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Screening of nutritional, phytochemical, antioxidant and antibacterial activity of *Chenopodium album* (Bathua)

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Abstract
Chenopodium album (L.), Bathua is a fast-growing weedy annual plant in the genus *Chenopodium*. In the present work, we have attempted to study different characteristics of bathua in different solvents (petroleum ether, dichloromethane, ethyl acetate, methanol, distilled water, and the mixture of all solvents in equal proportion), to understand its health benefits. Various studies were done to estimate phytochemicals like alkaloids, saponins, total flavonoids and total phenolics. Nutritional analysis proved it to be a potential source of energy, proteins, carbohydrates; ascorbic acid and beta carotene and minerals; potassium, sodium, calcium, phosphorous, magnesium, iron and zinc. The petroleum ether extract, methanol extract and aqueous extract of *C. album* revealed good antioxidant potential of ABTS and FRAP which proves them as potent electron and hydrogen donors. The methanol extract of *C. album* was found to be most active among all other extracts and inhibited *Staphylococcus epidermidis*, *Bacillus subtilis, Staphylococcus aureus*, *Escherichia coli.* The extract of a mixture of solvents was also found to be effective in inhibiting *Staphylococcus aureus*.

Keywords: *Chenopodium album*, Nutritional, Phytochemical, Antioxidant, Antibacterial, GC/MS, Bathua.

1. Introduction
Chenopodium album (L.) of the family *Chenopodiaceae* (Goosefoot family) belongs to the genus *Chenopodium.* It is also known as fat-hen, bathua, vastukah, chakvit. This weedy plant has various medicinal applications. It is a polymorphous, mealy white and erect herb which is 3.5m in height, and found wield in altitude of 4,700m. 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. The tender shoots are eaten raw in salad or with curd; they are also cooked as a vegetable or used as an ingredient in paratha. The dehydrated leaves of bathua can also be incorporated in various conventional food items as it can improve the nutritional quality of the product as well as add variety in the diet [1].
The dried herb is stored for future use. It is also used as fodder; pigeons consume the plant in large quantities [2]. Studies carried out in different parts of the world indicate that *C. album* is a rich source of nutrients, antioxidants and important dietary elements [3-4]. Vitamin C and β-carotene were detected from the young shoots and mature plants of *C. album*, indicating that these vegetables could constitute an important source of these vitamins in the diet [5].
The leaves of *C. album* are being used in traditional medicines. It has been found to have antipruritic and antinociceptic [6], sperm immobilizing agent [7], cryptomeridiol and 8-alpha-acetoxycyryptomeridiol as growth promoting activity. It has been found to have flavonoid as phenolic amide [8], hypotensive activity [9], saponin [10], rich in iron content [11], cinnamic acid amide [12], alkaloid chinoalbicin [13], apocortinoid [14], xyloside [15], phenols and lignans [16]. 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 poulticed leaves to soothe burns. The hepatoprotective activity of *C. album* against paracetamol-induced hepatotoxicity has been reported [17]. Also, a study by Jain *et al.* 2012, concluded the significant hepatoprotective activity of ethanol extract of *C. album* leaves against CCl_4-induced hepatotoxicity and suggests its use as potential therapeutic agents for liver diseases [18]. *C. album* extract was found to exhibit excellent antioxidant and free radical scavenging activity, when compared with ascorbic acid during *in vitro* studies [19]. A study by Laghari *et al.* 2011, revealed that the methanolic extracts of *C. album* from fruits and leaves have great potential as a source for natural health products [20]. *Chenopodium album* has significant antifungal potential against

phyto-pathogenic fungus *Ascochyta rabiei* [21]. The pharmacological studies reported by Agrawal Mona Y *et al.* confirm the therapeutic value of *Chenopodium album* [22]. The aim of the present study is to evaluate the nutritional, phytochemical constituents, antioxidant and antibacterial activity of *C. album.*

2. Material and methods

2.1 Materials

Chenopodium album was collected in the month of February, 2014 from a local market in New Delhi, India. The leaves of *C. album* were manually separated and thoroughly washed under tap water. The clean sample was dried in an oven at 40 °C and coarsely powdered using a mixer grinder, sieved and then stored in an air-tight, light resistant container for further use.

2.2 Reagents

Folin-Ciocalteu's Phenol reagent (SRL), Gallic Acid (HiMedia), Dimethyl Sulfoxide (SRL), Aluminium chloride (Fisher), Sodium Hydroxide (SRL), Ascorbic Acid (SRL), 2,2'-Azino-bis-(3-ethylbenzthiazoline-6-sulphonic acid) (Sigma), Trolox (Aldrich),TPTZ (Fluka), Ammonia solution (SRL), Ferrous Chloride (Thomas Baker), Petroleum Ether (Loba Chemie), Methanol (Thomas Baker), Dichloromethane (Fisher), Ethanol, Ethyl Acetate, Acetone, ICP Multielement Standard (Qualigens), Distilled water.

2.3 Extraction and fractionation

50 g sample of bathua was successively extracted with 100 ml of petroleum ether, dichloromethane, ethyl acetate, and methanol. The mixture was incubated at 60° at 150 rpm for 24 h in an incubator shaker. The mixture was filtered through whatman paper to obtain the filtrate. The marc left after the methanol extraction was macerated with distilled water for 24 h. The filtrates obtained were then transferred to separate beakers and covered with foil, with fine pores for the solvent to evaporate at 65 °C in a hot air oven to afford petroleum ether extract (PE), dichloromethane extract (DCM), ethyl acetate extract (EA), methanol extract (ME) and aqueous extract (WE). Along with these, an extract of a mixture of all the above solvents (MIX) in a ratio of 1:1:1:1:1 was also prepared.

2.4 Extract Yield (EY)

The yield of dried extracts based on their dry weights was calculated using the following equation:

Yield (g /100 g of dry plant material) = (W1 × 100) / W2

Where, W1 was the weight of the extract after the evaporation of solvent, and W2 was the weight of the dry plant material.

2.5 Nutritional Analysis

Macro Kjeldahl method was used for the estimation of crude protein content [23]. The powdered bathua sample was put in an oven at 105 °C for 24h. The difference in weight determines the moisture content [24]. Ash content was analyzed by AOAC method Ref. 942.05. The fat content of the samples was determined by using petroleum ether as a solvent. Total carbohydrate [23] and energy calorific value [23] were also calculated. Crude fiber content was evaluated by using AOAC method [25]. Mineral content was analyzed by inductively coupled plasma optical emission spectrometry (ICP-OES).

2.6 Phytochemical Analysis

2.6.1 Determination of Crude Alkaloids

2.5 g of the sample was weighed in a 250 ml beaker and 100 ml of 10% acetic acid in ethanol was added. It was covered and allowed to stand for 4h. This was filtered and the extract was concentrated on a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle, then the precipitate was collected and washed with dilute ammonium hydroxide and filtered again. The resulting alkaloid was dried and weighed [26].

2.6.2 Determination of Saponins

The method of Obadoni and Ochuko [27] was used to determine saponins. To 5 g of the powdered sample, 50 ml of 20% aqueous ethanol was added in a conical flask. The samples were heated over a hot water bath for 4h with continuous stirring at about 55 °C. The mixture was filtered and the residue re-extracted with another 50 ml of 20% ethanol. The combined extracts were reduced to 10 ml over a water bath at about 90 °C. The concentrate was transferred into a 250 ml separating funnel and 20 ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated. 15 ml of n-butanol was added. The combined n-butanol extracts were washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation the samples were dried in the oven to a constant weight and the saponin content was calculated as a percentage.

2.6.3 Determination of Total Phenolics

Folin Ciocalteu's reagent method was used to determine total phenols [28]. An aliquot (100 µl) of extract was mixed with 250 µl of Folin Ciocalteu's reagent and allowed to stand at room temperature for 5 min. Sodium bicarbonate (20%, 1.5 ml) was added to the mixture and incubated at room temperature for 120 min. Absorbance was measured at 765 nm using a spectrophotometer. A standard curve was plotted using different concentrations of gallic acid and the amount of total phenolics was calculated as gallic acid equivalents in µg/mg of dried extract.

2.6.4 Determination of Total Flavonoids

The total flavonoid content was also determined using the aluminium chloride colorimetric method, with Catechin as a standard. The sample extract (250 µl) was added to 4.5 ml distilled water, followed by 5% $NaNO_2$ (0.03 ml). After 5 min at 25 °C, $AlCl_3$ (0.03 ml, 10%) was added. After another 5 min, the reaction mixture was treated with 2 ml of 1M NaOH. Finally the reaction mixture was diluted to 10 ml with distilled water and absorbance was measured at 510 nm. The results were expressed as catechin equivalents (CE) in µg/mg of dried extract.

2.7 Determination of Secondary Metabolites

Secondary metabolites were determined by GCMS analysis. An Agilent 5975B mass spectrometric detector (MSD) was used in the scan mode (m/z 35-1050) for all the samples. Screening of volatiles and semi volatiles were performed using

the automatic RTL screener software in combination with the Agilent NIST'05 library. 1 µl of the sample from a 100 mg/ml stock was injected by split injection (1:20) at 280 °C.

2.8 Antioxidant activity

2.8.1 ABTS radical scavenging assay

ABTS radical scavenging activity of the hydrophilic fractions was determined by a procedure reported by Re *et al* [29]. The ABTS solution was prepared by mixing 8 mM of ABTS salt with 3mM of potassium persulfate in 25 ml of distilled water. The solution was held at room temperature in the dark for 16 h before use. The ABTS solution was diluted with ethanol in order to obtain an absorbance between 0.8 and 0.9 at 734 nm. Fresh solution was prepared for each analysis. Antioxidant or standard solutions, 10 µl were mixed with 990 µl of diluted ABTS solution and incubated for 10 min. The absorbance at 734nm was read. Ethanol was used as a blank. The Ascorbic acid was used as a standard. The concentration of extracts required to scavenge 50% of ABTS radicals, called inhibitory concentration (IC_{50}) was also calculated.

2.8.2 Ferric reducing antioxidant power (FRAP) assay

FRAP solution (900 µl) was mixed with a certain concentration of the extract (100 µl) and incubated at 37 °C for 4 min. The absorbance of the reaction mixture was measured at 593 nm BHT (butylated hydroxytoluene) was used as a standard.

2.9 Flavor and fragrance profiling

Thermal desorption system was used for profiling of volatile and semi-volatile compounds which are responsible for flavor and fragrance in the sample. Thermal desorption was done in Direct Sampling Mode under following Conditions**:** Presampling: Purge time: Up to 1min, Temperature of flow path: 120 ˚C, Carrier gas: Helium, Purge gas: Nitrogen, Carrier gas pressure: 5ml/min; Sampling: 100-200 mg weighed into empty glass tube or PTFE liner, Number of sampling cycle: 1, Pressurization time: 1 min, Flush: 1min, Sampling time: 3 min, Equilibration time: 3 min; Post sampling: Line purge: 1min, Pre tap fire purge: 1/min, TRAP: Tenax, Trap temperature: 100 ˚C, Maximum heating temperature: 300 ˚C, Hold up time: 3 min, Split: On, Separation column: GC column HP5MS, Analysis: GCMS. The conditions for GC were same as above mentioned except the oven was programmed from 60 ˚C (0 min) at 3 ˚C/min to 240 ˚C (6 min) at 5 ˚C/min to 280 ˚C (15 min). The compounds were eluted from GC in total ion chromatograph (TIC), which was searched against two databases: Agilent NIST'05 library developed by National Institute of Standards and Technology, Flavor 2 developed by Agilent.

2.10 Determination of antibacterial activity

Antibacterial activity was assessed using the agar well diffusion method against three gram positive bacteria (*Staphylococcus aureus, Staphylococcus epidermidis*, *Bacillus subtilis*) and two gram negative bacteria (*Proteus mirabilis, Escherichia coli*). Wells were punched onto the seeded nutrient agar plates with the help of 1ml micro pipette tips (6mm diameter). 100 µl of the samples were added into the wells under strict aseptic conditions and all the plates were incubated at 37 °C overnight. Antibacterial activity was determined by measuring the diameter of the zone of inhibition and the mean values were calculated.

3. Results and discussion

3.1 Nutritional analysis

In the present study, the potential benefits were shown by nutritional attributes of dried bathua (Table1). Moisture content and dry matter analysis, reporting during nutritional analysis is very important because it directly affects the nutritional content of vegetable. The moisture content was quite low (5.06%) which may be advantageous in view of increasing the sample's shelf life. Bathua was found to be rich in carbohydrates (40.84%). There was an appreciable amount of protein (28.69 % by weight) making it a good source of protein, while its fiber content is less. There is evidence that dietary fiber has a number of beneficial effects related to its indigestibility in the small intestine [30]. It has low amount of fat (4.41%) which makes it an ideal diet for overweight people. The energy value of bathua was calculated and the value obtained was 317.81 kcal. It was also found to contain potassium, sodium, calcium, magnesium, iron and zinc in high amounts followed by many other beneficial nutrients (Table 2).

Table 1: Nutritional analysis of dried *C. album*

Constituents	Dried bathua (%)
Moisture	5.06
Ash	21
Protein	28.69
Fat	4.41
Carbohydrate	40.84
Dietary fiber	0.1

Table 2: Concentration of minerals in *C. album*

Analyte	Concentration (µg/gm)
K	81252
Na	5739
Sb	ND
As	ND
Be	ND
Cd	ND
Ca	14389
Cr	ND
Co	ND
Cu	11.4
Fe	152
Pb	ND
Li	ND
Mg	13101
Mn	119
Mo	ND
Ni	0.9
P	4197
Se	ND
Sr	228
Tl	ND
Ti	ND
Sn	ND
V	ND
Zn	48.6

A high content of potassium can provide relief from stroke, blood pressure, heart and kidney disorders, also enhance muscle strength, water balance, electrolytic functions, and nervous system. Calcium can play a crucial role in providing rigidity to the skeleton besides its involvement in the neuromuscular functions, blood clotting, and many other

metabolic processes [31]. It also contains iron which is used against anaemia, tuberculosis and disorders of growth [32]. Zinc supplementation in diabetes mellitus proved to have antioxidant effect [33].

3.2 Phytochemical analysis of *C. album*

The phytochemical content of bathua was analyzed and was found to be very promising. The values of saponins and crude alkaloids were determined on dry weight basis (g/100 g) (Table 3). 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 providing beneficial health effects. Therefore, total phenolic and flavonoid content of different extracts of bathua were estimated (Table 4). The total phenolic content was found in high amounts in the ethyl acetate extract of bathua. So, ethyl acetate (mid polar solvent) is efficient for the extraction of phenolic compounds of *C. album*. 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 [34].

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 [35-37]. The flavonoid content of the different extracts was also found to be quite high for a mixture of solvents.

Table 3: Phytochemical analysis of *C. album*

Components	Concentration (g/100 g DM)
Crude alkaloids	9.7
Saponins	0.46

Table 4: Polyphenolic compounds of *C. album*

Sample	Total phenolics (µg GAE/mg extract)	Total flavonoids (µg CE/mg extract)
PE extract	2.7	24.26
DCM extract	18.9	15.68
EA extract	57	24.21
ME extract	12.24	11.26
WE extract	19.69	3.04
Mixture of solvents (PE+DCM+EA+ME+WE)	18.44	42.74

3.3 Antioxidant activity

The antioxidant capacity of different extracts of bathua was evaluated against ascorbic acid as percent inhibition of ABTS free radicals. ABTS radical is a blue chromophore produced by the reaction between ABTS and potassium persulfate. The antioxidant activity (IC_{50} value) as determined by ABTS assay is listed in Table 5.

In FRAP assay, reduction of the ferric-tripyridyltriazine to the ferrous complex forms an intense blue color which can be measured at a wavelength of 593nm. The intensity of the color is related to the amount of antioxidant reductants in the samples. FRAP activity is listed in Table 6.

Table 5: ABTS assay of different extracts of *C. album*

Sample	IC_{50} (mg/ml)
PE extract	34
DCM extract	-
EA extract	-
ME extract	11
WE extract	2.9
Mixture of solvents (PE+DCM+EA+ME+WE)	-

Table 6: FRAP assay of different extracts of *C. album*

Sample	BHTE equivalents(µg BHTE/mg extract)
PE extract	26.33
DCM extract	70.16
EA extract	146.66
ME extract	54.16
WE extract	23.16
Mixture of solvents (PE+DCM+EA+ME+WE)	150.83

3.4 Characterization of GC/MS analysis

The details of some identified compounds (Figure 1(A) and (B)) present in the PE and MIX extract of bathua are grouped according to their chemical nature (Table 7 and 8). In PE extract of bathua, many saturated fatty acids: Hexadecanoic acid (used in cosmetics, soaps, antioxidant), Octadecanoic acid (softening agent; in soaps and shampoos), Heptadecanoic acid, Tetradecanoic acid (cosmetic usage), Nonanoic acid, Octadecane, Eicosane, gamma-Tocopherol (anticancer, antioxidant, anti-inflammatory, cardioprotective) and vitamin E were found. The presence of phytol, stigmasterol and campesterol in PE extract which are well known for their medical, cosmetic, and functional food applications, may contribute towards the antimicrobial and antioxidant activities. They are also known for their saturated fat reducing and cholesterol lowering activity and thus, may reduce the risk of heart diseases [38]. Phytol can be used as a precursor for manufacturing synthetic forms of vitamin E and vitamin K. Phytol is also used in various industries like fragrance, cosmetics, shampoos, toilet soaps, household cleaners, and detergents. In MIX extract, Hexadecanoic acid, Silicic acid (has the ability to reduce aluminium uptake as well as cause renal excretion of aluminium), Thujone (used in perfumery and has menthol odor) were found.

Table 7: GCMS profiling of PE extract of *C. album*

Compound name	RT(min)	% Area	Cas #
Tetradecanoic acid	16.839	0.68	000544-63-8
1,4-Eicosadiene	17.928	1.19	1000131-16-3
3,7,11,15-Tetramethyl-2-hexadecen-1-ol	18.128	1.35	102608-53-7
Hexadecanoic acid, methyl ester	18.606	0.19	000112-39-0
n-Hexadecanoic acid	19.283	12.88	000057-10-3
9-Tetradecenal	19.539	0.95	053939-27-8
8-Octadecenal	19.539	0.95	056554-94-0
Heptadecanoic acid	20.005	0.84	000506-12-7
Cyclooctene, 3-ethenyl	20.161	0.22	002213-60-7
9,12-octadecadienoic acid, methyl ester	20.294	0.40	002566-97-4
9,12,15-Octadecatrienoic acid, methyl ester	20.361	0.36	000301-00-8
Phytol	20.472	1.35	000150-86-7
Octadecanoic acid	21.138	2.59	000057-11-4
10,12-Hexadecadien-1-ol acetate	21.616	3.29	1000130-89-5
Cyclooctene, 3-ethenyl	21.727	1.42	002213-60-7
3-Heptadecen-5-yne	22.749	0.47	074744-55-1
2- Chloropropionic acid, hexadecyl ester	23.671	0.38	086711-81-1
Octadecane	24.460	0.28	000593-45-3
Eicosane	24.460	0.28	000112-95-8
Nonanoic acid	25.371	7.93	055268-58-1
Cyclotetracosane	27.070	5.27	000297-03-0
gamma.-Tocopherol	28.926	0.23	007616-22-0
Stigmastan-3,5-diene	29.692	0.62	1000214-16-4
Vitamin E	30.147	0.16	000059-02-9
Campesterol	31.836	0.17	000474-62-4
Stigmasterol, 22,23-dihydro-	33.736	1.66	1000214-20-7
beta-Sitosterol	33.736	1.66	000083-46-5

Table 8: GCMS profiling of MIX extract of *C. album*

Compound name	RT(min)	% Area	Cas #
2-Hexadecene,3,7,11,15-tetramethyl	18.990	0.38	014237-73-1
Cyclotetradecane	19.114	1.31	000295-17-0
n-Hexadecanoic acid	20.258	11.01	000057-10-3
9,12-Octadecadienoic acid, methyl ester	21.906	4.12	002462-85-3
7-Tetradecyne	21.906	4.12	035216-11-6
9,12,15-Octadecatrienoic acid	21.962	18.19	000463-40-1
Cyclododecyne	22.063	12.92	001129-90-4
Silicic acid, diethyl bis (trimethylsilyl) ester	26.258	1.25	003555-45-1
Thujone	19.495	1.38	000546-80-5

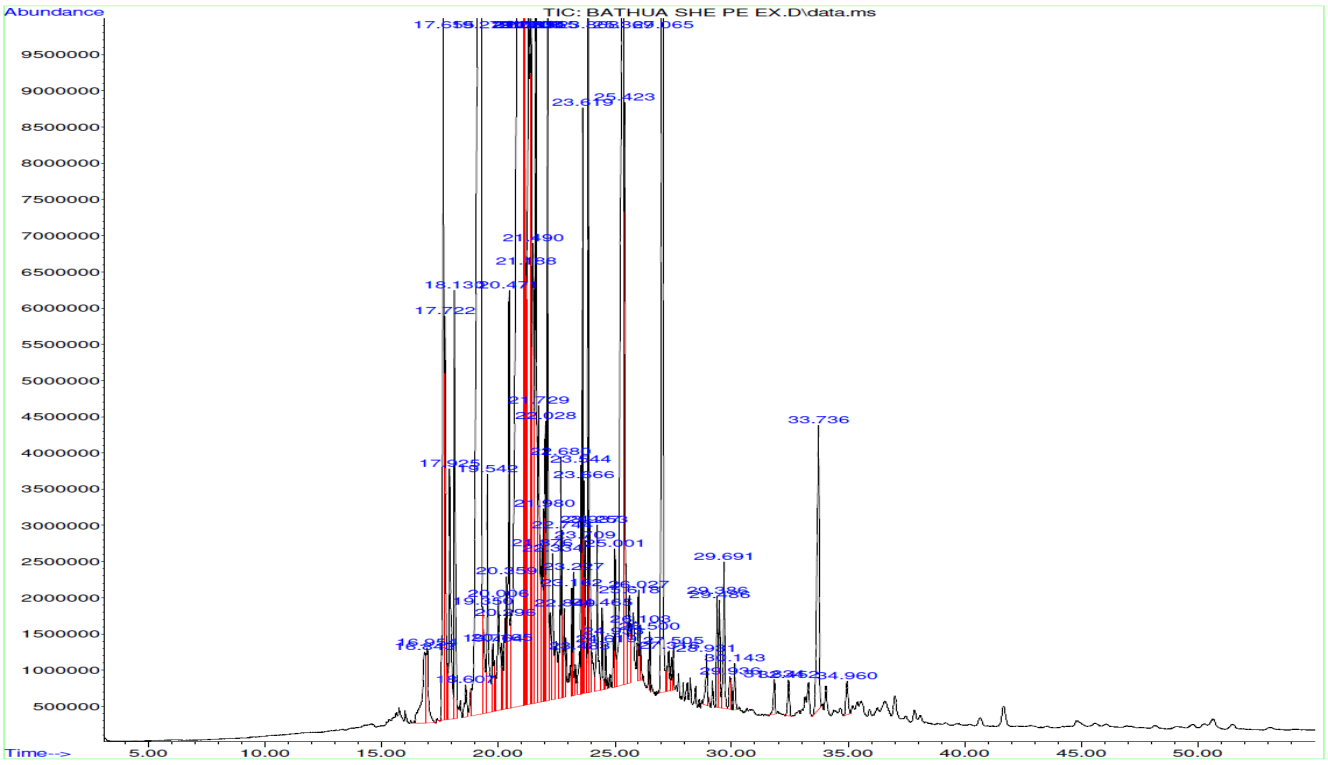

Fig 1(A): GC/MS chromatogram of PE extract of *C. album*

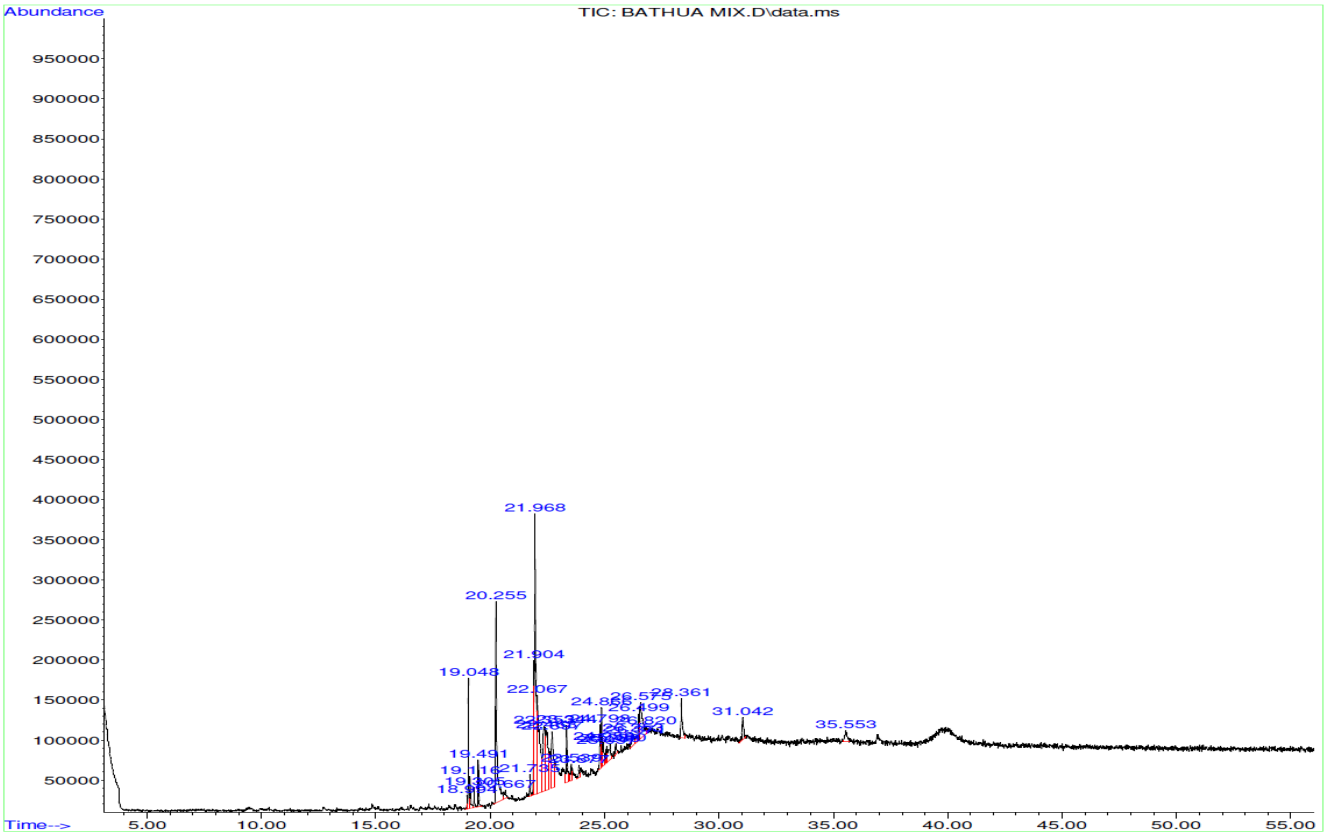

Fig 1(B): GC/MS chromatogram of MIX extract of *C. album*

3.5 Flavor and fragrance profiling of *C. album*

The flavor analysis of *C. album* (Figure 2) revealed the presence of compounds which are listed in Table 9.

Table 9: Flavor analysis of *C. album*

Compound name	RT(min)	% Area	Cas #
9-Tetradecenal, (Z)-	15.628	0.16	053939-27-8
1,13-Tetradecadiene	15.751	0.16	021964-49-8
Tetradecanoic acid	16.939	1.17	000544-63-8
1,4-Eicosadiene	17.928	1.03	1000131-16-3
Pentadecanoic acid, 14-methyl-, methyl ester	18.617	0.28	005129-60-2
n-Hexadecanoic acid	19.261	11.13	000057-10-3
Heptadecanoic acid	20.016	1.09	000506-12-7
Phytol	20.472	1.98	000150-86-7
9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	21.005	21.37	000463-40-1
Z,Z-10,12-Hexadecadien-1-ol acetate	21.616	4.17	1000130-89-5
Octadecane	24.460	0.55	000593-45-3
Cyclooctene, 3-ethenyl	25.626	0.86	002213-60-7
5,9,13-Pentadecatrien-2-one- 6,10,14-trimethyl	26.504	0.76	000762-29-8
Cyclotetracosane	27.048	4.92	000297-03-0
1-Nonadecene	28.103	0.25	018435-45-5
gamma.-Tocopherol	28.926	0.58	007616-22-0
Ergosta-4,6,22-trien-3-beta-ol	29.181	0.21	034026-93-2
Stigmastan-3,5-diene	29.692	0.68	1000214-16-4
Vitamin E	30.147	0.26	000059-02-9
Campesterol	31.836	0.29	000474-62-4
Stigmasterol	32.447	0.24	000083-48-7
beta-D-Mannofuranoside, farnesyl	33.291	0.44	1000155-15-5
Squalene	33.291	0.44	007683-64-9
beta-Sitosterol	33.736	1.61	000083-46-5

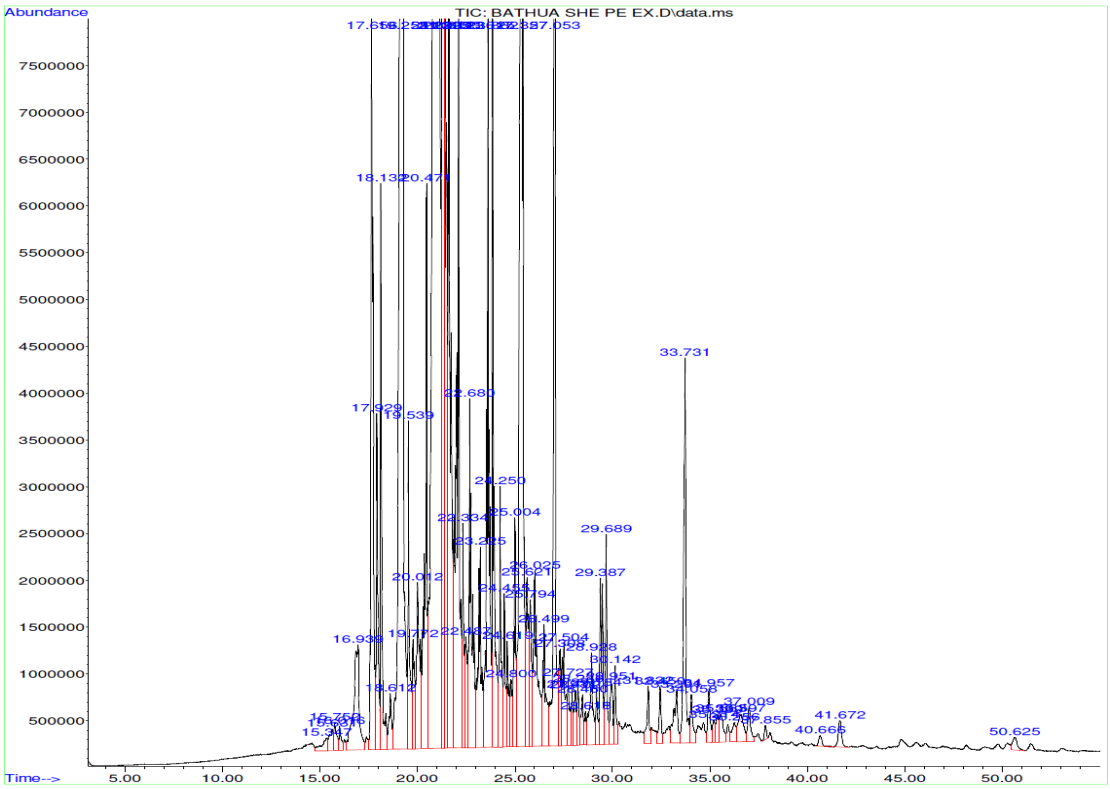

Fig 2: Chromatogram of flavor analysis of *C. album*

3.6 Antibacterial activity

Three gram positive (*Staphylococcus aureus, Staphylococcus epidermidis*, *Bacillus subtilis*) and two gram negative (*Escherichia coli, Proteus mirabilis*) bacteria were used to

evaluate the antibacterial activity of *Chenopodium album*. Agar well diffusion method was used to assess the activity against the bacteria by measuring zone of inhibition. Inhibiting concentrations used for all the extracts was 500 mg/ml. Methanolic extract of *C. album* was found to be most active among all other extracts and inhibits *Staphylococcus epidermidis*, *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*. The extract of a mixture of solvents was also found to be effective in inhibiting *Staphylococcus aureus*. Methanolic extract of *C. album* was found to give a most clear zone of inhibition. The antibacterial activity is attributed to the phenolic content present in the sample extract. The samples which had higher phenolic content were found to be better at inhibiting the growth of bacteria, hence giving zone of clearance of greater diameter. The zone of clearance or zone of inhibition of different extracts of *C. album* against various bacterial growths is listed in the Table 10.

Table 10: Antibacterial activity against various bacteria using the agar well diffusion method.

Bacterial strains	**Diameter of zone of inhibition(mm)**					
Gram positive	**PE extract**	**DCM extract**	**EA extract**	**ME extract**	**WE extract**	**MIX extract**
Staphylococcus aureus	-	-	-	20.5	-	16
Staphylococcus epidermidis	-	-	-	17.5	-	-
Bacillus subtilis	-	-	-	17	-	-
Gram negative						
Proteus mirabilis	-	-	-	-	-	-
Escherichia coli	-	-	-	18	-	-

4. Conclusion

"Bathua" was analyzed for nutritional, phytochemical, antioxidant and antibacterial activity for use as functional foods and nutraceutical and flavoring agents to provide health benefits. There are substantial anecdotal reports indicating that the consumption of bathua could ameliorate a wide range of illnesses. 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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6. References

1. Singh L, Yadav N, Kumar AR, Gupta AK, Chacko J, Parvin K, Tripathi U. Preparation of value added products from dehydrated bathua leaves (*Chenopodium album* L.). Natural Product Radiance 2007; 6:6-10.
2. Pande M, Pathak A. Preliminary Pharmacognostic Evaluations and Phytochemical Studies on Leaf of *Chenopodium album* (Bathua Sag). Asian Journal of Experimental Biological Sciences 2010; 1(1):91-95.
3. Afolayan J, Jimoh FO. Nutritional quality of some wild leafy vegetables in South Africa. International Journal of Food Sciences and Nutrition 2009; 60(5):424-431.
4. Hussain J, Khan AL, Rehman NU, Hamayun M, Shah T, Nisar M *et al*. Proximate and nutrient analysis of selected vegetable species: A case study of Karak region, Pakistan. African Journal of Biotechnology 2009; 8(12):2725-2729.
5. Gqaza BM, Njume C, Goduka NI, George G. Nutritional assessment of *Chenopodium album* L.(Imbikicane) Young Shoots and Mature Plant-Leaves Consumed in the Eastern Cape Province of South Africa. International Proceedings of Chemical, Biological and Environmental Engineering 2013; 53(19):97-102.
6. Dai Y, Ye WC, Wang ZT, Matsuda H, Kubo M, But PPH. Antipruritic and antinociceptive effects of *Chenopodium album* L. in mice. Journal of Ethnopharmacology 2002; 81: 245-250.
7. Kumar R, Mishra AK, Dubey NK, Tripathi YB. Evaluation of *Chenopodium ambrosioides* oil as a potential source of antifungal, antiaflatoxigenic and antioxidant activity. International Journal of Food Microbiology 2006; 115: 159-164.
8. Horio T, Yoshida K, Kikuchi H, Kawabata J, Mizutani J. A phenolic amide from roots of C*henopodium album*. Phytochemistry 1993; 33: 807-808.
9. Gohar AA, Elmazar MMA. Isolation of hypotensive flavonoids from *Chenopodium* species growing in Egypt. Phytotherapy Research 1997; 11: 564-567.
10. Lavaud C, Voutquenne L, Bal P, Pouny I. Saponins from *Chenopodium album*. Fitoterapia 2000; 71: 338-340.
11. Yadav SK, Sehgal S. In vitro and in vivo availability of iron from Bathua (*Chenopodium album*) and spinach (*Spinacia oleracea*) leaves. Journal of Food Science and Technology 2002; 39: 42-46.
12. Cutillo F, D'Abrosca B, Dellagreca M, Marino CD, Golino A, Previtera L, Zarrellia A. Cinnamic acid amides from *Chenopodium album*: effects on seeds germination and plant growth. Phytochemistry 2003; 64: 1381-1387.
13. Cutillo F, D'Abrosca B, DellaGreca M, Zarrelli A. Chenoalbicin, a novel cinnamic acid amide alkaloid from *Chenopodium album*. Chemistry and Biodiversity 2004; 1: 1579-1583.
14. DellaGreca M, DiMarino C, Zarrelli A, D'Abrosca B. Isolation and phytotoxicity of apocarotenoids from *Chenopodium album*. Journal of Natural Products 2004; 67: 1492-1495.
15. DellaGreca M, Previtera L, Zarrelli A. A new xyloside

from *Chenopodium album*. Natural Product Research 2005; 19: 87-90.
16. Cutillo F, DellaGreca M, Gionti M, Previtera L, Zarrelli A. Phenols and lignans from *Chenopodium album*. Phytochemical Analysis 2006; 17: 344-349.
17. Nigam V, Paarakh PM. Hepatoprotective activity of *Chenopodium album* L. against paracetamol induced liver damage. Pharmacologyonline 2011; 3: 312-328.
18. Jain NK, Singhai AK. Hepatoprotective activity of *Chenopodium album* L.: *in vitro* and *in vivo* studies. Journal of Experimental and Integrative Medicine 2012; 2(4): 331-336.
19. Baldi A, Choudhary NK. *In vitro* antioxidant and hepatoprotective potential of *Chenopodium album* extract. International Journal of Green Pharmacy 2013; 7(1):50-56.
20. Laghari AH, Memona S, Nelofar A, Khan KM, Yasmin A. Determination of free phenolic acids and antioxidant activity of methanolic extracts obtained from fruits and leaves of *Chenopodium album*. Food Chemistry 2011; 126: 1850–1855.
21. Jabeen K, Sherazi AZ, Iqbal S. Antifungal Potential of *Chenopodium album* L. against Chickpea Blight. Journal of Agricultural Science and Technology 2014; A(4):69-75.
22. Agrawal MY, Agrawal YP, Shamkuwar PB. Phytochemical and Biological Activities of *Chenopodium album.* International Journal of Pharm Tech Research 2014; 6(1):383-391.
23. Official methods of analysis, AOAC, Association of Official Analytical Chemists. Edn 16, Arlighton VA, USA, 1995.
24. Mattila P, Konko K, Eurola M, Pihlava JM, Astola J, Vanteristo L. Contents of vitamins, mineral elements, and some phenolic compounds in cultivated mushrooms. Journal of Agricultural and Food Chemistry 2001; 49:2343–2348.
25. Official Methods of Analysis, AOSC, Association of Official Analytical Chemists. Edn 12, Washington, D.C, 1975.
26. Herborne JB. Phytochemical methods. Chapman and Hall, London, 1973, 110−113.
27. Obadoni BO, Ochuko PO. Phytochemical studies and comparative efficacy of the crude extract of some homeostatic plants in Edo and Delta, States of Nigeria. Global Journal of Pure and Applied Sciences 2001; 8:203−208.
28. McDonald S, Prenzler PD, Antolovich M, Robards K. Phenolic content and antioxidant activity of olive extract. Food Chemistry 2001; 73:73-84.
29. Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C. Antioxidant activity applying an improved ABTS radical cation decolorisation assay. Free Radical Biology and Medicine 1999; 26:1231-1237.
30. Asp NG. Dietary carbohydrates: Classification by chemistry and physiology. Food Chemistry 1996; 7(1):9-14.
31. Campous D, Betalleluz I, Tauquino R, Chirinos R, and Pedreschi R. Nutritional and functional characterization of Andean chicuru (*Stangearhizanta*). Food Chemistry 2009; 112(1): 63−70.
32. Claude B, Paule S. The Manual of Natural Living. Edn 1, Biddles Limited, Guildford, Surrey, 1979, 98.
33. Roussel AM, Kerkeni A, Zouari N, Mahjoub S, Matheau JM, Anderson RA. Antioxidant effects of zinc supplementation in Tunisians with type 2 diabetes mellitus. Journal of American College of Nutrition 2003; 22(4):316-321.
34. Vaya J, Belinky PA, Aviram M. Antioxidant constituents from licorice roots: Isolation, structure elucidation and antioxidative capacity towards LDL Oxidation. Free Radical Biology Medicine 1997; 23:302-313.
35. Cook NC, Samman S. Flavonoids-chemistry, metabolism, cardioprotective effects, and dietary sources. Journal of Nutritional Biochemistry 1996; 7:66-76.
36. Havsteen BH. The biochemistry and medical significance of the flavonoids. Pharmacology and Therapeutics 2002; 96: 67-202.
37. Middleton E, Kandaswami C. Effects of flavonoids on immune and inflammatory cell function. Biochemical Pharmacology 1992; 43: 1167-1179.
38. Gabay O, Sanchez C, Salvat C, Chevy F, Breton M, Nourissat G, Wolf C, Jacques C, Berenbaum F. Stigmasterol: A phytosterol with potential anti-osteoarthritic properties. The American Journal of Clinical Nutrition 2010; 18(1):106−116