Scientific standardization of leaves of *Chenopodium album* L.

Mohit Kumar Pandey, Alok Kumar, Ravindra Singh and Manoj Tripathi

Abstract

India has a rich culture of medicinal herbs and spices, which includes Ayurvedic, Unani, Siddha and other traditional medicines but only very few have been studied chemically and pharmacognostically evaluated for their potential medicinal value. According to the World Health Organization, most populations still rely on traditional medicines for their psychological and physical health requirements. *Chenopodium album* L. (family Chenopodiaceae) belongs to the genus *Chenopodium*. It is also known as Fat-hen, Bathua, Vastukah, Chakvit. Bathua is a very famous herb used in splenic disorders, dysentery, bleeding piles and intrinsic hemorrhages. The result of the physic-chemical parameters viz. loss on drying (9.2%), ethanol soluble extractive value (6%), water soluble extractive value (40%), total ash value (13.6) and acid insoluble ash value (6.40%). The present paper provides a detailed account of the scientific evaluation of *Chenopodium album* L. leaves. The study includes macro and microscopic characters, powder microscopic characteristics, HPTLC fingerprinting, preliminary phyto -chemical screening and physicochemical parameters. The information generated by this particulars study provides relevant pharmacognostical and physicochemical data needed for proper identification and authentication of *Chenopodium album* leaves.

Keywords: *Chenopodium album*, Scientific evaluation, HPTLC fingerprints profile, Physico-chemical, Preliminary phyto-chemical evaluation.

1. Introduction

India is a rich source of plant and animal wealth, which is due to its varied geographical and agro-climatic regions. Besides it's varied biodiversity, it has a diverse cultural heritage. Though at present Indian health care system consists of both traditional and modern systems of medicines, traditional systems of medicine like Ayurveda, Siddha and Unani and unorganized systems like folk medicine have been flourishing well (Nedialkova et al. 2009, Agarwal et al. 2005). Ayurveda and Siddha are of Indian origin and accounted for about 60% health care system in general and 75% of rural Indian population. *Chenopodium album* (L.) of the family Chenopodiaceae belongs to the genus *Chenopodium*. It is also known as Fat-hen, Bathua, Vastukah, Chakvit. It is a polymorphous, mealy white and erect herb which is 3.5m in height. Leaves - simple, very variable, oblong or lanceolate, obtuse or acute, entire, stems - erect of ascending, often striped, flowers - clusters in spikes, fruits -membranous utricle, enclosed in the perianth, seed – smooth, shiny, compressed. Medicinally, this plant has been used to treat various symptoms attributable to nutritional deficiencies and found wild in altitude of 4,700m (Nishteswar and Hemadri 2005) [14]. The herb is a common weed during summer and winter in various symptoms attributable to nutritional deficiencies and found wield in altitude of 4,700m. It is also cultivated as a traditional leafy vegetable. Bathua is a very famous herb used in splenic disorders, dysentery, bleeding piles and intrinsic hemorrhages. Useful in vitiated conditions of *pitta* (cough). A tea prepared from green or dry leaves, used to relieve stomach pain. Consumption of plant as a vegetable with goat milk, help in bleeding piles. etc. (Panda 2005, Pramila 2006, Khare 2007, Singh 2007, Pande & Pathak 2010, Hussain et al. 2009, Patwardhan et al. 2004). The purpose of this standardization involves the safe, proper selection and handling of crude materials, ensure quality, safety, efficacy and stability of finished products. And another there are no reports of systematic pharmacognostic study of leaf of this plant. Keeping this aim into consideration, the present study was designed to scientific evaluation of Bathua leaves. The study includes macro and microscopic characters, powder microscopic characteristics, HPTLC fingerprinting, preliminary phytochemical screening and physicochemical parameters. The information generated by this particulars study provides relevant pharmacognostical and physicochemical data needed for proper identification and authentication of Bathua leaves.

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2. Materials and Methods
2.1 Collection and processing of plant material
The fresh plant leaves of Chenopodium album was collected from, Chitrakoot in the month of January. Samples were authenticated (Jain 1991, Kirtikar and Basu 1935, Verma et al.1993) [5, 11, 21] by taxonomist of Ayurveda Sadan (Research Laboratory), Deendayal Research Institute Chitrakoot. The voucher specimen (AD/AS/126/2016) prepared as per standard procedure (Jain and Rao 1977) and maintained in the herbarium of Department of Pharmacognosy, Ayurveda Sadan, (Research Laboratory, Deendayal Research Institute Chitrakoot for further reference. Fresh material was used for anatomical studies whereas shade dried material was powdered in electric grinder for physico-chemical study, phytochemical investigation and development of HPTLC fingerprint profile.

2.2 Macroscopic study
Macroscopic or organoleptic characters like appearance, colour, odour and taste were evaluated.

2.3 Microscopic study
Fresh stamen section was cut by free hand sectioning and numerous sections examined microscopically (Brain and Turner 1975 and Kokate 2006) [5, 12]. Photographs of the microscopical sections were captured with the help of Olympus trinocular research microscope CX-21lf with Digieye camera using Caliper plus version 4.2 software.

2.4 Powder microscopic study
The dried leaves was subjected to powdered and completely passes through 355 μm IS Sieve (old sieve number 44) and not less than 50% pass on through 180 μm IS Sieve (old sieve number 85). About 2 g of powder washed thoroughly with potable water, pour out the water without loss of material. Mounted a small portion in glycerin, warmed a few mg with chloral hydrate solution, wash and mounted in glycerin, treat a few mg with iodine solution and mount in glycerin, about 1 g of powder warmed over water bath with 50% con. Nitric acid till brown fumes appear, cool and wash with water thoroughly and mount a small portion in glycerin and seen under microscope at 40X x 10X magnification of the Trinocular Research Microscope (Evans 2003) [7].

2.5 Physico-chemical parameters
Physico-chemical parameters such as moisture content (loss on drying at 105 °C), water soluble extractive value, alcohol soluble extractive value, total ash value, acid insoluble ash value and water soluble ash were calculated (Anonymous 2000 and Anonymous 2007) [1, 2].

2.6 Fluorescence Studies
The fluorescence response of powdered drugs exposed to UV radiation (254 nm and 366nm wavelength) was studies using the standard procedure.

2.7 Preliminary phytochemical studies
Preliminary tests were carried out on ethanolic and water extract for the presence/absence of phyto-constituents like alkaloids, flavonoids, steroids, terpenoids, tannins, resins, carbohydrates, proteins and saponins (Tripathi and Sikarwar 2015, Tripathi et al. 2015) [21, 22].

2.8 High Performance Thin Layer
2.8.1 Chromatography (HPTLC)
For HPTLC, the powdered leaves 5 gm of sample was extracted with 100 ml of ethanol overnight, filtered and concentrated. It was applied by spotting extracted sample on precoated silica-gel aluminium plate 60 F254 (5x10 cm with 0.2 mm layer thickness Merk Germany) using Camag Linomat -5 sample applicator and a 100 μl Hamilton syringe. The samples, in the form of bands of length 6 mm, were spotted 15 mm from the bottom, 15 mm from left margin of the plate and 10 mm part. Plates were developed using mobile phase consisting of Hexane: Ethyl acetate (7:3 v/v). Linear ascending development was carried out in 10x10cm twin through glass chamber equilibrated with mobile phase. The optimized chamber saturation time for mobile phase was 30 min. at room temperature. The length of chromatogram run was 8 cm. 20 ml of the mobile phase. Subsequent to the development, TLC plates was dried with the help of Hot Air Oven. The peak area for samples and standard were recorded with Camera photo documentation system Camag Reprostar 3. Visualization of spot was made before and after derivatization (with 5% Vanillin - sulphuric acid reagent) at 254nm, 366nm and day light with Win cat software and Rf values noted (Ansari 2006, Choudhary et al. 2014). [1, 6]

3. Results and Discussion
3.1 Macroscopic characters
Chenopodium album is a polymorphous erect herb which is 3.5m in height Chenopodium album leaves powder colour is green, odour pleasant and taste sweet (Fig.1&2).

3.2 Microscopic characters
Transverse section of the leaf passing through the midrib. Detailed TS shows upper and lower epidermis of the the midrib and lamina covered with thin cuticle, the cells of the upper epidermis being bigger in size and bear plenty of simple and covering straight or bent short and long trichome, Stomata traversed throughout both the epidermiti being more on the lower side, a row of palisade runs underneath the upper epidermis of the lamina, discontinuous over the midrib, Midrib lies 2 to 3 rows of the collenchymatous tissue, the remaining cells of the ground tissue being parenchymatous, embedded with an arc of centrally located 4 vascular bundlesbeing located at upper side (fig.3).

3.3 Powder microscopic characters
Under microscope examined powder shows spiral, reticulate and annular thickening, sclereids, group of stone cells, group of fibres, pitted parenchymatous cells, Parenchyma filled with rosette crystals and cluster crystals of calcium (Fig. 4 to 11).

3.4 Physico-chemical analysis
The physico-chemical parameters such as extractive values are useful for the determination of exhausted or adulterated drug; ash values of the drug gave an idea of the earthy matter or the inorganic composition and other impurities present along with the drug. Physico-chemical results of the drug are given in (Table 1).

3.5 Preliminary phyto-chemical investigation
Qualitative phyto-constituents were screened in the extracts taken in water and ethyl alcohol. The screening exhibited presence of protein, carbohydrate, Alkaloid, Tannin, Flavonoid, and Saponin.
3.6 Fluorescence study
Fluorescence study was made and given in Table 2.

3.7 HPTLC finger print profile
High performance thin layer chromatography (HPTLC) study of the ethanolic extract two spots of the sample extracts applied in the TLC plate. Major spots R\textsubscript{f} values with colour were recorded under 254nm, 366nm, after derivatization 366nm. Chromatogram profile and R\textsubscript{f} values are given (Fig. 12, 13, 14 & Table 3). The macroscopic, microscopic and powder microscopic distinguished characters have been established to identify Chenopodium album Leaf. The pharmacognostic and physicochemical parameters can be used for checking the adulteration and purity of this drug. HPTLC finger print profile helps in identification of various phytochemical constituents present in the crude drug thereby substantiating and authenticating of crude drug. The TLC profile also helps to identify and isolate’s important phyto constituents. These finding could be helpful in identification and authentication.

Table 1: Physico-chemical analysis of the Chenopodium album-leaves

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Parameters</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Foreign matter</td>
<td>2%</td>
</tr>
<tr>
<td>2</td>
<td>Loss on drying at 105 °C</td>
<td>9.4%</td>
</tr>
<tr>
<td>3</td>
<td>Ethanol-soluble extractive</td>
<td>6%</td>
</tr>
<tr>
<td>4</td>
<td>Water-soluble extractive</td>
<td>40%</td>
</tr>
<tr>
<td>5</td>
<td>Total ash</td>
<td>13.6%</td>
</tr>
<tr>
<td>6</td>
<td>Acid-insoluble ash</td>
<td>6.40%</td>
</tr>
</tbody>
</table>

Table 2: Fluorescence study of Chenopodium album-leaves

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Powder + Chemical</th>
<th>Observation in day light</th>
<th>Observation in 366nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Powder</td>
<td>Green</td>
<td>Greenish yellow</td>
</tr>
<tr>
<td>2</td>
<td>Powder + 1N HCL</td>
<td>Greenish brown</td>
<td>Brown</td>
</tr>
<tr>
<td>3</td>
<td>Powder + 1N NaOH(water)</td>
<td>Green</td>
<td>Yellowish green</td>
</tr>
<tr>
<td>4</td>
<td>Powder + 1N NaOH(methanol)</td>
<td>Green</td>
<td>Dark green</td>
</tr>
<tr>
<td>5</td>
<td>Powder + 50% KOH</td>
<td>Green</td>
<td>Brown</td>
</tr>
<tr>
<td>6</td>
<td>Powder + 50% H2SO4</td>
<td>Green</td>
<td>Dark green</td>
</tr>
<tr>
<td>7</td>
<td>Powder + 50% HNO3</td>
<td>Greenish yellow</td>
<td>Dark brown</td>
</tr>
<tr>
<td>8</td>
<td>Powder + Acetic acid</td>
<td>Green</td>
<td>Yellowish green</td>
</tr>
<tr>
<td>9</td>
<td>Powder + Iodine water</td>
<td>Green</td>
<td>Green</td>
</tr>
<tr>
<td>10</td>
<td>Powder + Distilled water</td>
<td>Green</td>
<td>Green</td>
</tr>
<tr>
<td>11</td>
<td>Powder + Con. H2SO4</td>
<td>Black</td>
<td>Dark brown</td>
</tr>
<tr>
<td>12</td>
<td>Powder + Dil. HCL</td>
<td>Brownish green</td>
<td>Reddish yellow</td>
</tr>
<tr>
<td>13</td>
<td>Powder + Dil. NH3</td>
<td>Dark green</td>
<td>Yellowish green</td>
</tr>
<tr>
<td>14</td>
<td>Powder + Con. NH3</td>
<td>Light green</td>
<td>Greenish yellow</td>
</tr>
</tbody>
</table>

Table 3: R\textsubscript{f} value of HPTLC fingerprints profile of Chenopodium album-leaves

<table>
<thead>
<tr>
<th>R\textsubscript{f} values</th>
<th>Before derivatization</th>
<th>After derivatization</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>At 254nm</td>
<td>At 366nm</td>
</tr>
<tr>
<td>R1</td>
<td>0.21(black)</td>
<td>0.10</td>
</tr>
<tr>
<td>R2</td>
<td>0.39(black)</td>
<td>0.20</td>
</tr>
<tr>
<td>R3</td>
<td>-</td>
<td>0.30</td>
</tr>
<tr>
<td>R4</td>
<td>-</td>
<td>0.40</td>
</tr>
<tr>
<td>R5</td>
<td>-</td>
<td>0.50</td>
</tr>
<tr>
<td>R6</td>
<td>-</td>
<td>0.65</td>
</tr>
<tr>
<td>R7</td>
<td>-</td>
<td>0.75</td>
</tr>
<tr>
<td>R8</td>
<td>-</td>
<td>0.85</td>
</tr>
<tr>
<td>R9</td>
<td>-</td>
<td>0.92</td>
</tr>
</tbody>
</table>

Fig 1: whole plant
Fig 2: Leaves
Fig 3: TS leaf

Fig 4: Spiral & reticulate thickening

Fig 5: Spiral thickening

Fig 6: Annular thickening

Fig 7: Sclereids

Fig 8: Group of stone cells

Fig 9: Fibres

Fig 10: Pitted parenchyma

Fig 11: Parenchyma filled with rosette crystals and cluster crystals of calcium
4. Conclusion
The macroscopic, microscopic and powder microscopic diagnostic features have been established to identify *Chenopodium album* Linn. leaf. The pharmacognostic and physicochemical parameters can be used for checking the adulteration and purity of this drug. The extensive literature survey revealed that *Chenopodium album* is an important medicinal plants with diverse pharmacological spectrum. The plant shows the presence of many chemical constituents. It is known as a rich source of vitamins and iron, zink, flavonoids and glycosides present in *Chenopodium album* might be medicinally important and nutritionally valuable. The plant is rich in carbohydrates, oleic and stearic. HPTLC finger print profile helps in identification of various phytochemical constituents present in the crude drug thereby substantiating and authenticating of crude drug. The HPTLC fingerprints profile also helps to identify important phyto-constituents. These finding could be helpful in identification and authentication.

5. Acknowledgement
The authors are grateful to the Organizing Secretary Deendayal Research Institute, Chitrakoot, Satna (M.P.) for providing necessary facilities.

6. References