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Phytochemical and Pharmacognostical Analysis on *Chenopodium album:* A Plant with Immense Antioxidant and Nutritional Potential

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Abstract—*Chenopodium album belongs to family Amaranthaceae, genus Chenopodium commonly known as pigweed or goose foot or bathua (in Hindi) is a weedy plant found all over world and has gained renowned popularity these days due to its high nutritional composition especially amino acids. The leaves and seeds of all the members of this genus are edible and are consumed in cooked form mainly in combination with other species. It is an important medicinal plant in Ayurveda used in diseases of blood, heart, spleen, eye and in biliousness conditions, cough, abdominal pain, pulmonary obstruction and in nervous affections. The plant contains essential oils, besides alkaloids, trigonelline and chenopodine. Leaves are rich in potassium & vitamin C. In the present study the leaves of Chenopodium album were analysed for the phytochemical and pharmacognostical evaluation using standard methods. Phytochemical screening included qualitative chemical examinations and tests for determination of primary and secondary metabolites. Pharmacognostic evaluation included examinations of morphological characters, determination of leaf constant, ash value, powder analysis, and extractive values were carried out. The plant has effective pharmacological action. The medicinal properties of this plant were attributed to its variety of active phytochemical constituents. Although this plant had received interest for the phytochemical investigations since many years, more work has to be done on its isolation and characterization for exploring the immense medicinal potential of this plant.*

1. INTRODUCTION

Herbal medicines, also referred to as botanical medicine or phytomedicine, include herbs, herbal materials, herbal preparations and finished herbal products that contain parts of plants or other plant materials as active ingredients [1]. India has a rich cultural heritage of traditional medicines which chiefly comprised the two widely flourishing systems of treatments i.e. Ayurvedic and Unani systems since ancient times [2, 3]. These two systems of medicine use plants, minerals, metals and animals as source of drugs, in which plants being the major source. It is estimated that roughly 1500 plant species in Ayurveda and 1200 plant species in Siddha have been used for drug preparation[4, 5]. The use of medicinal plant is growing worldwide because of the increasing toxicity and allergic manifestations of the synthetic drugs. The World Health Organization (WHO) estimated that 80% of the population of developing countries relies on traditional medicines, mostly plant drugs, for their primary health care needs. Also, modern pharmacopoeia still contains at least 25% drugs derived from plants and many others which are synthetic analogues built on prototype compounds isolated from plants. Demand for medicinal is increasing in both developing & developed countries due to growing recognition of natural products, being non-narcotic, having no side effects, easily medicinal plant sector has traditionally occupied an important position in the socio cultural, spiritual and medicinal arena of rural & tribal lives of India.[6]

The several types of plant materials such as vegetables, fruits, leaves, oil seeds, cereals, crops, bark and roots act as crude plant drugs and are potential sources of antioxidants compounds [7]. There is currently immense interest in natural antioxidants and their role in human health and nutrition [8].*Chenopodium album* L. is herbaceous vegetable plant known as bathua sag in Hindi, pigweed in English and are distributed throughout world. About 21 species occur in India [2, 9], particularly in Western Rajasthan, Kulu valley and Shimla [10]. This plant is a polymorphous, mealy white, erect herb, up to 3.5m in height, and found wild in altitude of 4,700 meters. It is reputed to be a medicinal plant in scientific and folkloric literature and its medicinal values are well documented. It is cultivated as pot-herb and usually grown in gardens, but sometimes in corner of early grain fields. The plant has anthelminitic, laxative, diuretic, aphrodisiac action. It is also used in abdominal pains, eye disease, throat troubles, piles, diseases of the blood, heart and spleen and biliousness [11, 12]. The medicinal property of this plant is mainly present in leaves and seeds.

A Literature survey and screening of scientific data revealed that a large number of indigenous drugs have already been investigated as regards their botany and chemistry is concerned, however a systematic standardization including pharmacognostical and physico-chemical study is still lacking.

The present investigation *Chenopodium album* is therefore taken up to to know phytochemicals present for its antioxidant action and to determine its pharmacognostical properties. Further, the study will greatly help in quality assurance of finished products of herbal drugs [11, 12]

2. MATERIAL AND METHODS

2.1 Procurement and authentication of plant material

Chenopodium album leaves were collected from local market of Greater Noida, in month of November-December 2014 and authenticated by Botanist of Sharda University, Greater Noida, Uttar Pradesh.

2.2 Preparation of leaves extracts by successive extraction method

Leaves were air dried at room temperature for 3 weeks to get consistent weight. The dried leaves were later ground to crude powder. Fifty grams of crude powder of leaves was taken in soxhlet apparatus. Successive extraction with different solvents (Petroleum ether, Benzene, Chloroform, Ethyl Acetate and Ethanol) was carried out. Extracts were filtered using a Buckner funnel and Whatman No 1 filter paper. Each filtrate was concentrated to dryness under reduced pressure at 40°C through evaporator and stored at 4°C for further studies.

2.3 Qualitative estimation

For the quantitative estimation 100 g of powdered leaves was successively extracted in a soxhlet apparatus with various solvents like petroleum ether, chloroform, ethyl acetate, ethanol and water [13].The extracts were dried on water bath, weighed and colour of the extracts was also observed. The different extracts were subjected to qualitative estimation for the presence of various phytoconstituents [14].

2.4 Phytochemical Profiling

Preliminary screening for the presence of phytoconstituents (Primary and Secondary metabolites) of all the extracts was carried out using standard conventional procedures [14].

2.5 Macroscopic Studies

Macroscopic characters were examined according to standard procedures [13, 14]. The parameters included surface constants like stomatal number, stomatal index, veins, veins-islets and vein terminations.

2.6 Pharmacognostical and fluorescence analysis

Pharmacognostical parameters such as foreign organic matter, loss on drying, total ash, acid insoluble ash, water soluble ash, moisture content and crude fibres contents were performed as per Indian Pharmacopoeia [15, 16]. Fluorescence analysis of the leaves powder [13] was carried out with different chemical reagents in day (254 nm) and UV light (365 nm).

3. RESULTS AND DISCUSSION

3.1 Yield of extracts from successive extraction of *Chenopodium album* leaves

The yield of successive extracts (g) is shown in Table 1. The amount of the pet ether extract obtained from the extraction was 16g (16 % w/w yield), benzene extract was 12.15g (12.15 % w/w yield), chloroform extract 12g (12 % w/w yield), ethyl acetate extract 5.5g (5.5 % w/w yield), ethanol extract 25g (25 % w/w yield) and aqueous extract was 15g (15 % w/w yield).

Table 1: Phyto-profile and Yield of extracts from succesive extraction of *Chenopodium album* leaves (100g)

S. No	Name of extracts	Polarity Index	Colour	Consistency	Nature	% yield (w/w)
1	Pet ether	0.0	Light Green	Oily	Semi-Solid	16%
2	Benzene	2.7	Dark Green	Oily	Solid	12.15%
3	Chloroform	4.1	Green	Dry	Solid	12%
4	Ethyl Acetate	4.4	Yellowish-Green	Sticky	Solid	5.50%
5	Ethanol	5.2	Brownish-Green	Sticky	Semi-Solid	25%
6	Aqueous	9.0	Dark Brown	Dry	Crisp-Solid	15%

3.2 Phytochemical screening of successive extracts of *Chenopodium album* leaves

The presence or absence of different phytoconstituents viz. carbohydrate, glycoside, protein, tannins, saponins, flavonoids and terpenoids were detected by the phytochemical screening methods with different chemical reagents. Phytochemical components are responsible for both pharmacological and toxic activities in plants. These metabolites are said to be useful to a plant itself but can be toxic to animals, including man. The presence of these chemical constituents in this plant is an indication that the plant, if properly screened, could yield drugs of pharmaceutical significance. This is better supported by the fact that members of the family of this plant have been

known to be involved in ethnomedicine in the management of various ailments [17-20].

The result showed the presence of alkaloids, phenolics, flavonoids, saponin, tannin, lignin, protein, carbohydrates, suberin, glucoside, flavin, and traces amount of oil & sugars in the successive extract of *Chenopodium album* leaves and the result of phytochemical test is presented in Table 2. Crude alkaloids were present in high amount and saponins were found in lesser quantity. Alkaloids are good spasmolytic and anesthetic agents while saponins help in boosting the immune system, in lowering cholesterol levels in the blood and reducing the risk of getting intestinal cancer. Various reports have shown that phenolic compounds contribute to quality and nutritional value in terms of modifying color, taste, aroma and flavor and also in providingbeneficial health effects [21]. Phenolics provide the plants with defense mechanisms to neutralize reactive oxygen species (ROS) in order to survive and prevent molecular damage and damage by microorganisms, insects, and herbivores [22]. Flavonoids show a wide range of biological activities such as inhibition of cell-proliferation, induction of apoptosis, inhibition of enzymes and other antibacterial and antioxidant effects [23, 24]. The flavonoid content of the different extracts was also found to be quite high for a mixture of solvents.All these phytochemicals possess good antioxidant activities and has been reported to exhibit multiple biological effects including anti-inflammatory and antitumor activities.

Table 2: Qualitative phytochemical screening of successive extracts of *Chenopodium album* leaves

Plant Constituents	Tests Performed	Name of extracts					
		PE	Ben	Chl	EA	Et	Aq
Proteins and Amino Acids	Millon's Test	+	+++	-	++	+	+++
	Biuret Test	-	++	-	++	+	++
	Ninhydrin Test	-	+	-	++	+	++
	Xanthoproteinic Test	-	+	-	++	+	++
Carbohydrates	Molish's Test	+	+	+	+++	++	+
	Benedict's Test	+	-	+	+++	++	++
Oils and Fats	Stain Test	+++	+++	+	++	+	+
	Soap Test	+++	+++	-	-	+	-
Alkaloids	Dragendroff's Test	+++	+++	+	++	+	+
	Mayer's Test	+++	++	+	++	-	++
Saponins	Olive oil Test	++	++	++	-	+	-
	Frothing Test	++	+	+	-	+	-
Terpenoids	Salowski Test	+++	++	+	++	+	+
Tannins	Ferric Chloride Test	-	+++	-	++	+	++
Steroids	Liebermann-Burchard's Test	++	++	++	+	++	+++
Poly phenols	Folin's Test	+	+++	++	+	+++	++
Phenols	Ferric Chloride Test	++	+++	++	+	+	-
Pholobatanins	Hydrochloride Test	-	-	-	-	+	++
Cardiac Glycosides	Killer Killani Test	+++	+++	+	+	++	+
Flavonoids	Lead Acetate Test	-	+++	+	+	+++	++
	Ammonia Test	-	++	++	+	+++	++
Phytosterols	Acid Test	++	+++	++	+	+	-

(–): absent; (+): weak; (++): moderate; (+++): strong **Abbreviations**: **PE**: Pet Ether; **Ben**: Benzene; **Chl**: Chloroform; **EA**: Ethyl Acetate; **Et**: Ethanol; **Aq**: Aqueous

3.3 Organoleptic Characters and Macromorphologyof *Chenopodium album* leaves

The macroscopic study is the morphological description of the plant parts which are seen by naked eye or magnifying lens. Organoleptic evaluation can be done by means of sense organs, which provide the simplest as well as quickest means to establishthe identity and purity to ensure quality of a particular drug. Organoleptic characters such as shape, size, colour, odour, taste and fracture of stem bark, leaf structure like margin, apex, base surface, venation and inflorescence, etc are evaluated [25].

The plant was an erect or ascending, green or reddish, herb, upto 3.50 meter in height. Stem was angular, rarely slender often striped green or dark green in colour. Leaves were variable in size, shape and dark green in colour. These were rhomboid, deltoid to lanceolate, upper entire, lower toothed or regularly lobed; petioles long slender, often equal or longer than the blade, petiole is 12.10±1.02 cm long; leaf was 1.30-4.00 x 5.00-7.54 cm^2 (Table 3). Flowers in clusters were forming complex or lax paniculate often mealy spikes in axils; utricles with round, compressed, shining black seeds, and containing sharp margins. The spikes were more in number. The stomatal no 80.25±1.23 mm^2, stomatal index 15.23%,

veinislet no 8-11, veinislet termination no 8.18±2.45 and palisade ratio 9.5-12.5 values were observed in fresh leaves (Table 4). The number of stomata and epidermal cells, vein-islets and vein termination number per unit area, palisade ratio, stomatal index etc. give constant structure for different species of plants.

Table 3: Organoleptic charactersof *Chenopodium album* leaves

S. No.	Variables	Results obtained
1.	Leaf Colour	Dark Green
2.	Leaf Shape	Rhomboid
3.	Stem colour	Light Green, angular
4.	Seed Colour	Black
5.	Odour	Aromatic
6.	Taste	Acidic
7.	Height of Plant (m)	3.50±0.02
8.	No. of leaves/ plant	250.52±0.17
9.	Leaf Area (cm2)	16.12±1.52
10.	Spikes/ plant	25.29±0.82
11.	Petiole (cm)	L=12.10±1.02
12.	Lamina (cm)	L=4.52±0.92 W=2.12±0.12

Table 4: Macroscopic charactersof *Chenopodium album* leaves

S. No.	Variables	Values (in 1 mm2 area)
1.	Stomatal number	80.25±1.23
2.	Stomatal index	15.23%
3.	Veinislet number	25±2.07
4.	Veinlet Termination No	8.18±2.45
5.	Palisade ratio	9.5-12.5

3.4 Pharmacognostical Evaluation:

Identification and evaluation of plant material using various analysis techniques is one of the simplest and cheapest methods to establish the correct identity of the source materials.The parameters which are studied are moisture content, loss on drying, total ash, acid-insoluble ash, alcohol and water-soluble extractive values etc. [13-15]. The proximate analysis revealed that water soluble extractive values of leaves was 27.52, alcohol soluble extractive values of leaves was 31.81, total ash value was 13.07, acid insoluble ash was found to be 1.59 and sulphated ash was 30.12. Loss on drying was 24.52, foreign organic matter was found to be 8.91 and pH was slight acidic, 5.9 were observed in fresh leaves (Table 5).

Estimation of ash values is also significant parameter for the detection of nature of material, which is added to the drug for the purpose of adulteration, impurities and determination of authencity, quality and purity of test sample. The ash values usually represents the inorganic salts present in the test sample and is the residue after incineration. Total ash values of leaves indicate the inorganic composition or earthy materials and other impurities present along with the plant material. Ash values are used to determine quality and purity of crude drug. It indicates presence of various impurities like carbonate, oxalate and silicate. The water soluble ash is used to estimate the amount of inorganic compound present in drugs. The acid insoluble ash consist mainly silica and indicate contamination with earthy material [25].

Estimation of extractive values determines the amount of the active constituents in a given amount of plant material when extracted with a particular solvent. The extractions of any crude drug with a particular solvent yield a solution containing different phytoconstituents. The compositions of these phytoconstituents depend upon the nature of the drug and the solvent used. Extractive values determination was useful for identification of exhausted drugs [26]**.** The amount of the extract that drug yields in solvent is often an approximate measure of the amount of certain constituents that the drug contains. The water soluble extractive value indicated the presence of organic and inorganic compounds and the alcohol soluble extractive values indicated the presence of secondary metabolites which are polar in nature like phenols, steroids, terpenoids and flavonoids [26].

Determination of moisture content (higher or lower percentage) of drugs is important as it gives information about conditions of climate, whether drug is restored in humid, wet or dry climate. Higher moisture content if present would be favouring more growth of micro-organisms, thus favouring contamination that may result in deterioration of drug. The moisture content was quite low which may be advantageous in view of increasing the sample's shelf life [25]. Percentage of loss of weight on drying indicates loss of volatile compounds along with water. Less moisture content could prevent microbial (bacterial, fungal or yeast) growth. The determination of pH value is necessary inorder to know absorption mechanism in body [13].

Table 5: Physicochemical Analysis of *Chenopodium album* leaves

S. No.	Parameters	Values obtained w/w on dry weight basis
1.	Total Ash value	13.07±0.12*
2.	Water soluble ash	27.52±1.25**
3.	Acid Insoluble ash	1.59±0.97*
4.	Alcohol soluble ash	31.81±0.82*
5.	Sulphated ash	30.12±0.02*
6.	Loss on Drying	24.52±0.17**
7.	Foreign organic Matter	8.91±1.20*
8.	pH	5.9±0.12

Significant Level: *= 0.01%, **= 0.1%

3.5 Fluorescence Analysis of leaves and its powder

The result of fluorescence studies of leaves powder using different reagents are given in Table 6 and that of the extracts is compiled in Table 7. Fluorescence is an important phenomenon exhibited by various chemical constituents present in plant material. Many phytochemical fluorescence are seen when suitably illuminated. The fluorescence colour is specific for each compound. A nonfluorescent compound may fluorescent if mixed with impurities that are fluorescent. Some constituents show fluorescence in the visible range in day light. The ultra violet light produces fluorescence in many natural products (e.g. alkaloids like berberine), which is not visible in day light. If the substances themselves are not fluorescent, they may often be converted into fluorescent derivatives after reacting with different reagents hence some crude drugs are often assessed qualitatively in this way and it is an important parameter of pharmacognostical evaluation [13, 25]. The results of fluorescence analysis of leaves powder showed their characteristic fluorescent colour.

Table 6: Florescence characteristicof powder of *Chenopodium album* leaves in different means

Leaves powder + Treatments	Visible Light	UV Short (254nm)	UV long (366nm)
Powder as such	Dark Green	Fluorescent green	Fluorescent green
Distilled Water	Dark Green	Fluorescent green	Fluorescent green
Glacial acetic Acid	Light Green	Fluorescent green	Fluorescent green
Picric Acid	Yellowish-Green	Yellow	Yellow
1N HCL	Brown	Brown	Dark Brown
1N H2SO4	Dark Brown	Red	Dark Red
Conc. HNO3	Dark Red	Light Green	Light Green
1N NaOH	Dark Red	Dark Green	Fluorescent green
Ferric Chloride	Red-Brown	Red	Red
Iodine Solution	Bluish	Dark Green	Bluish-Green
Ammonia Solution	Green-Yellow	Green-Yellow	Light Green
Potassium Dichromate	Orange	Brown	Brown
Ethanol	Dark Green	Light Green	Light Green
Methanol	Dark Green	Light Green	Light Green

The colours mentioned in the table are based on the colour identification chart, Royal Botanica Garden, Edinburg (1969)

Table 7: Florescence characteristicof different extracts of *Chenopodium album* leaves

S. No	Name of Extracts	Under Ordinary Light	Under UV (366nm) light
1	Pet ether	Light Green	Fluorescent green
2	Benzene	Dark Green	Fluorescent violet
3	Chloroform	Green	Fluorescent green
4	Ethyl Acetate	Yellowish-Green	Yellow
5	Ethanol	Brownish-Green	Brown
6	Aqueous	Dark Brown	Black

4. CONCLUSION

In order to determine the quality of medicinal plants with regard to its authenticity phyto-pharmacognostical characters viz. macroscopical, powder analysis, chemical analysis, fluorescence behaviour, extractive values and ash values are very important. These proves very useful for individual identification of plants and the authenticity of plant drugs. They provide evidences concerning relationship of groups such as families or help to establish affinities of genera of uncertain taxonomic status. The medicinal properties of this plant were attributed to its variety of active phytochemical constituents. Although this plant had received interest for the phytochemical investigations since many years, more work has to be done on its isolation and characterization. The pharmacological studies reported in this review confirm the therapeutic value of *Chenopodium album*. However very less information is available regarding the phytoanalytical properties of this plant. Phytochemical studies have been reported but still it needs to progress. If the ethnobotanical claims are sufficiently evaluated, then it can provide good remedies and can help the mankind for various ailments.This study establishes not only pharmacognostic and phytochemical characterizations of leaves but also microscopic and fluorescence characters. These characteristics can be used further as identification and authentication parameters of the leaves. The leaves studied here can be seen as a potential source of useful therapeutics. Further studies are going on these leaves in order to isolate, identify, characterize and elucidate the structure of bioactive compounds along with their pharmacological activity.In addition, it can be used as a food ingredient to make processed products like raita, paratha. It can be used as animal feed as it does not contain any toxic compound. These results also support beneficial health claims. Thus, there is enormous scope for future research and further pharmacological investigation on *C. album.*

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