Evaluation of phytochemical constituents and antioxidant activity of *Chenopodium album* of Bundelkhand region

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**ABSTRACT**

*Aim:* *Chenopodium album* L. is a vegetable plant commonly known as Bathua belongs to the family Chenopodiaceae, which is an important medicinal plant in Ayurveda that cures leukoderma, biliousness, epilepsy, salivation, fever, cough, and abdominal pain. Keeping in view of the above beneficial effects of *C. album*, we sought to analyze the phytochemicals present in the methanolic as well as in aqueous extracts. **Materials and Methods:** Aqueous and methanolic extraction of *C. album* was done for screening of different kinds of bioactive compounds, namely: Saponins, carbohydrate, reducing sugar, cardiac glycosides, protein and amino acid, glycosides, alkaloids, tannins, flavonoids, terpenoids, and steroids using standard phytochemical screening methods. Thin-layer chromatography (TLC) and antioxidant activity were also evaluated. **Results:** There were variable results for the alkaloid test with different methods. Molisch and Barfoed’s test for carbohydrate shows a negative result for all the extracts. Fehling test and Keller–Killani test are negative in the quath and methanolic extract. **Conclusions:** Mostly, tests for flavonoids, glycosides, tannin, and phenolics showed the presence of these secondary metabolites in all the extract. Saponin test showed very impressive results. Biuret test is negative in all the extracts. Terpenoids and steroids are present in quath as well as in aqueous extraction, whereas these metabolites are absent in methanolic extraction. All the extraction procedures show five bioactive components. Further, the antioxidant activity was observed in all the extracts. Mostly, all the phytochemicals are present in the leaves extract of Bathua. Further, five bioactive components were observed by TLC. Antioxidant activity observed in the leaves extract is may be due to the presence of flavonoids and phenolic components.

**KEY WORDS:** Antioxidant activity, *Chenopodium album*, Extracts, Medicinal plant, Phytochemical analysis

**INTRODUCTION**

According to the WHO, medicinal plants would be the best source to obtain a variety of drugs. About 80% of individuals from developed countries used traditional medicine, which has compounds derived from medicinal plants.¹ Medicinal plants besides therapeutic agents are also a big source of information for a wide variety of chemical constituents which could be developed as drugs with precise selectivity. Herbal products are suitable for treating a wide range of infections and diseases. *Chenopodium album* L. is a vegetable plant commonly known as Bathua belongs to the family Chenopodiaceae. *C. album* is a fast-growing weedy annual plant. Plants native in Eastern Asia are included under *C. album* but often differ from European specimen.² The species are cultivated as a grain or vegetable crop (such as in lieu of spinach), as well as animal feed in Asia³ and Africa, whereas in Europe and North America, it is commonly regarded as a weed in places such as potato fields.⁴ The leaves and young shoots may be eaten as a leaf vegetable, either steamed in its entirety or cooked like spinach, but should be eaten in moderation due to high levels of oxalic acid.⁵ *C. album* is very rich in various nutrients such as proteins, vitamins (Vitamin A and Vitamin C), and also minerals such as iron, potassium, phosphorus, and calcium. Due to its high nutritive value and medicinal properties, *C. album* is used in the preparation of many traditional medicines. In India, Bathua is used to prepare many edible items such as bathua saag, bathua paratha, and curd bathua. *C. album* is widely distributed in tropical and subtropical parts of India. It is commonly found in Gujarat, Haryana, Uttar Pradesh, Himachal Pradesh, Madhya Pradesh, Karnataka, Maharashtra, Rajasthan, West Bengal, Sikkim, and Jammu and Kashmir. A number of uses have been reported for *C. album*. The leaves may be taken in the form of an infusion or decoction as a laxative and anthelmintic. It has

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also been recommended for the treatment of hepatic disorders and splenic enlargement.\cite{9} The finely powdered leaf is used as a dusting powder to allay irritation about the external genitalia of children.\cite{10} It is also used in abdominal pains, eye disease, throat troubles, piles, diseases of the blood, heart and spleen, and biliousness.\cite{11}

Medicinally, this plant has been used to treat various symptoms attributable to nutritional deficiencies. It is also said to have sedative and refrigerant properties, and people have used the poultice leaves to soothe burns. The hepatoprotective activity of \textit{C. album} against paracetamol-induced hepatotoxicity has been reported.\cite{9} Furthermore, a study by Jain \textit{et al.} concluded the significant hepatoprotective activity of ethanol extract of \textit{C. album} leaves against CCl\textsubscript{4}-induced hepatotoxicity and suggests its use as potential therapeutic agents for liver diseases.\cite{10} \textit{C. album} extract was found to exhibit excellent antioxidant and free radical scavenging activity when compared with ascorbic acid during \textit{in vitro} studies.\cite{11} A study by Laghari \textit{et al.} revealed that the methanolic extracts of \textit{C. album} from fruits and leaves have great potential as a source for natural health products.\cite{12} Further, it has a significant antifungal potential against phyto-pathogenic fungus \textit{Ascochyta rabiei}.\cite{13} The pharmacological studies reported by Agrawal \textit{et al.} confirm the therapeutic value of bathua.\cite{14}

Free radicals such as superoxide anions, hydrogen peroxide, and hydroxyl radicals can trigger reactions damaging essential biomolecules in living organisms such as lipids, proteins, and nucleic acids. Diabetes and degenerative diseases, cancer, heart and vascular diseases, neurological, and ocular disorders, besides the aging process itself, are some of the consequences of their action.\cite{13,15} Antioxidants, either as nutraceuticals, pharmaceutical supplements, or as food additives, can terminate radical reactions. Current concerns about potential mutagenic activity and possible carcinogenic effects of synthetic antioxidants, such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole, have encouraged the search for naturally occurring antioxidants.\cite{17,18}

From the study done so far, it has been elucidated that flavonoids are major anti-inflammatory agents. Some of them act as phospholipase inhibitors, and some have been reported as TNF-\alpha inhibitors in different inflammatory conditions. Biochemical investigations have also shown that flavonoids can inhibit both cyclooxygenase and lipoxygenase pathways of arachidonic acid metabolism depending on their chemical structures.\cite{21,22} Alkaloids in asserted skeletal type based on pyridine ring system have been reported to have striking anti-inflammatory activity, and ex. berberine from Berberis is traditional remedy against rheumatism.\cite{23} Terpenoids significantly inhibit the development of chronic joint swelling. Terpenoids may affect different mechanism relevant to inflammations arising in response to varied etiological factors.\cite{24} However, still many herbal medicines for inflammation and rheumatism have not undergone through scientific investigations. Hence, it is a need of time that all such herbal medicines should consider for the determination of their pharmacological activities, isolation of single entity responsible for anti-inflammatory activity, and development of suitable formulation which would be beneficial against inflammatory disorders. Therefore, in the present study, phytochemical constituents and anti-oxidant activity have been evaluated.

\section*{Study Area}

Bundelkhand region covers a total of 13 districts, of which 7 of Uttar Pradesh and 6 districts of Madhya Pradesh. It is fallen under 23° 8–26° 31 and 78° 11–81° 31 latitude and longitude, respectively. The region is characterized as hot semi-arid eco-region along with growing period of 90–150 days. The annual rainfall ranges from 838.6 to 1251 mm over the region which is often erratic.\cite{25} The main occupation of this region is agriculture, and mostly, people are involved in labor work and are very poor because of unemployment and education. However, the region is rich in ecological and biodiversity and also rich in medicinal plants. Some of the medicinal plants are mainly found in this region. Therefore, to improve the economic status of the farmers, villagers, an extensive research work is required for their use.

\section*{MATERIALS AND METHODS}

\subsection*{Collection of Plant Materials}

The plant of \textit{C. album} was collected in January from local market of Jhansi (U.P.). First, the collected plant material was washed with tap water for 3–4 times and then with deionized water for 2 times. After washing, plants were kept in the dark for drying at room temperature and under the constant observation to avoid any contamination. Dried leaves were crushed with the help of electric grinder. Powdered sample was stored for the further use.

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Extraction Procedure
Extraction was done by three methods, i.e., aqueous, quath, and methanolic extraction.

Aqueous Extract
Different concentration of dry powder, i.e., 5 g and 10 g was taken in conical flasks having equal amount (100 ml) of deionized water. Both the flasks were heated at 90°C in water bath for 1 h. After 1 h, flasks were taken out from water bath and kept at room temperature for cooling purpose. Then, the extract was filtered with the help of filter paper and stored at 4°C.

Quath Extraction
For quath extraction, fresh leaves were used. First, leaves washed carefully and then crushed in automatic grinder to make paste. 100 ml of paste was mixed with 300 ml of distilled water in the beaker and heated at 100°C until the final volume remains 100 ml. Cool down the quath extract, filtered with a muslin cloth, and stored at 4°C.

Methanolic Extract
The powdered material was extracted with absolute 80% methanol using Soxhlet apparatus. Different concentration of plant material and solvent were taken. After filling the Soxhlet apparatus with plant material and solvent, it was run at 60°C–80°C until it gets colorless and continuously flow of water to cool down the condenser. Finally, the extract was collected in airtight bottles and stored at 4°C.

Phytochemical Analysis
Detailed phytochemical analysis was carried out for all the three extracts, i.e., leaves, developed bulbs, undeveloped bulbs, and roots as per the standard methods[26,27] with some of the modifications.

Thin Layer Chromatography (TLC)
Each of the extracts was to begin with, checked by TLC on analytical plates over silica gel-G of 0.2 mm thickness. These plates were developed in butanol:acidic acid:water having a ratio of 2:1:1. The developed TLC plates were air-dried followed by hot air oven for 20 min. Freshly prepared 0.2% ninhydrin solution was used to detect the bands on the TLC plates.

The movement of the spots was expressed by its retention factor (Rf).

\[ Rf = \frac{\text{Distance traveled by solute}}{\text{Distance traveled by solute}} \]

Antioxidant Activity
The total antioxidant capacity (TAC) of the methanol extract of different parts of Allium sativum was evaluated by the phosphomolybdenum reduction assay method according to the procedure described by Prieto et al.[28] The assay is based on the reduction of Mo (VI) to Mo (V) by the methanol extract of different part of garlic, and subsequent formation of green phosphate/Mo (V) complex at acid pH. 1 mL of various concentrations (3–21 μg/mL) of extract was combined with 1 mL of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate) and incubated at 95°C for 90 min. BHT was used as a standard. A typical blank solution contained 3 ml of reaction mixture and the appropriate volume of the same solvent used for the samples/standard. The absorbance of the reaction mixture was measured at 695 nm using a spectrophotometer.

RESULTS
C. album L. leaves were screened for the presence of phytochemical components by different extraction methods such as quath, aqueous, and methanolic extraction. Different phytochemical tests were performed by distinctive reagent, for example, Mayer’s test, Wagner’s test, and Hager’s test, all were performed for the detection of alkaloids. Alkaloids are a class of nitrogenous organic compounds of plant origin which have diverse and important physiological effects on humans and other animals. Well-known alkaloids include morphine, strychnine, quinine, ephedrine, and nicotine. Quath extraction shows positive results with Mayer’s and Hager’s test while methanolic extraction shows the presence of alkaloids by Wagner’s test. Carbohydrate is absent in all the extract by all the extraction methods. Reducing sugar is detected by Benedict’s test in all the extracts. Flavonoids are hydroxylated polyphenolic compounds that carry out important functions in plants, including attracting pollinating insects; combating environmental stresses, such as microbial infection; and regulating cell growth. There are six major subclasses of flavonoids, namely, anthocyanidins, flavan-3-ols, flavonols, flavanones, flavones, and isoflavones; flavonols are the most widespread in the human diet. Tests for flavonoids and glycosides show positive results for all the tests we performed. Cardiac glycosides are class of organic compounds that increase the output force of the heart and decrease its rate of contraction by acting on the cellular Na-K ATPase pump. Keller–Killani test for cardiac glycosides showed positive results mainly with aqueous extraction.

Phenolic compounds are any compounds derived from the phenol group and contribute to the color, structure, and astringency. Tannins are large molecular weight compounds resulting from polymerization reaction of smaller phenolic compounds. Test for tannin and phenolics by different methods showed the presence
of their compounds in all the extraction procedures we conducted. Saponins are glycosides with foaming characteristics and have many health benefits. Saponins are present in all the extract. Ninhydrin tests showed mostly positive results with quath and aqueous extraction while methanolic extraction shows negative results with both the tests, i.e., ninhydrin and biuret test. Plant terpenoids are used extensively for their aromatic quality and play a role in traditional herbal remedies [Table 1]. There are different terpenoids found in different plants, and one of the most studied is curcumins found in turmeric and mustard seeds. Terpenoids and steroids are present in the quath as well as in aqueous extraction.

Aqueous extraction of bathua, (5 and 10 g) show 5 spots having Rf values 020, 050, 058, 065, and 080 (5 g) and Rf 0.20, 0.50 0.55, 0.65, and 0.75 (10 g), respectively. Similarly, methanolic extraction also shows 5 spots having Rf 0.30, 0.60, 0.68, 0.78, and 0.90 [Figure 1].

The TAC test is based on the reduction of Mo (VI) to Mo (V) by the extract and formation of green phosphate/Mo (V) complex at acidic pH. TAC of the phosphomolybdenum model evaluates both water-soluble and fat-soluble antioxidant capacity. Extracts were subjected for the evaluation of antioxidant activity. Antioxidant activity was observed in vitro.

DISCUSSION

The presence or absence of different phytoconstituents, namely, carbohydrate, glycoside, protein, tannins, saponins, flavonoids, and terpenoids was 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.[29,30]

The leaves of *C. album* contained various anti-nutrients such as alkaloids, flavonoids, glycosides, saponins, and tannins.[31] Our results also show the presence of alkaloids, phenolic, flavonoids, saponin, tannin, protein, carbohydrates, and glucoside, in the extract of *C. album* leaves, and the result of phytochemical test is presented in Table 1. Therefore, the phytochemical content analyzed and was found to be very promising. Further, 4 spots were found in the methanolic extract of *C. album*, and approximately, similar results (4–8 spots) were reported by other authors.[32] Further, all the extraction procedure reveals the antioxidant activity.

Alkaloids are good spasmylytic and anesthetic agents while saponin helps in boosting the immune system, in lowering cholesterol levels in the blood and reducing the risk of getting intestinal cancer. Adedapo et al. reported that the alkaloid content in leaves of *C. album* as 1.8 mg/100 g.[33] Gupta and Wagle reported 0.90% saponin content in leaves of *C. album*.[34] This variation in result from other workers might be due to different varieties which lead to genetic variability, time of harvest, stage of harvest, analytical procedure applied, and climatic conditions. Bhargava et al. revealed that saponin content in leaves of quinoa varies in different growing stages, and low saponin content was found in branching stage and higher in blooming stage.[35] From the nutritional or pharmacological point of view, saponin could also have some important value. They can increase membrane permeability, thus enabling use for increased food intake at the intestinal level or even for drug assimilation.[36]

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 providing beneficial health effects. Therefore, total phenolic and flavonoid content of different extracts of bathua was estimated [Table 1]. The total phenolic content was found in all the extracts of bathua, i.e., quath, aqueous, and methanolic. Phenolic provide the plants with defense mechanisms to neutralize reactive oxygen species (ROS) to survive and prevent molecular damage and damage by microorganisms, insects, and herbivores.[37] Besides exhibiting negative effects, tannin also provide some health benefits including antioxidant and radical scavenging properties, anticarcinogenic, antibacterial, and anti-enzymatic effects[38] and thus has gained popularity in today’s scenario. Tannins have been found to form irreversible complexes with proline-rich protein,[39] resulting in the inhibition of cell protein synthesis. Tannins are known to react with proteins to
provide the typical tanning effect which is important for the treatment of inflamed or ulcerated tissues. Polyphenols and particularly flavonoids derivatives behave as reducing agents, mostly donating hydrogen and quenching singlet oxygen. They seem to have additive effects on endogenous scavenging compounds.\[^{42}\]

Flavonoids are versatile bioactive secondary metabolites present in almost all plant species. Most representative family members include flavones, flavanones, flavonols, catechins, and anthocyanidins. Their antioxidant potential toward ROS depends on structural characteristics such as the number and substitution pattern of hydroxyl groups and the extent at which these groups are glycosylated.\[^{43}\]

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.\[^{44,45}\] The flavonoids 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 have been reported to exhibit multiple biological effects including anti-inflammatory and antitumor activities.

**CONCLUSION**

Plants that are rich in secondary metabolites, called medicinal plants, are widely used in traditional medicine to combat and cure various ailments. There were not much differences in the quath and aqueous extraction in terms of phytochemical constituents. Furthermore, all the extraction procedures show five bioactive components. Further, the antioxidant activity was observed in all the extracts. The presence of flavonoids and phenolic compounds in the leaves extract could be considered responsible for conferring antioxidant ability.

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