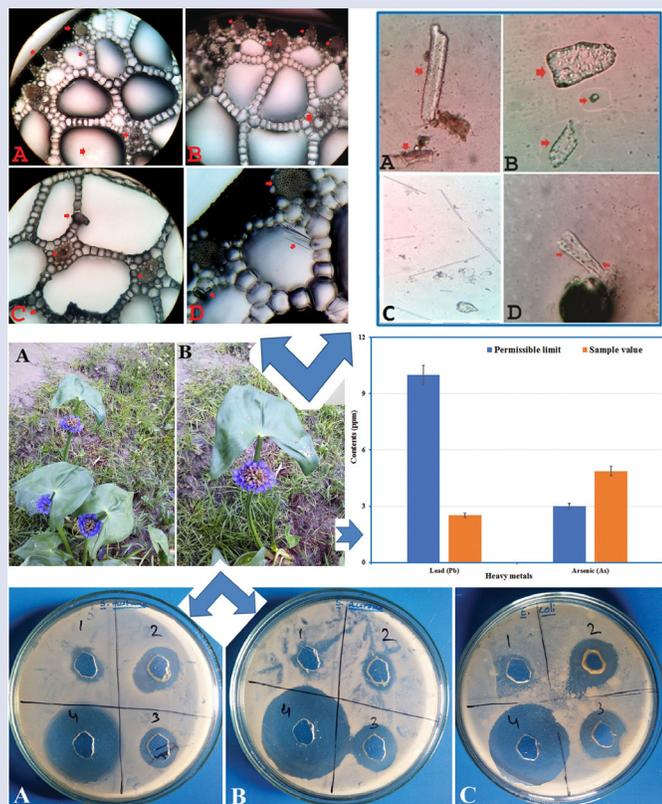
*M. hastata* (L.) Solms is an evergreen perennial aquatic herb growing in wide geographical range. Though the plant has been used by certain ethnic communities in Asia, but the characterization of active principles have not been done so far. The present research article focuses on the evaluation of pharmacognostic identity and quality parameters to be used as herbal drug and thus validating its ethno-medicinal use to cure boils, gastritis, hepatopathy and as laxative.

## REFERENCES

- Unnikrishnan PM, Suneetha MS. Linking medicinal plants, traditional knowledge and community health United Nations University-International Institute of Global Health, Malaysia. *J Tradit. Folk Pract.* 2012;04(1):100-03.
- Dharmani P, Kuchibhotla VK, Maurya R, Srivastava S, Sharma S, Palit G. Evaluation of anti-ulcerogenic and ulcer-healing properties of *Ocimum sanctum* Linn. *J Ethnopharmacol.* 2004;93(2):197-206.
- Kumar M, Sheikh MA, Bussmann RW. Ethno-medicinal and ecological status of plants in Garhwal Himalaya, India. *J Ethnobiol. Ethnomed.* 2011;7(1):32.
- Das P, Rahman I. Medicinal Plants Traditionally used by Scheduled Caste Community of Lakhimpur District, Assam. *J Frontl. Res. Art. Sci.* 2011;1:54-57.
- Datta T, Patra AK, Dastidar GS. Medicinal plants used by tribal population of Coochbehar district, West Bengal, India- an Ethnobotanical survey. *Asi. Pac. J Trop. Biomed.* 2014;4(1):S478-82.
- Chowdhury M, Das AP. Inventory of some Ethno-medicinal plants in wetlands areas in Maldah district of West Bengal. *Pleione.* 2009;3(1):83-8.
- Chowdhury M, Mukherjee R. Wild edible plants consumed by local communities of Maldah district of West Bengal, India. *Ind. J. Sci. Res.* 2012;3(2):163-70.
- Mitra S, Mukherjee SK. Some less known plants from Malda district of West Bengal used for the treatment of arthritis, rheumatism and gout. *Int. J Pharmaceut. Res. Bio. Pharmaceut. Res. Bio-sci.* 2013;2(4):337-45.
- Saha MR, De Sarker D, Kar P, Gupta PS, Sen A. Indigenous knowledge of plants in local healthcare management practices by tribal people of Malda district, India. *J Intercult. Ethnopharmacol.* 2014;3(4):179-85.
- Saha MR, Kar P, Sen A, De Sarker D. Ethnobotany of Chanchal Block of Malda District of West Bengal (India): plants used in local healthcare. *Pleione.* 2014;8(2):381-90.
- Saha MR, Rai R, Kar P, Sen A, De Sarker D. Ethnobotany, traditional knowledge and socioeconomic importance of native drink among the Oraon tribe of Malda district in India. *J Intercult. Ethnopharmacol.* 2015;4(1):34-9.
- Pullaiah T. Medicinal Plants in Andhra Pradesh, India. New Delhi: Regency Publications. 2002.
- Das NJ, Saikia SP, Sarkar S, Devi K. Medicinal plants of North-Kamrup district of Assam used in primary healthcare system. *Ind. J Trad. Knowl.* 2006;5(4):489-93.
- Nair DD, Gupta AK, Das HB, Chakraborti A. Medicinal Plants of Tripura a Photo Descriptive Field Manual of 203 Medicinal Species. Medicinal Plants Board of Tripura and New Delhi: Concept Publishing Company. 2009.
- Gupta AK. The IUCN Red List of Threatened Species 2011: *Monochoria hastata*. e.T168865A6543142. <http://dx.doi.org/10.2305/IUCN.UK.2011-1.RLTS.T168865A6543142.en>.
- Chowdhury A, Das AP. Aquatic and semi-aquatic macrophytic diversity of the river Karala at Jalpaiguri, West Bengal, India and their growth form analysis. *Int. J Pharma. Bio. Sci.* 2013;4(4):1336-43.
- Mandal CK, Nandi NC. Distribution of Leech faunal diversity in fresh water wetlands of West Bengal and Tamil Nadu. *Proceedings of Taal, the 12<sup>th</sup> World Lake Conference; 2007.* In: Sengupta M., Dalwani, R, Editors. 2008;1831-39.
- Nivedithadevi D, Somasundaram R. Pharmacognostical and qualitative phytochemical studies on the aerial parts of *Tephrosia purpurea* L. *Int. J Res. Biol. Sci.* 2012;2(2):48-53.
- Kadiri AB, Agboola OM, Fashina FO. Ethnobotanical survey and phyto-anatomical studies of some common plants used for the treatment of epilepsy in some rural areas of South west Nigeria. *J Pharmacog. Phytochem.* 2013;2(4):175-82.
- Anonymous. Indian Herbal Pharmacopoeia (Volume 2). Delhi: Regional Research Laboratory (CSIR) Jammu tawi and Indian Drugs Manufacturer's Association. 1998.
- Azajudin, Saraf S. Evaluation of physicochemical and phytochemical properties of safoff-E-Sana, a Unani polyherbal formulation. *Pharmacog. Res.* 2010;2(5):318-22.
- Goswami P, Islam R, Khongmalai E, Ray Sarkar B, Sen S, Dey BK. Pharmacognostical and preliminary phytochemical screening of leave of *Polygonum muricatum*. *Int. J Res. Pharm. Pharmaceut. Sci.* 2017;2(3):06-10.
- Bele AA, Khale A. Standardization of herbal drugs, a review. *Int. J Pharm.* 2011;2(12):56-60.
- Pimenta AM, Montenegro MC, Ara Ujo AN, Mart'inez JC. Application of sequential injections analysis to pharmaceutical analysis. *J Pharmaceut. Biomed. Anal.* 2006;40(1):16-34.
- Burow M, Wittstock U, Gershenzon J. Sulfur-containing secondary metabolites and their role in plant defense. In: Hell R, Dahl C, Knaff D, Leustek T, Editors. *Sulfur Metabolism in Phototrophic Organisms*, AIPH. Springer. 2008;27:201-222.
- Kamble VM, Pawar SG. Characterization of *Eclipta prostrata* (L.) L. leaves by FTIR spectroscopy method, CHNS and ICP-MS analysis techniques. *Int. Res. J Biol. Sci.* 2007;6(2):30-35.
- Jasrotia S, Kansal A, Mehra A. Performance of aquatic plant species for phytoremediation of arsenic-contaminated water. *Appl. Wat. Sci.* 2017;7(2):889-96.
- Kulhari A, Sheorayan A, Bajar S, Sarkar S, Chaudhury A, Kali RK. *Spr. Pl.* 2013;2:676.
- Anonymous. Childhood Lead Poisoning. Geneva: World Health Organization. 2010.
- Anonymous. WHO Guidelines for Assessing Quality of Herbal Medicines with Reference to Contaminants and Residues. Geneva: World Health Organization. 2006.
- Tagg TR, Dajani AS, Wannamaker LW. Bacteriocin of Gram positive bacteria. *Bacter. Rev.* 1976;40(3):722-56.
- Gupta S. The Ayurvedic system of medicine occurring in Charka, Sushruta. New Delhi: Neeraj Publishing House. 1984:2.
- Yunus M, Yunus D. Systematic bark morphology of some tropical trees. *Bot. J Linn. Soc.* 1990;103(4):367-77.
- Mehlhorn H. Nature helps. How plants and other organisms contribute to solve health problems. Germany: Springer. 2011;12.
- Pizzolato P. Histochemical recognition of calcium oxalate. *J Histochem. Cytochem.* 1964;12(5):333-6.
- Evans WC. Trease and Evans' Pharmacognosy, 16th ed. London: Elsevier Ltd. 2009.
- Lala PK. Lab Manuals of Pharmacognosy, 5(10). Calcutta: CSI Publishers and Distributors. 1993;5:38-48.
- Ahmad RV, Sharma RK. Evaluation of drug for standardization. *Proceedings of WHO training cum-workshop.* Ghaziabad: Pharmaceutical lab for Indian medicine, MoHFW, Gol. 2001.
- Anonymous. The Indian Pharmacopoeia. Delhi: The controller of publication, CSIR. 1996;2(2).
- Anonymous. Indian Pharmacopoeia. Ghaziabad: The Indian Pharmacopoeia Commission, Central Indian Pharmacopoeia Laboratory, MoHFW, Gol. 2007;1.
- Mandal M, Misra D, Ghosh NN, Mandal V. Physicochemical and elemental studies of *Hydrocotyle javanica* Thunb. for standardization as, herbal drug. *Asi. Pac. J Trop. Biomed.* 2017;7(11):979-86.
- Freund JE. Modern Elementary Statistics. 3rd ed. New Delhi: Prentice Hall of Indian Private Limited. 1977.
- Singh S, Manchawal L, Chauhan MG. Pharmacognostic study of male leaves of *Trichosanthes dioica* Roxb. with special emphasis on microscopic technique. *J Pharmaceut. Phytother.* 2010;2(5):71-5.

44. Kunle OF, Egharevba HO, Ahmadu PO. Standardization of herbal medicines - A review. *Int. J Biodiver.Conserv.* 2012;4(3):101-12.
45. Reddy SL, Fayazuddin M, Adeel A, Reddy N, Rao PS, Reddy GS, *et al.* Characterization of bringaraj and guduchi herbs by ICP-MS analysis, optical absorption and EPR spectroscopic methods. *Spectrochim. Act. Part A: Mol. Biomol. Spect.* 2008;71(1):31-38.
46. Anonymous. 2007. [http://www.qcin.org/documents/Certification\\_Scheme\\_AYUSH/](http://www.qcin.org/documents/Certification_Scheme_AYUSH/).
47. Panigrahi S. Groundwater Arsenic Contamination: A Study of Malda District of West Bengal, India. *Imp. J Interdiscip. Res.* 2016;2(8):742-7.

### GRAPHICAL ABSTRACT



### SUMMARY

- *M. hastata* (L.) Solms is an evergreen perennial aquatic herb growing in wide geographical range. Though the plant has been used by certain ethnic communities in Asia, but the characterization of active principles have not been done so far. The present research article focuses on the evaluation of pharmacognostic identity and quality parameters to be used as herbal drug and thus validating its ethno-medicinal use to cure boils, gastritis, hepatopathy and as laxative.

### ABOUT AUTHORS

**Debabrata Misra** is a Ph. D. Research Scholar at the Plant and Microbial Physiology and Biochemistry Laboratory, Department of Botany, University of Gour Banga, Malda in West Bengal. He is currently working on purification and characterization of antibacterial compounds from folklore aquatic plant for their use in gastrointestinal micro flora.

**Manab Mandal** is a Ph. D. Research Scholar at the Plant and Microbial Physiology and Biochemistry Laboratory, Department of Botany, University of Gour Banga, Malda. He is currently engaged in the research on high altitude plant resource as antibacterial agents.

**Narendra Nath Ghosh** is a Ph. D. Research Scholar at the Department of Chemistry, University of Gour Banga, Malda. His research focuses on the theoretical and computational chemistry of fluorescent dyes.

**Vivekananda Mandal**, Professor of Department of Botany, University of Gour Banga, Malda has more than ten years experience in the area of probiotics and antibiotic research. Dr Mandal had published more than 50 research papers in national and international journals and conferences.

**Cite this article:** Misra D, Mandal M, Ghosh NN, Mandal V. Pharmacognostic Standardization of an Ethnomedicinal Aquatic Herb, *Monochoria hastata* (L.) Solms for its Antibacterial Potentiality. *Pharmacog J.* 2018;10(3):533-40.