



Research Article

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Phytochemical screening and TLC studies of different extracts of *Chenopodium album*

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ABSTRACT

Chenopodium album (*C. album*), extensively exploit in folk medicine in various parts of world. The plant possesses various therapeutic properties such as antiphlogistic, mildly laxative, anthelmintic, odontalgic properties and antirheumatic. In the present research, Histological examination, phytochemical screening and Thin layer chromatographic identification of leaf and extracts has been studied extensively and provided diagnostic key to detect the presence of adulterant. It is concluded that our study provides the data which helps to isolate and characterize the different medicinal potential of the *C. album* such as diuretic, antiparasitic, hepato-protective, laxative and sedative.

Keywords: *Chenopodium album*, Morphology, Microscopy, Physicochemical, Phytochemical, TLC.

INTRODUCTION

Chenopodium album L., (*C. album*) commonly known as Bathua is having family Chenopodiaceae. The leaves possess various therapeutic properties such as antiphlogistic, mildly laxative, anthelmintic, odontalgic properties and antirheumatic [1]. Moreover, in rheumatism, its aerial parts with alcohol were used in the form of decoction [2]. Different phytoconstituents such as flavonoids [3,4], apocarotenoids and Cinnamic acids amides [5] has been isolated from plants. Flavonoid, polyphenol and flavonone present in from *C. album* have reported to possess significant antioxidant potential, anti-inflammatory and NF kappa B inhibition potential responsible for antirheumatic activity [6]. In this study the attempt was made to establish the Histological examination, phytochemical screening and Thin layer chromatographic identification of leaf and its extracts respectively.

MATERIAL AND METHODS

Identification and authentication of plant

The aerial parts of *C. album* Linn. was collected locally and taxonomic authentication was done by the Botany Department, Rashtrasant Tukadoji Maharaj Nagpur University, Nagpur. A voucher specimen of the plant material has been preserved and deposited with specimen number RA 9576 in the Herbarium of Botany Department for future reference.

Morphological and microscopical evaluation

The fresh leaves and stem of the *C. album* were procured and examined for various morphological characters such as colour, taste and odour of leaves. The other external characters like venation, surface, base, margin, size and shape of leaves were also studied. The microscopical examination of *C. album* was done with the help of Motic Image plus 2.0 microscope. The air-dried plant material was then, pulverized into a coarse powder and used for research work.

Determination of Physicochemical constants

Physico-chemical constants of *C. album* aerial parts were determined for Ash and Extractive value as per the method described in Pharmacopoeias and reported previously [7].

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Preparation of extract

Aerial parts were dried and milled to a coarse powder. One kg of fresh plant material was grounded and defatted using petroleum ether. Defatted material is then extracted subsequently with chloroform, ethyl acetate, acetone and methanol in a Soxhlet apparatus followed by maceration with 50 % methanol for 7 days. The organic solvents were evaporated by rotary vacuum evaporator to get the chloroform extract (CHCA), ethyl acetate extract (EACA), acetone extract (ACCA), methanolic extract (MECA) and 50 % methanolic extract (HACA) respectively. Phytochemical and TLC studies of these extracts were undertaken.

Phytochemical Screening

The Phytochemical study of the extracts were assessed to detect the occurrence of different phytoconstituents such as alkaloids, flavanoids, saponins, triterpenoids, steroids, carbohydrate, tannin, coumarins, phenols, carboxylic acid, amino acid and proteins by performing chemical tests described previously [8,9].

TLC of extracts

One gram of extracts were dissolved in methanol, filtered and utilised for TLC studies. About Six µl of extracts of *C. album* in 6 mm of bandwidth were applied on aluminium plates pre-coated with silica gel G F254 using CAMAG Linomat 4 (Muttenez, Switzerland) TLC applicator [10]. The plate was developed in different solvent system in CAMAG twin trough chamber. Visualisation and documentation of developed plates were done in short and long UV using CAMAG Photodocumentation unit [11].

RESULTS AND DISCUSSION

Morphological and microscopical evaluation

The fresh leaves and stem of the *C. album* were procured and examined for various morphological characters such as colour, taste and odour of leaves. The other external characters like venation, surface, base, margin, size and shape of leaves were also studied. The following morphological and microscopical characters were observed in the plant.

Morphology of *C. album* aerial parts

The morphological data showed the colour of leaves was found to be dark green with smooth undersurface. The shape of leaves was extremely variable as simple, deltoid to lanceolate, upper entire, rhomboid, lower toothed or irregularly lobed. The size is about 11-14 cm long; petioles were of 1 to 1.3 cm in length and as long as thick blade, lanceolate to oblong. Its length varies from 9 to 4.5 cm broad having dentate margin.

Microscopical features of *C. album* aerial parts

The microscopical study of *C. album* stem and leaf was done with the help of Motic Image plus 2.0 microscope.

Stem: Transverse section of mature stem shows a well-built periderm containing 8 to 11 coatings of tangentially extended cork cells, 3 to 5 covering of phelloderm and cortex were having parenchymatous cells. These parenchymatous cells showed the group of thick walled pericyclic and lignified phloem fibres with ample starch grains and

sclerocymatous tissues. Endodermis is indistinct, steller region consist of rings of vascular bundles encircled by pericyclic fibers (Figure 1).

Leaf: The transverse section of the leaves shows that *C. album* leaf is a dorsiventral leaf with palisade cells towards the upper epidermal layer. The palisade cells are long and cover nearly half portion of the leaflet. Upper epidermis showed the presence of thick walled parenchymatous cells whereas in lower epidermis the cells were replaced by round thin walled collenchymas. Collenchymatous cells are present at the mid rib region below the upper epidermis interrupting the palisade layer. Thick walled parenchymatous cells showed the presence of spiral and annular vessels near the vascular bundles. The upper epidermis consists of cells that are flattened while the lower epidermal cells are rounded. The vascular bundles are arranged in the midrib region below the collenchymatous cells as like the bunch of grapes. Xylem vessels are 12-15 in number (Figure 2).

Determination of Physicochemical constants

The substance left behind after ignition of the crude drug is called as ash. The ash and acid insoluble ash value was found to be 11.56 % and 0.55 % respectively. It varies within fixed limits conferring to the soils. It may also contain inorganic impurities mixed as deliberate type of adulteration. The total ash usually consists mainly of carbonates, phosphates, silicates and silica. The acid insoluble ash indicates the amount of total ash insoluble in dilute hydrochloric acid. Its higher limits reflect contamination with the earthy material. The alcohol soluble and water soluble extractive value was found to be 6.67 % and 29.36 % respectively representing the existence of polar phytoconstituents [12]. The extractive values are indicative of approximate measures of their chemical constituents, these values are used to determine the quality of drugs since many times, drugs are identified as of substandard quality due to either faulty collection or improper storage.

Extraction of plant material

The air dried plant part was defatted using petroleum ether and the subsequent material was then extracted successively with solvents of increasing polarity viz. chloroform, ethyl acetate, acetone, methanol and hydro-ethanol (1:1) to obtain the nonpolar, semipolar and polar constituents of the plants. The appearance and yield of different extracts were mentioned in table 1. The maximum yield was found as 13.58 % and 12.56 % in MECA and HACA extracts respectively. These findings are in accordance with the results of extractive value determination. The extracts obtained are indicative of approximate measures of their chemical constituents by exhausting plant materials with specific solvents. In absence of appropriate assay method, this specific method is used as estimation parameter. [13]

Preliminary phytochemical screening of extracts

Preliminary phytochemical investigations of extracts were assessed to reveal the presence of different secondary metabolites. Petroleum ether extract (PECA) extract revealed the presence of steroids and triterpenoids; CHCA extract revealed the presence of triterpenoids; EACA extract revealed the presence of triterpenoids, carbohydrates and flavonoids; ACCA showed the presence of flavonoids, tannins and carbohydrates. Whereas MECA and HACA extracts indicated the presence of flavonoids, carbohydrates, saponins, proteins, alkaloids and tannins respectively (Table 2).

Table 1: Yield of extracts obtained from successive extraction of aerial parts of *Chenopodium album*

Plant	Type of Extract	Appearance/ State	Yield (% w/w)
<i>Chenopodium album</i> aerial parts	PECA	Dark Brown / Semisolid	1.7
	CHCA	Dark Brown-black/ Semisolid	2.0
	EACA	Dark Brown-black/ Semisolid	3.9
	ACCA	Dark Brown-black/ Semisolid	4.79
	MECA	Dark Brown-black/ Semisolid	13.58
	HACA	Dark Brown-black/ Semisolid	12.76

PECA, Petroleum ether extract of *C. album*; CHCA, chloroform extract of *C. album*; EACA, ethyl acetate extract of *C. album*; ACCA, acetone extract of *C. album*; MECA, methanolic extract of *C. album*; HACA, hydroalcoholic extract of *C. album*

Table 2: Preliminary phytochemical screening of extracts of aerial parts of *Chenopodium album*

Chemical tests	<i>Chenopodium album</i> aerial parts extracts					
	Name of the extracts					
	PECA	CHCA	EACA	ACCA	MECA	HACA
Proteins & Amino acid	-	-	-	+	+	+
Carbohydrate	-	-	-	-	+	+
Sterol	+	+	-	-	-	-
Terpenoids	+	+	-	-	-	-
Saponin	-	-	-	-	+	+
Flavonoid	-	-	+	+	+	+
Alkaloid	-	-	-	-	+	+
Tannin	-	-	+	+	+	+

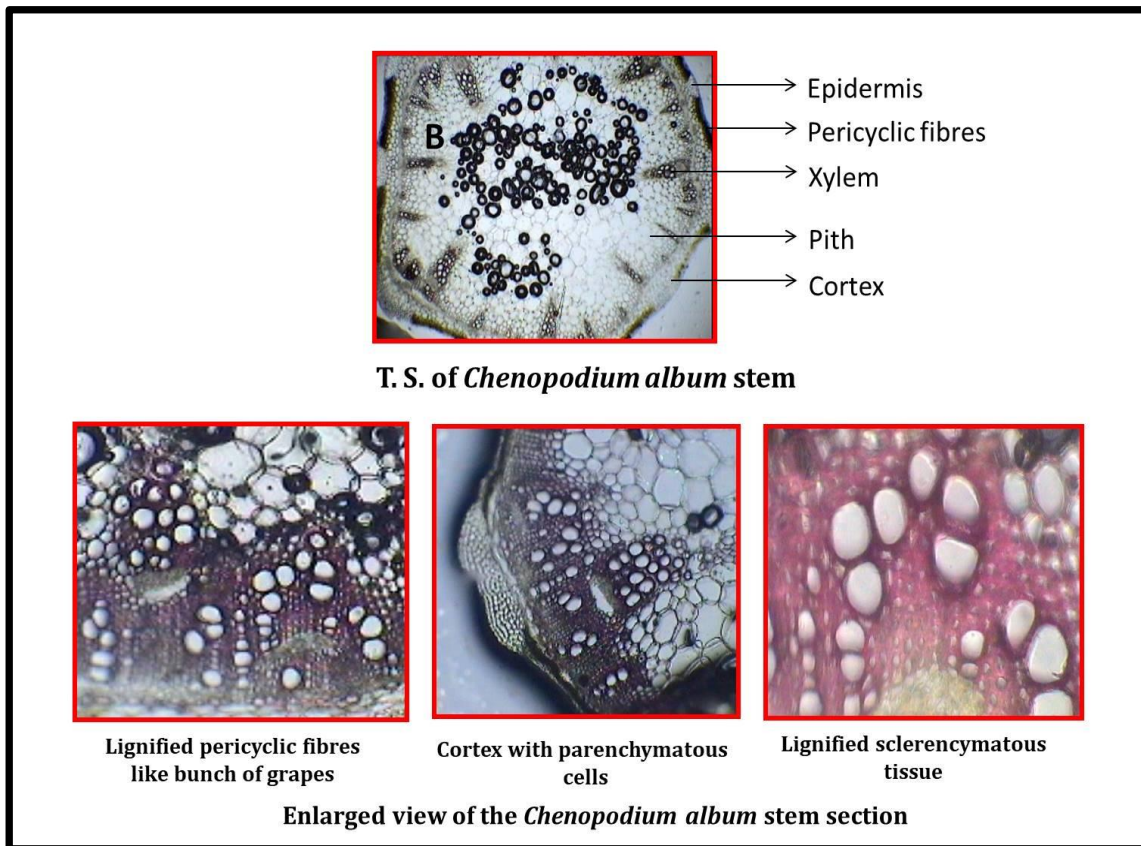
+ indicates present and – indicates absent

PECA, Petroleum ether extract of *C. album*; CHCA, chloroform extract of *C. album*; EACA, ethyl acetate extract of *C. album*; ACCA, acetone extract of *C. album*; MECA, methanolic extract of *C. album*; HACA, hydroalcoholic extract of *C. album*

Table 3: Mobile phase for TLC studies of extracts of *Chenopodium album* plant

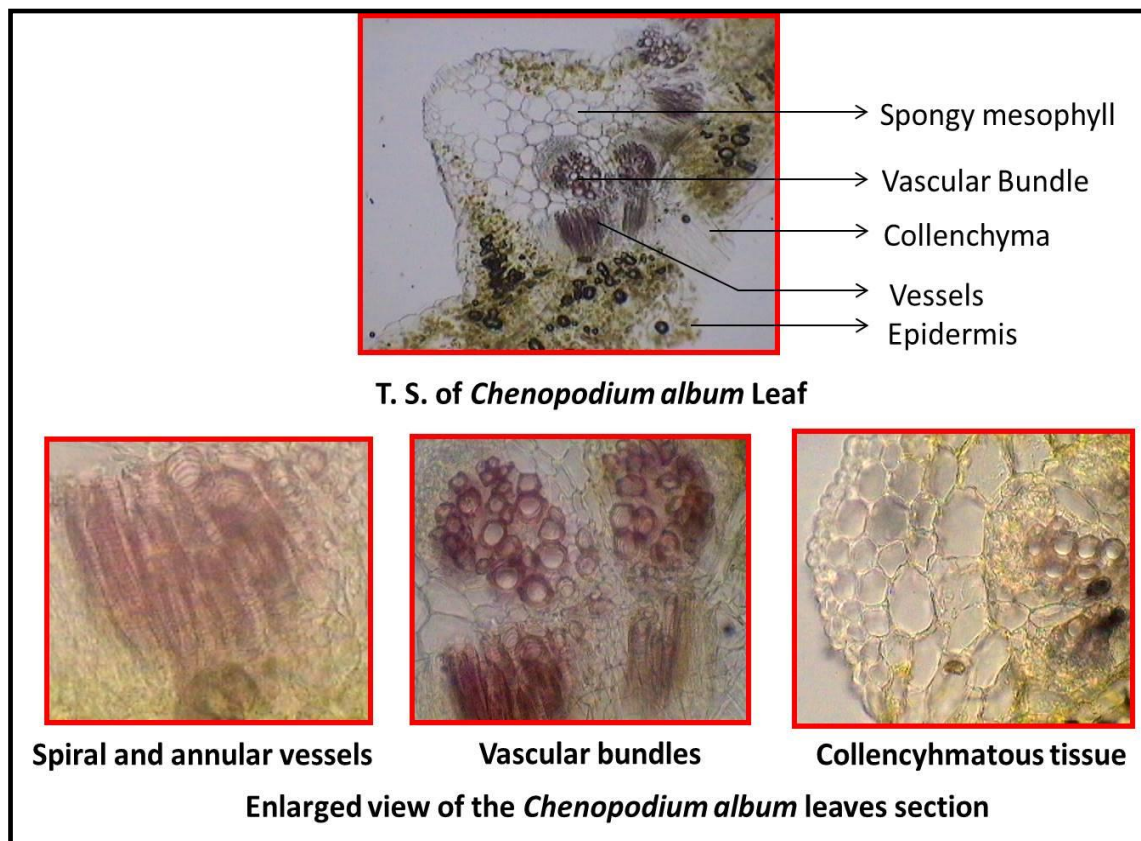
Sr.no.	Test extract	Solvent system	Number of Bands
01	PECA	Toluene: Chloroform (1:1)	05
02	CHCA	Toluene: Methanol (9:1)	07
03	EACA	Ethyl Acetate : Methanol (8:2)	06
04	ACCA	Ethyl Acetate : Methanol (7.5:2.5)	07
05	MECA	Ethyl Acetate: Methanol: Triethylamine (7.5:2.5:0.5)	07
06	HACA	Ethyl Acetate: Methanol: Glacial acetic acid (7:2.2:0.8)	10

PECA, Petroleum ether extract of *C. album*; CHCA, chloroform extract of *C. album*; EACA, ethyl acetate extract of *C. album*; ACCA, acetone extract of *C. album*; MECA, methanolic extract of *C. album*; HACA, hydroalcoholic extract of *C. album*



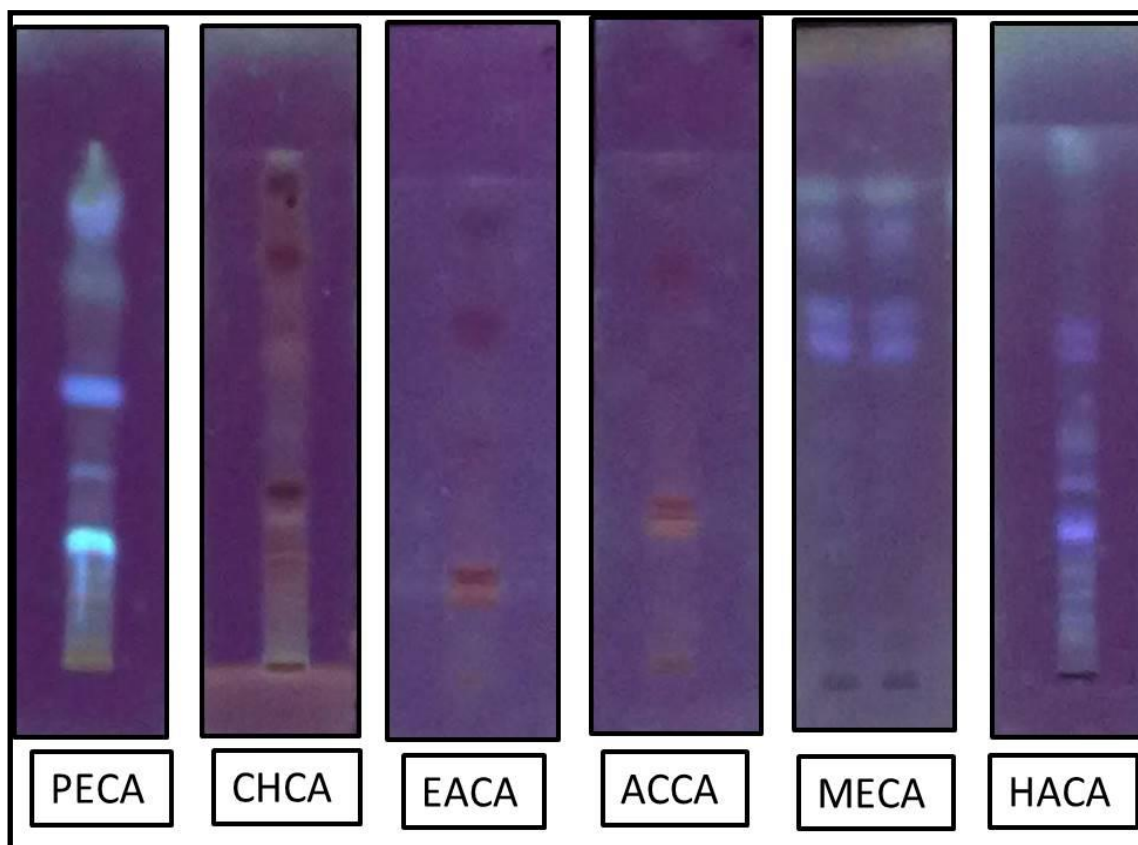
Microscopical study of *C. album* stem indicating presence of characteristic features as lignified pericyclic fibres like bunch of grapes and lignified Sclerenchymatous tissue

Figure 1: Microscopical study of *Chenopodium album* stem



Microscopical features of *C. album* leaf indicating presence of characteristic features as spiral and annular vessels.

Figure 2: Microscopical study of *Chenopodium album* leaf



TLC Photodocumentation of PECA, Petroleum ether extract of *C. album*; CHCA, chloroform extract of *C. album*; EACA, ethyl acetate extract of *C. album*; ACCA, acetone extract of *C. album*; MECA, methanolic extract of *C. album*; HACA, hydroalcoholic extract of *C. album* observed under long UV at 366 nm

Figure 3: TLC study of *Chenopodium album* leaf extracts

TLC studies of extracts

The extracts were undertaken for TLC profiling to assess the nature of phytochemicals present in it. Since, Chromatography is the most widely used technique for separation of constituents present in a mixture using stationary and mobile phase. Furthermore, this method helps in isolation, quantification and identification of separated components.^[14] Separations are based on differences in migration rates among the sample components. A number of developing solvent systems were tried for all the extract and fractions. The solvent system, which gave best resolution, was considered optimised, valid and useful. The satisfactory resolution was obtained in the mobile phase mentioned in table 3 and photo documentation was shown in figure 3. However, presence of phytoconstituents in particular extracts and fractions were confirmed by spraying TLC plates with different spraying reagents.

CONCLUSION

The present work deals with the study of plants *C. album* for Pharmacognostic characterization, determination of their physiochemical parameters, phytochemical screening and TLC studies of the crude extracts. The selected plants were authenticated and the macroscopic studies were performed as the first step towards establishing their identity and purity. The microscopic study was carried out to determine basic cellular composition of the leaf petiole, stem and the type of stomata etc. These microscopic characteristics of particular species were treated as standard for identification of the plant species. Physicochemical studies were carried out as per Ayurvedic and Indian Pharmacopoeia (I.P., 1996) such as ash value, acid insoluble ash values

and extractive values. The important phytoconstituents were present as depicted in phytochemical screening which are well known for their medicinal potentials which was further characterized by TLC. Thus it is concluded that our study provides the data which helps to isolate and characterize the different medicinal potential of the *C. album* such as diuretic, antiparasitic, hepato-protective, laxative and sedative.

Authors' contribution

Sumit Arora contributed in procurement and authentication of plant material, carried out the laboratory work and their analysis. The concept study was conducted, designed and supervised by Prakash Itankar. Subhash Yende prepared the draft of the manuscript. All the authors agreed the text for submission.

Source of Support

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Conflict of Interest

None declared.

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