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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 spleenic 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 imnsoluble 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) [15, 4]. 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 wield in altitude of 4,700m (Nishteswar and Hemadri 2005) [14]. The herb is a common weed during summer and winter in waste places and in the field of wheat, barley, mustard and gram, and reduces their yield. In India, it is also cultivated as a traditional leafy vegetable. Bathua is a very famous herb used in spleenic 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) [16, 17, 13, 20, 19, 8, 18]. 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.

2. Materials and Methods

2.1 Collection and processing of plant material

The fresh plant leaves of *Chenopodium album* was collected from the, Chitrakoot in the month of January. Samples were authenticated (Jain 1991, Kirtikar and Basu 1935, Verma *et al*.1993) [9, 11, 23] 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- 21*l* 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 ^{0}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 F_{254} (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 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 R_f values noted (Ansari 2006, Choudhary *et al.* 2014). [3, 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 epidermii 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_f values with colour were recorded under, 254nm, 366nm, after derivatization 366nm. Chromatogram profile and R_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

S. No.	Parameters	Values
	Foreign matter	2%
1	Loss on drying at 105 ^{0}C	9.4%
2	Ethanol-soluble extractive	6%
3	Water- soluble extractive	40%
4	Total ash	13.6%
5	Acid-insoluble ash	6.40%

Table 2: Fluorescence study of *Chenopodium album*-leaves

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

Table 3: R_f value of HPTLC fingerprints profile of *Chenopodium album*-leaves

R_f values	Before derivatization		After derivatization
	At 254nm	At 366nm	At 366nm
R_f1	0.21(black)	0.10 (red)	0.08 (pink)
R_f2	0.39(black)	0.20 (red)	0.10 (sky blue)
R_f3	-	0.30 (red)	0.20 (pink)
R_f4	-	0.40 (red)	0.30 (pink)
R_f5	-	0.50 (red)	0.40 (pink)
R_f6	-	0.65 (red)	0.50 (pink)
R_f7	-	0.75 (red)	0.60 (sky blue)
R_f8	-	0.85 (red)	0.85 (pink)
R_f9	-	0.92 (red)	0.92 (pink)

Fig 1: whole plant

Fig 2: Leaves

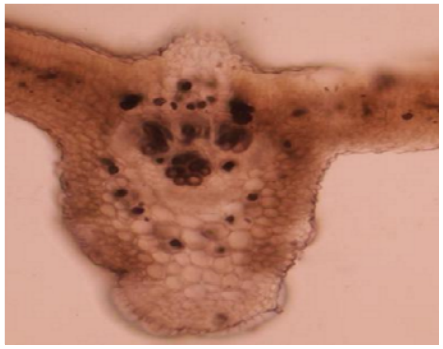


Fig 3: TS leaf

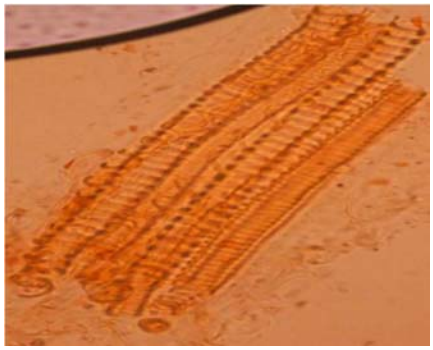


Fig 4: Spiral & reticulate thickening

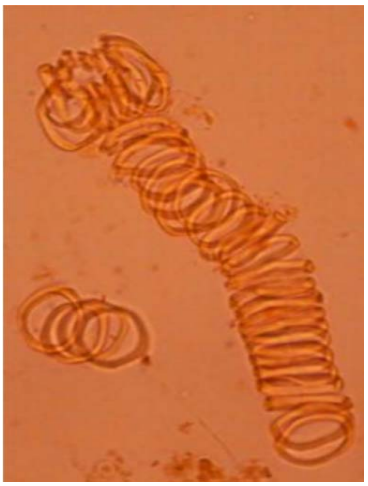


Fig 5: Spiral thickening

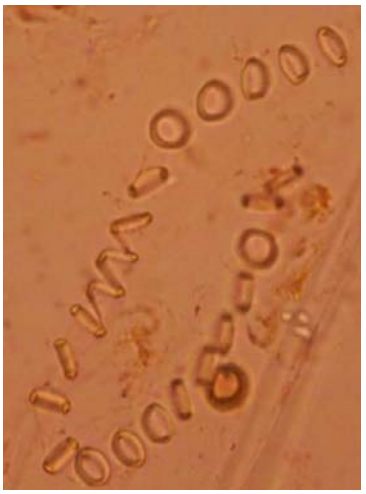


Fig 6: Annular thickening

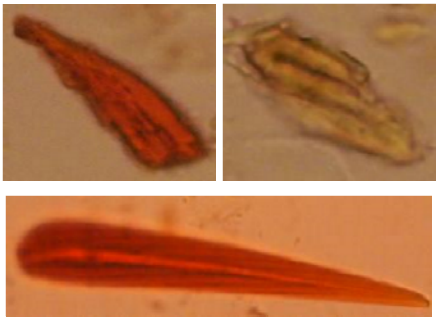


Fig 7: Sclereids

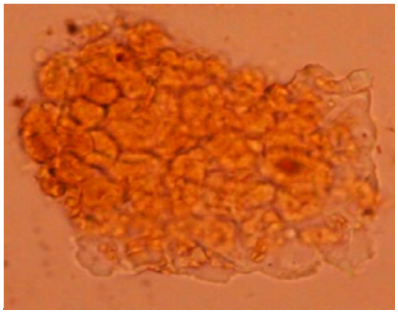


Fig 8: Group of stone cells

Fig 9: Fibres

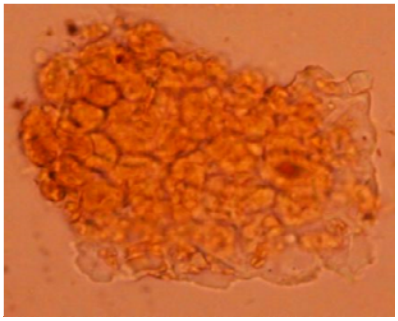


Fig 10: Pitted parenchyma

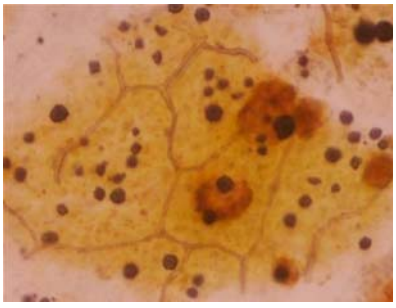
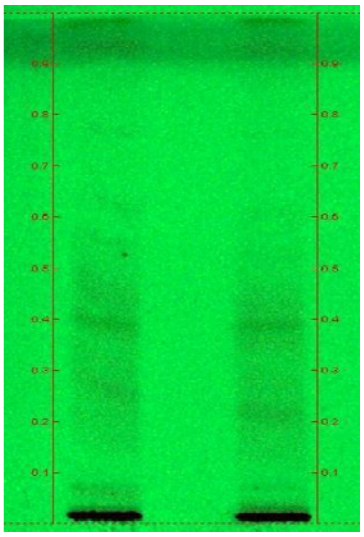
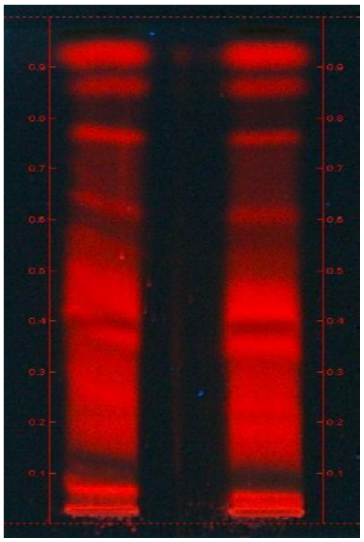


Fig 11: Parenchyma filled with rosette crystals and cluster crystals of calcium



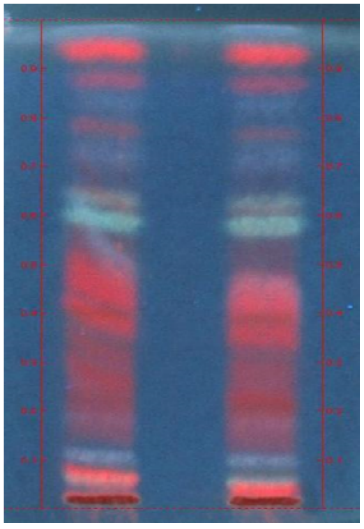
A B

Fig 12: HPTLC fingerprint profile at 254 nm



A B

Fig 13: HPTLC fingerprint profile at 366 nm



A B

Fig 14: HPTLC fingerprint profile at 366 mm after derivatization

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.

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