Indian Journal of Animal Sciences **91** (3): 239–244, March 2021/Article
https://doi.org/10.56093/ijans.v91i3.114152

Evaluation of nutritional value of prickly chaff flower (*Achyranthes aspera*) as fish feed ingredient

NEELESH KUMAR[1], JAIGOPAL SHARMA[1], GUDDU KUMAR[2], AVANISH KUMAR SHRIVASTAV[1],
NEHA TIWARI[1], AJIMA BEGUM[3] and RINA CHAKRABARTI[2]✉

Delhi Technological University, Bawana Road, New Delhi 110 042 India

Received: 26 June 2021; Accepted: 12 July 2021

ABSTRACT

The nutritional value of seeds and leaves of *Achyranthes aspera* as fish feed ingredients was evaluated. The protein and lipid contents were higher in seeds and ash content was higher in leaves. In seeds, total 18 essential and non-essential amino acids were present; total 17 amino acids were found in leaves, cysteine was absent. The essential and non-essential amino acid contents were higher in seeds compared to leaves except, leucine, tryptophan, valine, alanine and tyrosine. Taurine content was higher in leaves. Higher saturated, monounsaturated and *n*-6 polyunsaturated fatty acids (*n*-6 PUFA) contents were observed in seeds, whereas, *n*-3 PUFA level was higher in leaves. In seeds, oleic and linoleic acid levels were 1863.34±9.25 and 3342.05±8.05 mg/100 g, respectively; α-linolenic acids level in leaves was 1058.07±3.71 mg/100 g. Vitamins A, D_2, E, B_1, B_2, B_6, B_{12} and C were present in seeds; vitamins A, D_2, E, B_2 and B_{12} were found in leaves. In seeds and leaves, four macro, nine trace and four ultra-trace minerals were found and contents were higher in leaves, except zinc. In seeds and leaves, iron contents were 76.82±4.15 and 293.73±11.40 μg/g, respectively. Thus, seeds and leaves are rich sources of nutrients.

Keywords: α-linolenic acid, *Achyranthes aspera*, Amino acids, Linoleic acid, Minerals, Vitamins

Achyranthes aspera, the prickly chaff flower (AmeranthECeae), occupies a significant position in Ayurveda, Unani and folk medicines of India. The herb grows profusely in the tropical climate of Asia, South America and Africa. In India, the plant grows throughout the year. The plant has several medicinal applications, viz. for the treatment of diabetes, dysentery, fever, hypertension, asthma (Girach-Aminuddin and Khan 1992, Tang 1992, Liersch 1992). The roots are applied for the treatment of diarrhoea and cold in children; leaves are effective to treat asthma and seeds have emetic and hydrophobic properties (Bishit and Sandhu 1990, Borthakur and Goswami 1995, Singh 1995). The aqueous extract of the plant stimulates immune system of mice (Vasudeva *et al.* 2002). Even the roots, leaves and seeds of the plant improve the immune system of fishes and provide protection against bacterial pathogens (Rao *et al.* 2004, Rao *et al.* 2006, Rao and Chakrabarti 2005, Chakrabarti and Rao 2006, Chakrabarti and Rao 2012, Chakrabarti *et al.* 2012, Kumar *et al.* 2019, Sharma *et al.* 2019, Singh *et al.* 2019). The seeds protect fishes from harmful UV-B irradiation (Singh *et al.* 2013a, Singh *et al.* 2013b). Feeding of fish with *A. aspera* seeds and leaves enriched feeds always enhances the growth of fishes.

The scattered information are available on the composition of various parts of the plant. In seeds of *A. aspera*, two glycosides of oleanolic acid and two homogeneous saponins, saponin A and B are found (Hariharan and Rangaswami 1970). The seeds contain amino acids, fatty acids, different oleonic acid, saponins and ecdysterone (Varuna *et al.* 2010). The calorific value of seeds is 3.92/g (Goyal *et al.* 2007). In petroleum ether fraction of seeds, linolenic, oleic, palmitic and stearic acids are found; ecdysterone has been reported in the alcohol and aqueous alcohol extracts of the seeds (Chakrabarti *et al.* 2012). In root, stem and leaves extracts alkaloids, saponin, tannin, steroid reducing sugars, glycosides, phenolic compounds and protein are present (Dhale and Bhoi 2013). In leaves, 13 chemical compounds are isolated through HPLC and alkaloids are the dominant one (Meles *et al.* 2017). The proximate and mineral compositions of roots and leaves are documented (Joy *et al.* 2017, Rana *et al.* 2019). Review of literature shows the pharmatological importance of the plant and most of these studies are conducted in isolation. The present investigation has been designed to evaluate the nutritional value of seeds and leaves of *Achyranthes aspera* as fish feed ingredient. A holistic approach has been taken for their proper neutraceutical applications.

Present address: [1]Delhi Technological University, Bawana Road, New Delhi; [2]University of New Delhi, Delhi. [3]National Institute of Plant Genome Research (NIPGR), Aruna Asaf Ali Marg, New Delhi. ✉Corresponding author email: aquaresearchlab @yahoo.co.in

MATERIALS AND METHODS

Biochemical composition of leaves and seeds: A. aspera were cultivated in a specialized nursery in the outdoor facility (Fig. 1a, b). The mature seeds and leaves were collected. After air-drying, ground and powder was stored at 4°C for various assays. The proximate composition of leaves and seeds were analyzed following standard methods (AOAC 2000). Three replicates were used for each assay. Moisture content was estimated after drying the sample in an oven at 105°C. Oven dried sample was kept at 550°C for 8 h for the determination of ash contents of leaves and seeds. The crude protein content was analyzed using an automated Micro-Kjeldahl apparatus (N × 6.25). Crude lipid contents of leaves and seeds were assayed gravimetrically (Folch *et al.* 1957).

Fig. 1a. *Achyranthes aspera* with juicy leaves and young flowers. The plants were grown in the outdoor facility.

Fig. 1b. *Achyranthes aspera* with maturing seeds. The plants were grown in the outdoor facility.

Amino acids: Hitachi L-8900 Automatic Amino Acid Analyser (Tokyo, Japan) was used for estimation of amino acids present in the leaves and seeds. The first step of estimation was hydrolysis of the sample. The dried powder of leaves and seeds were hydrolysed with HCl (6 N) for estimation of all amino acids, except methionine, cysteine and tryptophan. The hydrolysis was performed at 110°C for 22 h. Methionine and cysteine were treated with performic acid and hydrobromic acid (48%); methanesulfonic acid (4N) and 3-(2-aminoethyl) indole was used for tryptophan estimation. In the second step, the hydrolysed sample was dried in a nitrogen evaporator and after drying, HCl (0.02 N) was added in the sample. The protein content of sample was 0.5 mg/mL. Finally, the sample was kept in the Auto sampler and 20 µL sample was injected for analysis. All amino acids were monitored at 570 nm except, proline and hydroxyproline; these were measured at 440 nm. Amino acids were quantified with Standards solution (WPCI, Japan).

Fatty acids: The leaves and seeds were analyzed for fatty acid profile study with Gas Chromatograph (GC)-Flame Ionization Detector, Clarus 580 (PerkinElmer, Waltham, USA). Total lipid was extracted from leaves and seeds samples (Folch *et al.* 1957). The fatty acid methyl esters, FAME (1 mg/mL) was prepared from the extracted lipid by transesterification using sulphuric acid in methanol at 50°C for 16 h (Christie 2003). After extraction and purification of FAME (Tocher and Harvie 1988), 1 mL sample was kept in a glass vial of Auto sampler of GC. In GC column (60 m ZB-wax, Phenomenex, UK), the sample was separated and quantified. The injector temperature was 260°C and nitrogen was used as a carrier gas (2 mL/min). The following conditions were set for oven ramping: initial setting at 150°C and hold for 3 min, then raising of temperature @ 2°C/min to 180°C hold for 2 min, again raising of temperature to 220°C @ 1°C/min and hold for 15 min. The data were collected from pre-installed programme software (TotalChrom Workstation Ver6.3, PerkinElmer, USA). Standard (Sigma-Aldrich, USA) was used for the identification of fatty acids.

Vitamins: Vitamin contents of leaves and seeds were analyzed with UHPLC (Thermo Fisher Scientific, USA) with C18 column (3 µM, 150×4.6 mm). The fat soluble vitamins were analyzed (Qian and Sheng 1998). In an extraction tube (10 mL), 4 mL of hexane was added to 1 g sample and flushed with nitrogen gas; then centrifuged at 1,500× g for 5 min. Supernatant (1 mL) was transferred in a vial (10 mL) and evaporated under nitrogen evaporator. The residue was dissolved in *n*-butanol (0.3 mL) and injected to the UHPLC. Mobile phase used for fat soluble vitamins was methanol and the flow rate was 1 mL/min. The vitamin levels were recorded at 290 nm.

The water soluble vitamins were analyzed (Sami *et al.* 2014). The dry sample (2 g) was hydrolyzed with 0.1 N H_2SO_4 at 121°C for 30 min for the estimation of thiamine (B_1), riboflavin (B_2), pyridoxine (B_6) and cobalamin (B_{12}). After bringing at room temperature, 2.5 M sodium acetate was mixed with the hydrolyzed sample and pH of the sample was 4.5. The enzyme takadiastase (50 mg) was added to the sample and incubated at 35°C for 12 h. Then the sample was filtered with Whatman filter paper (no. 4) and distilled water was added to make the volume 50 mL. Sample (2 g) was treated with a solution of metaphosphoric acid (0.3 M) and acetic acid (1.4 M) for the estimation of vitamin C. The sample was centrifuged at 9000 × g for 15 min and filtered with Whatman filter paper. Mobile phase used for water soluble vitamins was a mixture of 0.1 M potassium acetate (33 mL, pH 4.9) and 67 mL acetonitrile with water

(1:1); the flow rate was 1 mL/min. The absorbance was recorded at 254 nm at 25°C. The micropore (0.45 µm) syringe filter was used to filter the samples before injection; then placed into the UHPLC vial. Then 20 µL sample was injected automatically in UHPLC system.

Minerals: The ICP-MS (Inductively Coupled Plasma Mass Spectrometer, Agilent 7900, USA) was used for the study of mineral composition of leaves and seeds. The following conditions were used for the operation of the equipment - Nebulizer and auxillary gas flow were 1 L/min; plasma gas flow was 15 L/min, helium gas flow inside the reaction cell was 0.2 mL/min. Forward and reflected powers were set at 1500 W and 45 W, respectively. Analyzer vacuum pressure was maintained at 6×10^{-5} Pascal. In a closed digestion vessel, 150 mg sample (leaves/seeds powder) was taken and mixed with 8 mL of suprapure (69%) nitric acid (Merck, USA); then the sample was kept in a Microwave (Multiwave PRO; Anton Paar, Austria) for digestion. The digestion programme in the Microwave was as follows - first ramping at 1200 W for 15 min and then 30 min holding at 1200 W. The digested sample after cooling was taken in a separated tube and the volume was made 40 mL with Milli-Q water. The sample was filtered through syringe filter (0.2 µm, Thermo Scientific, USA) and the filtrate was collected in a glass vial. A 20 µL sample was injected automatically in the ICP-MS. Standard solution (Agilent Technologies, USA) for each mineral was diluted with Milli-Q water (containing 1% HNO_3) to make concentrations of 20, 40, 60, 80, 100, 250, 500, 1000 µg/L. The calibration (standard) curve was prepared. The Milli-Q water served as blank.

Statistical analysis: Data were expressed as mean ±SEM with a significance value using t-test statistic to compare the differences between leaves and seeds. Significance level was accepted at P<0.05.

RESULTS AND DISCUSSION

Biochemical analysis: Protein and lipid contents were significantly higher in seeds of *A. aspera* compared to the leaves. Significantly higher ash content was found in leaves compared to the seeds (Table 1).

A marked variation was found between the composition of seeds and leaves. In the present assay, plants are grown in a well planned nursery and used for various biochemical studies. So the results are reproducible–this is the advantages of the present investigation over the assays conducted with wild collections. It was interesting to see that protein and lipid levels were higher in seeds compared to the leaves, whereas maximum ash content was found in leaves. In Bangladesh, plants were collected from wild source and proximate composition of the leaves was assayed. The protein, lipid, ash and carbohydrate contents were 18.13±1.67, 1.88±0.20, 21.43±0.33 and 39.91±1.85 mg/100 g (DW), respectively in the leaves (Rana *et al.* 2019). In the present investigation, protein and lipid contents were higher and ash content was lower in nursery grown leaves compared to the wild collection in Bangladesh. Joy *et al.* (2017) reported the protein, lipids and ash levels as 25.76±3.71, 4.89±0.41 and 3.73±0.04 g/100 g, respectively in raw leaves. Therefore, a variation in the biochemical composition of leaves was recorded in different studies. The composition may vary depending on the environmental conditions, where the plant was grown.

Table 1. Proximate composition of *A. aspera* seeds and leaves (dry matter g/100 g)

Parameter	Seeds	Leaves
Protein	29.68±0.27^a	23.05±0.05^b
Lipid	4.36±0.01^a	2.50±0.08^b
Ash	2.74±0.05^b	15.47±0.12^a

Means (±SEM) sharing different letters in the same row are significantly (P<0.05) different (n=3).

Amino acids composition: Among essential amino acids, histidine, isoleucine, lysine, methionine and arginine were significantly higher in seeds compared to leaves. Leucine, tryptophan and valine levels were significantly higher in leaves compared to the seeds. The phenylalanine and threonine contents of leaves and seeds were not significantly different. All non-essential amino acids (except, alanine and tyrosine) contents were significantly higher in seeds compared to leaves. Alanine content was significantly higher in leaves compared to the seeds. The tyrosine level of leaves and seeds was not significantly different. Cysteine was absent in leaves (Table 2). Among free amino acids, phosphoserine and glutamine were present in seeds and absent in the leaves; α-amino adipic acid and hydroxylysine present in leaves were absent in seeds. Taurine was found in both leaves and seeds and the amount was significantly higher in leaves compared to the seeds. β-alanine and hydroxyproline levels were significantly higher in seeds compared to leaves, whereas β-amino isobutyric acid and γ-amino-n-butyric acid levels were significantly higher in leaves compared to the seeds. Total essential, non-essential and free amino acids contents were higher in seeds compared to the leaves (Table 2).

Essential and non-essential amino acids compositions were same in both seeds and leaves, except cysteine was absent in the leaves; the amount of individual amino acid was higher in seeds compared to leaves except, leucine, tryptophan and valine. These amino acids were higher in leaves. It was interesting that free amino acid taurine content was higher in leaves compared to the seeds of the plant. The amino acids like, leucine, isoleucine, phenylalanine and valine contents of *A. aspera* seeds were similar to Bengal gram; methionine and cysteine contents in seeds were higher than most pulses (Goyal *et al.* 2007). The present investigation confirmed the earlier findings of amino acid profile of seeds.

Fatty acid profile: The comparative study of fatty acid profiles of seeds and leaves showed that total saturated fatty acids (SFAs) and monounsaturated fatty acids (MUFAs) were around 2 and 9-fold higher in seeds compared to the leaves (Table 3). Among SFAs, carproic, caprylic, capric

Table 2. Amino acid profile of *A. aspera* (g/100 g dry weight)

Amino acid	Seeds	Leaves
Essential amino acids		
Histidine (His)	0.92±0.01^{a}	0.55±0.03^{b}
Isoleucine (Ile)	1.38±0.01^{a}	1.31±0.07^{b}
Leucine (Leu)	2.06±0.01^{b}	2.21±0.13^{a}
Lysine (Lys)	1.73±0.04^{a}	1.33±0.07^{b}
Methionine (Met)	0.61±0.02^{a}	0.50±0.04^{b}
Phenylalanine (Phe)	1.54±0.09^{a}	1.52±0.08^{a}
Threonine (Thr)	1.35±0.01^{a}	1.34±0.05^{a}
Tryptophan (Trp)	0.17±0.17^{b}	0.71±0.00^{a}
Valine (Val)	1.53±0.01^{b}	1.80±0.11^{a}
Arginine (Arg)	3.08±0.57^{a}	1.83±0.06^{b}
Σ Essential amino acids	14.37±1.92^{a}	13.11±0.64^{a}
Non-essential amino acids		
Alanine (Ala)	1.17±0.02^{b}	1.54±0.08^{a}
Aspartic acid (Asp)	3.03±0.02^{a}	2.67±0.14^{b}
Cysteine (Cys)	0.48±0.08^{a}	–
Glutamate (Glu)	7.22±0.01^{a}	3.44±0.19^{b}
Glycine (Gly)	2.83±0.02^{a}	1.61±0.09^{b}
Proline (Pro)	1.35±0.05^{a}	1.18±0.06^{b}
Serine (Ser)	1.59±0.01^{a}	1.16±0.03^{b}
Tyrosine (Tyr)	1.33±0.05^{a}	1.33±0.06^{a}
Σ Non-essential amino acids	18.99±0.66^{a}	12.93±1.34^{b}
Free amino acids		
Phosphoserine (p-ser)	0.13±0.02^{a}	–
Taurine (Tau)	0.08±0.01^{b}	0.13±0.01^{a}
Phospho ethanolamine (PEA)	0.04±0.01^{a}	0.04±0.02^{a}
Glutamine (GluNH$_2$)	0.01±0.00^{a}	–
Cystathionine (Cysthi)	0.30±0.01^{b}	0.32±0.05^{a}
β-Alanine (β-Ala)	0.26±0.01^{a}	0.18±0.01^{b}
β-Amino isobutyric acid (β-AiBA)	0.17±0.04^{b}	0.19±0.12^{a}
γ-Animo-n-butyric acid (γ-ABA)	0.13±0.07^{b}	0.19±0.01^{a}
1-Methylhistidine (1-Mehis)	0.09±0.01^{a}	0.11±0.02^{a}
Hydroxyproline (Hypro)	1.48±0.04^{a}	0.10±0.01^{b}
α-Amino adipic acid (α-AAA)	–	0.01±0.00^{a}
Hydroxylysine (Hylys)	–	0.04±0.01^{a}
Σ Free amino acids	2.67±0.23^{a}	1.31±0.53^{b}

Means (± SEM) sharing different letters in the same row are significantly ($P< 0.05$) different (n=3).

Table 3. Fatty acid profile of *A. aspera* seeds and leaves (mg/100 g)

Fatty acid	Formula	Seeds	Leaves
Saturated fatty acids			
Caproic acid	6:0	7.88±0.02^{a}	–
Caprylic acid	8:0	18.05±0.16^{a}	–
Capric acid	10:0	25.56±0.29^{a}	–
Lauric acid	12:0	5.50±0.37^{a}	–
Myristic acid	14:0	22.82±0.16^{a}	6.81±0.32^{b}
Palmitic acid	16:0	1780.41±8.14^{a}	910.71±2.32^{b}
Stearic acid	18:0	1.97±0.20^{b}	4.59±0.83^{a}
Arachidic acid	20:0	–	6.24±0.18^{a}
Lignoceric acid	24:0	22.07±1.37^{a}	10.92±0.81^{b}
Σ SFA		1884.28±7.84^{a}	939.27±3.66^{b}
Monounsaturated fatty acids			
Palmitoleic acid	16:1 n-7	–	45.83±0.83
Oleic acid	18:1 n-9	1863.34±9.25^{a}	164.81±0.62^{b}
Gondoic acid	20:1 n-9	104.25±0.88^{a}	–
Erucic acid	22:1 n-9	61.42±0.11^{a}	13.94±0.80b
Σ MUFA		2029.01±8.33^{a}	224.58±1.69^{b}
n-6 Polyunsaturated fatty acids			
Linoleic acid	18:2 n-6	3342.05±8.05^{a}	604.87±2.58^{b}
Σ n-6 PUFA		3342.05±8.05^{a}	604.87±2.58^{b}
n-3 Polyunsaturated fatty acids			
α-Linolenic acid	18:3 n-3	29.66±0.50^{b}	1058.07±3.71^{a}
Σ n-3 PUFA		29.66±0.50^{b}	1058.07±3.71^{a}

Means (±SEM) sharing different letters in the same row are significantly ($P < 0.05$) different (n = 3).

and lauric acids were present in seeds, absent in leaves. Arachidic acid was present in leaves and absent in the seeds. Palmitic acid was the dominant SFA in both seeds and leaves and maximum amount was present in the former one. Palmitoleic and gonodoic acids were absent in seeds and leaves, respectively. In seeds, oleic acid content was 11-fold higher compared to the leaves. Higher levels of linoleic acid, the *n*-6 polyunsaturated fatty acid (*n*-6 PUFA) and α-linolenic acid, the *n*-3 polyunsaturated fatty acids (*n*-3 PUFA) were found in seeds and leaves, respectively. In seeds, linoleic acid content was 3342.05±8.05 mg/100 g and in leaves α-linolenic acid level was 1058.07±3.71 mg/100 g.

Higher saturated, monounsaturated and *n*-6 PUFA contents were observed in seeds, whereas, *n*-3 PUFA level was higher in the leaves. Daulatabad and Ankalgi (1985) recorded the fatty acid composition of seeds. They reported that oleic (22.6%) and linoleic acids (49.4%) were dominant unsaturated fatty acids and palmitic (18.6%) acid was the major constituent of the saturated fatty acids; lauric (0.4%), myristic (1.2%), stearic (4.4%), arachidic (1.6%) and behenic (1.8%) acids were present in small amount in the seeds. The fatty acid profile of seeds of the present investigation is comparable with the previous data. In the present assay, oleic, linoleic and palmitic acids contents were 25.57, 45.87 and 24.44%, respectively. The arachidic and behenic acids were absent in seeds. In the present study, the arachidic acid was found in the leaves.

Vitamin contents: The retinol (vitamin A) content was same in seeds and leaves (Table 4). Vitamin D$_2$ (ergocalciferol) and vitamin E (tocopherol) levels were significantly higher in leaves compared to the seeds. Among water soluble vitamins, thiamine (B$_1$) and pyridoxine (B$_6$) were absent in leaves and present in the seeds; riboflavin (B$_2$) and cobalamin (B$_{12}$) contents were significantly higher in seeds compared to leaves. Vitamin C was present in seeds and absent in the leaves.

There were some differences in the vitamin composition between seeds and leaves, like, vitamin D$_2$ and vitamin E were higher in leaves compared to seeds; vitamins B$_1$ and B$_{12}$ were higher in seeds. The absence of vitamins B$_1$, B$_6$ and C in leaves was conspicuous. Fatima *et al*. (2014) measured various vitamins in the whole plant collected from

Table 4. Vitamin contents of seeds and leaves of *A. aspera* (mg/100 g, dry weight)

Vitamin	Seeds	Leaves
Fat soluble		
Retinol (A)	0.26 ± 0.08^{a}	0.26 ± 0.09^{a}
Ergocalciferol (D_2)	1.49 ± 0.01^{b}	69.53 ± 0.53^{a}
Tocopherol (E)	0.62 ± 0.07^{b}	34.92 ± 0.40^{a}
Water soluble		
Thiamine (B_1)	2.16 ± 0.15^{a}	–
Riboflavin (B_2)	137.43 ± 6.17^{a}	25.81 ± 2.75^{b}
Pyridoxine (B_6)	2.93 ± 0.19^{a}	–
Cobalamin (B_{12})	79.83 ± 8.40^{a}	1.34 ± 0.06^{b}
Ascorbic acid (C)	166.40 ± 3.41^{a}	–

Means (±SEM) sharing different letters in the same row are significantly ($P<0.05$) different (n=3).

Table 5. Minerals composition of *A. aspera* seeds and leaves (dry powder)

Mineral	Seeds	Leaves
Macro minerals (*mg/g*)		
Sodium (Na)	0.06 ± 0.01^{b}	0.15 ± 0.01^{a}
Potassium (K)	6.35 ± 0.04^{b}	27.50 ± 0.32^{a}
Calcium (Ca)	0.17 ± 0.01^{b}	2.10 ± 0.12^{a}
Magnesium (Mg)	2.18 ± 0.01^{b}	5.70 ± 0.17^{a}
Trace minerals (*μg/g*)		
Molybdenum (Mo)	0.28 ± 0.02^{b}	0.66 ± 0.10^{a}
Manganese (Mn)	30.20 ± 0.63^{b}	43.09 ± 1.58^{a}
Aluminium (Al)	41.07 ± 4.16^{b}	333.36 ± 16.62^{a}
Iron (Fe)	76.82 ± 4.15^{b}	296.73 ± 11.39^{a}
Zinc (Zn)	41.77 ± 0.18^{a}	36.05 ± 2.18^{b}
Copper (Cu)	7.67 ± 0.19^{b}	9.49 ± 0.57^{a}
Strontium (Sr)	3.39 ± 0.26^{b}	58.10 ± 1.54^{a}
Cadmium (Cd)	0.10 ± 0.04^{b}	0.62 ± 0.34^{a}
Lead (Pb)	0.21 ± 0.02^{b}	1.32 ± 0.06^{a}
Ultra-trace minerals (*μg/g*)		
Chromium (Cr)	2.18 ± 0.38^{b}	8.43 ± 1.39^{a}
Cobalt (Co)	0.09 ± 0.01^{b}	0.23 ± 0.04^{a}
Nickel (Ni)	1.35 ± 0.44^{b}	3.03 ± 0.23^{a}
Tin (Sn)	0.18 ± 0.04^{b}	0.38 ± 0.03^{a}

Means (±SEM) sharing different letters in the same row are significantly ($P<0.05$) different (n=3).

the local market. In the whole plant, vitamins B_1, B_2, B_3 and B_6 contents were 0.27, 0.28, 0.58 and 0.27 mg/100 g, respectively and vitamin B_9 content was 39 μg/100 g. These values were lower compared to the present study.

Mineral composition: All macro, trace and ultra-trace minerals contents (except, zinc) were significantly higher in leaves compared to seeds (Table 5). Zinc content was significantly higher in the latter compared to former one. In leaves, sodium, potassium, calcium and magnesium levels were 0.15±0.01, 27.50±0.32, 2.10±0.12 and 5.70±0.17 mg/100 g, respectively. Iron contents were 76.82±4.15 and 296.73±11.40 μg/100 g of seeds and leaves, respectively. Significantly higher level of cobalt (0.23±0.04 μg/g) was found in leaves compared to the seeds.

Like ash, most of the minerals (except zinc) were higher in the leaves compared to the seeds in the present assay. In the whole plant, different minerals, viz. calcium, sodium, potassium, magnesium, iron, copper, zinc etc. were observed (Fatima *et al*. 2014). Total 14 minerals were reported in the leaves of the plant; Ca and K were absent (Joy *et al*. 2017). The present assay showed presence of 17 minerals (four macro, nine trace and four ultra-trace minerals) in both leaves and seeds.

In conclusion, the present investigation helps to understand the nutritional and pharmaceutical values of the seeds and leaves of *A. aspera*. Seeds and leaves are rich sources for amino acids, fatty acids, vitamins and minerals and have ample scope for application in fish feed industry.

ACKNOWLEDGEMENTS

Authors are thankful to Department of Biotechnology, DBT for financial support to carry out this study (Project: 102/IFD/SAN/4260/2014-2015). NK expresses his sincere thanks to DST, the Department of Science and Technology, Government of India for providing him Inspire Fellowship (DST/INSPIRE Fellowship/2017/IF170573).

REFERENCES

Association of Official Analytical Chemists (AOAC). 2000. Official Methods of Analysis. 17th edn. Association of Official Analytical Chemists Inc, Gaithersburg, MD, USA.

Bishit G and Sandhu H. 1990. Chemical constituents and antimicrobial activity of *Achyranthes bidentata*. *Journal of the Indian Chemical Society* **67**: 1002–08.

Borthakur S K and Goswami N. 1995. Herbal remedies from Dimoria of Kamrup district of Assam in northeastern India. *Fitoterapia* **66**: 333–40.

Chakrabarti R and Vasudeva R Y. 2006. *Achyranthes aspera* stimulates the immunity and enhances the antigen clearance in *Catla catla*. Int. Immunopharmacol. **6**: 782–90.

Chakrabarti R and Rao Y V. 2012. *Achyranthes aspera* enhances immunity and antigen clearance in common carp, *Cyprinus carpio* L. *Journal of Fish Diseases* **35**: 389–92.

Chakrabarti R, Srivastava P K, Kundu K, Khare R S and Banerjee S. 2012. Evaluation of immunostimulatory and growth promoting effect of seed fractions of *Achyranthes aspera* in common carp *Cyprinus carpio* and identification of active constituents. *Fish Shellfish Immunology* **32**: 839–43.

Christie W W. 2003. *Lipid Analysis*, fourth edn. Bridgewater, Oily Press, United Kingdom.

Daulatabad C D and Ankalgi R F. 1985. Minor seed oils, 2: Fatty acid composition of some seed oils. *Fette Seifen Anstrichm* **87**: 196–97.

Dhale D A and Bhoi S. 2013. Pharmacognostic characterization and phytochemical screening of *Achyranthes aspera* Linn. *Current Agriculture Research Journal* **1**: 51–57.

Fatima N, Dar N G, Imran H, Sohail T, Asghar U, Yaqeen Z and Jamil K. 2014. Evaluation of nutritional and sub-acute toxicological study of plant based supplement of *Achyranthes aspera*. *Pakistan Journal of Pharmaceutical Sciences* **27**: 1199–1202.

Folch J, Lees M and Stanley G S. 1957. A simple method for the isolation and purification of total lipids from animal tissues. *Journal of Biological Chemistry* **226**: 497–509.

Girach R D and Khan S A. 1992. Ethnomedicinal uses of *Achyranthes aspera* L. in Orissa (India). *International Journal of Pharmacognsy* **30**: 113–15.

Goyal B R, Goyal R K and Mehta A A. 2007. Plant review phytopharmacology of *Achyranthes aspera:* A review. *Pharmacognsy Reviews* **1**: 143–50.

Hariharan V and Rangaswami S. 1970. Structure of saponins A and B from the seeds of *Achyranthes aspera*. *Phytochemistry* **9**: 409–14.

Joy J K, Aswini C and Siddhuraju P. 2017. Studies on mineral profile and antioxidant potential of differentially processed lesser known green leafy vegetables (*Achyranthes aspera* L. and *Talinum triangulare* L.). *International Journal of Food Science and Nutrition* **2**: 36–44.

Kumar N, Sharma J, Singh S P, Singh A, Krishna V H and Chakrabarti R. 2019. Validation of growth enhancing, immunostimulatory and disease resistance properties of *Achyranthes aspera* in *Labeo rohita* fry in pond conditions. *Heliyon* **5**: e01246.

Liersch B K H R. 1992. Achyranthes (Eds) Haensel R, Kelelr K, Rimpler G and Schneider G. Hagers handbuch der pharmazeutischen praxi. Springer-Verlag Berlin Heidelberg, New York, pp. 54–59.

Meles D K, Wurlina W and Adnyana D P A. 2017. Measurement of alkaloids *Achyranthes aspera* Linn level using thin layer chromatography method and high-performance liquid chromatography. *KnE Life Sciences* **2017**: 378–85.

Qian H and Sheng M. 1998. Simultaneous determination of fat-soluble vitamins A, D and E and pro-vitamin D2 in animal feeds by one-step extraction and high-performance liquid chromatography analysis. *Journal of Chromatography A* **825**: 127–33.

Rana Z H, Alam M K and Akhtaruzzaman M. 2019. Nutritional composition, total phenolic content, antioxidant and α-amylase inhibitory activities of different fractions of selected wild edible plants. *Antioxidants* **8**: 203.

Rao Y V and Chakrabarti R. 2005. Dietary incorporation of *Achyranthes aspera* seed influences the immunity of common carp *Cyprinus carpio*. *Indian Journal of Animal Sciences* **75**: 1097–102.

Rao Y V, Romesh M, Singh A and Chakrabarti R. 2004. Potentiation of antibody production in Indian major carp *Labeo rohita*, rohu, by *Achyranthes aspera* as a herbal feed ingredient. *Aquaculture* **238**: 67–73.

Rao Y V, Das B K, Jyotyrmayee P and Chakrabarti R. 2006. Effect of *Achyranthes aspera* on the immunity and survival of *Labeo rohita* infected with *Aeromonas hydrophila*. *Fish Shellfish Immunology* **20**: 263–73.

Sami R, Li Y, Qi B, Wang S, Zhang Q, Han F and Jiang L. 2014. HPLC analysis of water-soluble vitamins (B_2, B_3, B_6, B_{12}, and C) and fat-soluble vitamins (E, K, D, A, and β-carotene) of okra (*Abelmoschus esculentus*). *Journal of Chemistry* **2014**: 831357.

Sharma J, Kumar N, Singh S P, Singh A, HariKrishna V and Chakrabarti R. 2019. Evaluation of immunostimulatory properties of prickly chaff flower *Achyranthes aspera* in rohu *Labeo rohita* fry in pond conditions. *Aquaculture* **505**: 183–89.

Singh V. 1995. Traditional remedies to treat asthma in North West and Trans-Himalayan region in J. & K. state. *Fitoterapia* **66**: 507–09.

Singh M K, Sharma J and Chakrabarti R. 2013a. Effect of UV-B radiation on the defence system of *Labeo rohita* (Actinopterygii: Cypriniformes: Cyprinidae) larvae and its modulation by seed of devil's horsewhip, *Achyranthes aspera*. *Acta Ichthyologica et Piscatoria* **43**: 119–26.

Singh M K, Sharma J and Chakrabarti R. 2013b. Impact of UV-B radiation on the physiology of freshwater carp *Labeo rohita* larvae and evaluation of UV-B protective properties of seeds of *Achyranthes aspera* and vitamin C. *Agriculture Research* **2**: 166–71.

Singh A, Sharma J, Paichha M and Chakrabarti R. 2019. *Achyranthes aspera* (Prickly chaff flower) leaves-and seeds-supplemented diets regulate growth, innate immunity, and oxidative stress in *Aeromonas hydrophila*-challenged *Labeo rohita*. *Journal of Applied Aquaculture* **32**: 250–67.

Tang W and Eisenbrand G. 1992. Chinese Drugs of Plant Origin: Chemistry, Pharmacology, and Use in Traditional and Modern Medicine. Springer Berlin Heidelberg, New York.

Tocher D R and Harvie D G. 1988. Fatty acid compositions of the major phosphoglycerides from fish neural tissues; (*n*–3) and (*n*–6) polyunsaturated fatty acids in rainbow trout (*Salmo gairdneri*) and cod (*Gadus morhua*) brains and retinas. *Fish Physiology and Biochemistry* **5**: 229–39.

Varuna K M, Khan M U and Sharma P K. 2010. Review on *Achyranthes aspera*. *Journal of Pharmaceutical Research* **3**: 714–17.

Vasudeva R Y, Duddukuri G R, Sunil B G and Athota R R. 2002. Immunomodulatory activity of *Achyranthes aspera* on the elicitation of antigen-specific murine antibody response. *Pharmaceutical Biology* **40**: 175–78.