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Full Length Research Paper

Phytochemical screening, mineral and proximate composition of *Asteracantha longifolia* leaf extracts as a quality livestock feed

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Fractions of hot water extract of *Asteracantha longifolia* Nees leaf and aerial parts were subjected to qualitative and quantitative screening of different phytochemicals and for quantitative determination of different micro and macro elements. Dried leaf powder was analyzed for proximate composition and quantitative determination of different micro and macro elements. Proximate composition of the leaf revealed 14.212±0.375% of ash, 18.413±0.258% of protein, 3.401±0.081% of ether extract and 41.205±0.573% of fiber. Qualitative phytochemical screening of fractions of hot water extract of leaves revealed the presence of alkaloids, carbohydrates, proteins, phenolic compounds and flavonoids in all the fractions in addition to presence of proteins containing aromatic amino acids in aqueous fraction. Total phenolic compounds and total antioxidants in all the three fractions were in the range of 0.094 to 0.14 mg of gallic acid equivalent mg^{-1} extract and 40.95 to 317.5 µM of ascorbic acid equivalent mg^{-1} extract, respectively. Relatively low concentration of total antioxidant was found in residual fraction. Flavonoid concentrations were found in the range of 0.17±0.03 to 3.17±0.04 mg of quercetin equivalent g^{-1} extract, highest being the aqueous fraction and lowest the residual fraction. The residual fraction was found to contain a higher concentration of iron (Fe), cobalt (Co), copper (Cu), zinc (Zn), calcium (Ca), magnesium (Mg) and phosphorus (P). The level of sodium (Na) and potassium (K) were higher in methanolic fraction and hot water extract, respectively. The incorporation of *A. longifolia* can be a potential source of antioxidant, macro and micro minerals for manufacturing nutraceutical and improving livestock feed.

Key words: Qualitative, quantitative, phytochemical screening, mineral, antioxidant, phenolics.

INTRODUCTION

Phytochemicals are naturally occurring and non–nutritive chemical compounds in plants having protective or disease preventive properties. They are found in fruits, vegetables, beans, grains, and other plants. Scientists have identified thousands of phytochemicals, although only a small fraction has been studied closely (Djeridane et al., 2006). Some of the more commonly known phytochemicals include beta carotene, ascorbic acid (vitamin C), folic acid and vitamin E. Antioxidants protect the cell against free radicals and they are essential in

obtaining and preserving good health. Antioxidants have been widely used as food additives to provide protection against oxidative degradation of foods and to extend their shelf-life (Djeridane et al., 2006). Antioxidants may act as free radical scavengers, reducing agents, chelating agents for transition metals, quenchers of singlet oxygen molecules and/or activators of antioxidative defense enzyme systems to suppress the radical damages in biological systems (Yu et al., 2002; Prior et al., 2005). Hence, in recent days much attention is being focused on the use of antioxidants to protect the cells from biological damages like free radicals (Kiselova et al., 2006). Plant originated polyphenols and flavonoids have powerful anti-oxidant activities. These phytochemicals are able to scavenge a wide range of reactive oxygen species, including hydroxyl radicals, peroxyl radicals, hypochlorous radicals and superoxide radicals inhibiting lipid peroxidation in human tissues (Sugihara et al., 1999; Rice-Evans et al., 2007). Polyphenols in plants include simple phenols, phenolic acids (both benzoic and cinnamic acid derivatives), coumarins, flavonoids, stilbenes, hydrolyzable and condensed tannins, lignans, and lignins (Dixon and Paiva, 1995).

The genus *Asterocantha*, perennial angiospermic plant of family Acanthaceae, is a commonly found herb in India being used as vegetable in some states like Odisha, Chhattisgarh and West Bengal. Boiled aerial parts of succulent plant of pre-flowering and flowering stages are used extensively to increase the haemoglobin status by the rural people of these states. This herbal remedy is devoid of any side effects with proven effectiveness. *Asteracantha longifolia* Nees [Synonym(s) *Hygrophila spinosa* T. Anders] contains various groups of phyto-constituents viz. phytosterols, fatty acids, minerals, polyphenols, proanthocyanins, mucilage, alkaloids, enzymes, amino acids, carbohydrates, hydrocarbons, flavonoids, terpenoids, vitamins, glycosides, etc. and is useful in the treatment of anasaraca, diseases of urinogenital tract, dropsy of chronic Bright's disease, hyperdipsia, vesical calculi, flatulence, diarrhea, dysentery, leucorrhoea, gonorrhoea, asthma, blood diseases, gastric diseases, painful micturition, menorrhagea, etc. (Rastogi and Mehrotra 1993; Annonymous, 2002; Sharma et al., 2002; Asolkar et al., 2005; Nadkarni, 2007). In the rural areas of West Bengal, feeding the hot water infusion of succulent aerial parts of pre-flowering and flowering plant to pregnant women is a common practice to prevent anaemia.

In the era of organic farming, *A. longifolia* Nees has attracted the special attention in the treatment of anaemia and ethanolic extract of the aerial part was reported to increase hemopoietic parameters (Dasgupta et al., 2001). Pre-flowering plant extracts are reported to demonstrate better haematinic effect than the post-flowering extract in induced anemic Wistar rats (Mandal, 2008; Thakur, 2008). *H. spinosa* hot water extract alone exhibited a better effect than conventional haematinic mixture and long term use of *H. spinosa* hot water extract and in combination with haematinics could better ameliorate the condition of Wistar rats with induced anemia (Mandal, 2008). *A. longifolia* is also reported to be equally effective as haematinic in goats (Basir, 2009). Due to paucity of literature on composition of hot water extract of *A. longifolia* and its various fractions, the present study was undertaken to evaluate its proximate composition, qualitative phytochemical screening, quantitative estimation of minerals and various biochemical constituents.

MATERIALS AND METHODS

Collection of leaf, identification and proximate composition of leaf powder

Fresh leaf and aerial parts of *A. longifolia* Nees at its pre-flowering stage were collected from waterlogged area of the College of Veterinary Science and Animal Husbandry, Indira Gandhi Krishi Viswa Vidyalaya, Anjora, Durg, Chattishgarh, India. The plant was authenticated by Dr. Smt. Ranjana Shrivastava, Professor and Head, Department of Botany and Microbiology, Government VYT PG Autonomous College, Durg, having specimen no. 04/EVM/Durg and a voucher specimen was preserved for further references. The leaf and areal parts were cleaned, shade-dried and ground to fine powder form using domestic mixer grinder and the leaf powders were subjected to proximate analysis as per AOAC (1984).

Preparation of extract and fractionation

Finely ground powder (80 g) was dispersed in 1 L of boiled distilled water and cooled at room temperature in a closed glass container. The content was filtered with the help of double-layered muslin cloth to obtain hot water extract. To free crude material, filtrate was centrifuged at 5000 rpm for 10 min and then supernatant was filtered using Watman's filter paper No 40. The extract obtained after filtration was dried using a rotary vacuum evaporator at 60°C temperature and low pressure. After complete evaporation of the water, the weight of the extract was noted and the percent (%) of recovery of extract was recorded on dry weight basis. The hot water extract thus obtained was subjected to fractionation according to solubility in methanol and triple distilled water at room temperature. Briefly, the methanol soluble fraction was removed from the hot water extract followed by aqueous fraction. The leftover fraction of the hot water extract (primary extract) was also collected as third fraction (residual fraction) and the resulted fractions were subject to drying at 60°C and percent of recovery was noted on dry weight basis of hot water extract. The pH of the hot water extract solution and residue solution followed by separation of methanolic and residual fractions were also determined. At each step of separation, the change in pH was also recorded by using digital pH/mV meter (MSW- 552, MAC, India).

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Table 1. Percentage yield of crude extract and different fractions of *A. longifolia* leaf and aerial parts on dry weight basis.

Name of fraction	Percentage of yield
Crude fraction (Hot water extract)	6.3 ± 0.75
Methanolic fraction	36.56 ± 0.87
Aqueous fraction	57.76 ± 1.77
Residual fraction	5.68 ± 1.98

Each value is expressed as mean ± standard error (n = 9).

Phytochemical screening

The different qualitative chemical tests for detection of alkaloids (Wagner, 1993; Wagner et al., 1996; Evans, 1997), carbohydrates and glycosides (Ramakrishnan and Rajan, 1994), saponins (Kokate, 1999), proteins (Yasuma and Ichikawa, 1953; Rasch and Swift, 1960; Fisher, 1968; Ruthmann, 1970; Gahan, 1984), phenolic compounds (Mace, 1963; Evans, 1997) and flavonoids (Alkaline reagent test) were performed for establishing the profile of extracts for its chemical composition (phyto-constituents). Phenolic compounds are a class of antioxidant agents which act as free radical terminators (Shahidi and Wanasundara, 1992) and are considered as a major group of compounds that contribute to the antioxidant activities of plant materials because of their scavenging ability on free radicals due to their hydroxyl groups (Djeridane et al., 2006). Flavonoids are a group of polyphenolic compounds with known properties of free radical scavenging, antibacterial and anti-inflammatory action (Balasundram et al., 2005).

Quantification of total antioxidant, phenolics and flavonoids

The total antioxidant capacity of plant extracts was evaluated by the method of Prieto et al. (1999). The assay was based on the reduction of Mo (VI) to Mo (V) by the extract and subsequent formation of a green phosphate / Mo (V) complex at acid pH. Briefly describing, an aliquot of 0.1 ml of sample solution was combined with 1.0 ml of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). For the blank, 0.1 ml of tripple distilled water was used in place of sample. The tubes were capped and incubated in a boiling water bath at 95°C for 90 min. After the samples were cooled to room temperature, the absorbance of the aqueous solution of each sample was measured at 695 nm against a blank (ʎ- 25 UV-visible spectrophotometer, Perkin -Elmer, USA). Antioxidant capacity of samples was expressed as equivalents of ascorbic acid (µM mg^{-1} of extract). The concentration of phenolics in the extracts was determined by the method of Singh et al. (2002) and results were expressed as gallic acid equivalents (mg mg^{-1} of extract). Sample (0.2 ml) was mixed with 1.0 ml of 10-fold diluted Folin–Ciocalteu reagent and 0.8 ml of 7.5% sodium carbonate solution. After standing for 30 min at room temperature, the absorbance was measured at 765 nm (ʎ25 UV-visible spectrophotometer, Perkin -Elmer, USA). In place of plant sample, 0.2 ml of triple distilled water was taken as blank. Total flavonoid content was determined (Ordon et al., 2006) with slight modification. Briefly, 0.5 ml of 2% ethanolic solution of aluminium chloride was added to 0.5 ml of sample. After incubation for 1 h at room temperature, the absorbance was measured at 420 nm (ʎ- 25 UV-visible spectrophotometer, Perkin -Elmer, USA). Flavonoid concentration of the extract was expressed as mg g^{-1} equivalent of quercetin.

Mineral concentration

Trace minerals namely Cu, Co, Fe, and Zn were estimated in concentrate hot water extract of *A. longifolia* leaf and resultant three fractions by using an atomic absorption spectrophotometer (AAS 4141, ECIL-Elements, India) and macro- minerals like Na and K were measured by using flame photometer (Model no. 1381, ESPIO, Japan). The accurately weighed amount of samples (0.215, 0.215, 0.215, 0.214 and 0.114 g, respectively) were digested by 10 ml of Tri acid solution (Nitric acid: sulphuric acid: perchloric acid-9:2:1) and then volume was made up to 25 ml by adding triple distilled water to the digested solution. Ca was estimated by Arsenazo method [Kit supplied by Reckon Diagnostics Pvt. Ltd, India (Chempak)], inorganic phosphorus (Pi) by phosphomolybdate complex method [kit supplied by Chemelex, S.A., India]. Magnesium in the hot water extract and its fractions was estimated by kit supplied by Lab-Care Diagnostics (India) Pvt. Ltd.(Accucare™ Magnesium Xylidyl Blue). For estimation of Mg in leaf powder, leaf powder was digested in tri-acid solution and was estimated using an atomic absorption spectrophotometer (ECIL-Elements AAS 4141). All the results were expressed as µg mg^{-1} of extract.

Statistical analysis

Data obtained from the present study was subject to one way ANOVA using SPSS software (Version SPSS, 1999, SPSS I User's Guide; release 10.0.1 ed. SPSS Inc. USA, 1999) and the results were expressed as Mean ± SEM. Significant differences among means were found out using Duncan's Multiple Range Test (DMRT) at level less than or equal to 5% ($P \leq 0.05$).

RESULTS AND DISCUSSION

Percent recovery of powder and fractions and their pH

The yield of leaf and aerial parts of *A. Longifolia* Nees plant was observed to be 12.12% at preflowering stage which was slightly higher compared to dry matter 10.3% in leaves and shoots combindly (Banerjee and Matai, 1990). The hot water extract yielded 6.3% of dry weight of leaf and was in conformation with the findings of Mandal (2008) and Thakur (2008). Percent recoveries of methanolic, aqueous and residual fractions of hot-water extract were 36.56±0.87, 57.76±1.77 and 5.68±1.98, respectively (Table 1). The pH of solution after separation of each fraction revealed that the methanolic fraction, residual fraction and aqueous fraction were acidic, alkaline and slightly acidic in nature, respectively (Table 2). pH of crude extract was found to be significantly lower than its fractions ($p \leq 0.05$) but all have pH values nearer to the physiological pH.

Table 2. pH of crude extract and pH during fractionation of *A. longifolia* leaf and aerial parts.

Name of extract or stage of fractionation	pH
Crude fraction (hot water extract)	6.60±0.007[a]
Aqueous fraction + residual fraction (after removal of methanolic fraction)	7.01±0.08[b]
Aqueous fraction (after removal of both methanolic and residual fraction)	6.92±0.07[b]

Each value is expressed as mean ± standard error (n = 6). Means with the different letters differ significantly (P < 0.05).

Proximate composition of leaf

The proximate analysis values of leaf on dry matter basis in the present study were found to be crude protein (18.41±0.26%), ash (14.21±0.38%), ether extract (3.40±0.08%), crude fiber (22.77±0.37%) and nitrogen free extract (41.21±0.57%) which differ from the values of crude protein (20.6%), ash (18.3%), crude fat (3.4%), crude fiber (21.3%) and nitrogen free extract (36.4%) (Banerjee and Matai, 1990). This variation might be attributed to different stage of harvest and variation in soil nutrient due to different agro climatic zone.

Phytochemical screening of *A. longifolia* fractions

The phytochemical screening revealed the presence of alkaloids, carbohydrates, proteins, phenolic compounds and flavonoids in all the fractions. But a positive result with Millon's test was found only with aqueous fraction which indicates the presence of protein containing aromatic amino acids (Table 3). Our findings were in support with the studies conducted by Mandal et al. (2010) who reported the presence of alkaloids, carbohydrates and phenolic compounds in hot water extract, proteins in petroleum ether extract; alkaloids and proteins in chloroform extract; alkaloids, carbohydrates, proteins, steroids, tannins and phenolic compounds in alcoholic extract; carbohydrates, proteins, amino acids, tannins and phenolic compounds in aqueous extract (Patra et al., 2009). The present study demonstrated presence of phenolics and flavonoids in methanolic extract of leaves, having promising antioxidant activity as confirmed by Sawadogo et al. (2006).

Total antioxidant, phenolics and flavonoid concentration

Hot water extract and fractions were subjected to quantitative estimation of phenolic compounds, total antioxidant and flavonoid content (Table 4), revealed the presence of significantly high concentration of phenolic compounds in hot water extract, aqueous fraction and residual fraction ($P \leq 0.05$) and a significantly low concentration of phenolic compounds in methanolic fraction ($p \leq 0.05$) compared to all other fractions as reported to be 17.75±0.03 g $100g^{-1}$ (0.17 mg mg^{-1}) of aqueous acetone extract of leaf and stem taken combindly (Sawadogo et al., 2006). Total antioxidant (µM of ascorbic acid equivalent mg^{-1} of extract) of hot water extract and all the three fractions ranged from 40.94±1.74 to 317.50±3.73 µM. A significantly low concentration of total antioxidant content in the present study was found in residual fraction ($P \leq 0.05$). Dasgupta and De (2007) found the total antioxidant activity of hot water extract of whole plant to be 67.7 µg ascorbic acid equivalent / mg of dried plant. Flavonoid concentration ranged from 0.17±0.03 to 3.17±0.04 mg quercetin equivalent /g with significantly highest in aqueous fraction and lowest in residual fraction ($P \leq 0.05$) which is in line with the findings of Sawadogo et al. (2006) (0.033±0.002 gQE/100 g) who have recorded total flavonoid content of the same plant using 80% acetone as solvent. Similarly, Dasgupta and De (2007) reported the flavonoid content in the hot water extract of whole plant as 12.6 µg catechin equivalent / mg of dried plant material.

Mineral concentration

Mineral concentration of hot water extract and three fractions (Table 5) evidenced that residual fraction contained a significantly higher ($P \leq 0.05$) concentration of Fe, Co, Cu, Zn, Ca, Mg and P than hot water extract as well as other fractions, but Na and K concentrations were found significantly higher ($P \leq 0.05$) in methanolic fraction and hot water extract, respectively, compared to others. The iron content in hot water extract and in different fractions were much lower compared to that obtained in ethanolic extract 622 µg 50 mg^{-1} (Pawar et al., 2010). Minerals viz. Mn, Mg, Zn, Ca, Fe, Ni, Cr, Na, K, Al, and Sr were also isolated by Sondhi and Agarwal (1995) from the plant using flame photometer, atomic absorption spectrometer and inductively coupled plasma and the presence of Fe, Cu, and Co in the plant extract

Table 3. Phytochemical screening of fractions of hot water extract of *A. longifolia* leaf and aerial parts (n = 3).

Test used to detect phytochemicals	Methanolic fraction	Aqueous fraction	Residual fraction
Alkaloids			
Wagner's reagent	+	+	+
Hager's reagent	+	+	+
Mayer's reagent	+	+	+
Saponins			
Foam test	-	-	-
Carbohydrates			
Molish's test	+	+	+
Barfoed's test	+	+	+
Benedict's test	+	+	+
Glycosides			
Borntrager's test	-	-	-
Legal's test	-	-	-
Proteins			
Biuret reagent	+	+	+
Mllon's reagent	-	+	-
Aminoacids			
Ninhydrin test	-	-	-
Phenolic compounds			
Ferric chloride test	+	+	+
Gelatin test	+	+	+
Lead acetate test	+	+	+
Flavonoids			
Alkaline reagent test	+	+	+

+, Positive; -, negative.

Table 4. Total phenolics, total antioxidant and flavonoids of crude extract and fractions of *A. longifolia*.

Name of the fraction	Total antioxidant (µM of ascorbic acid equivalent mg^{-1} of extract)	Total phenolics (mg of gallic acid equivalent mg^{-1} of extract)	Flavonoids (mg of quercetin equivalent g^{-1})
Crude fraction (hot water extract)	317.50 ± 3.73^{c}	0.1425 ± 0.002^{b}	2.67 ± 0.05^{c}
Methanolic fraction	306.74 ± 4.05^{bc}	0.0940 ± 0.001^{a}	0.36 ± 0.02^{b}
Aqueous fraction	300.00 ± 5.42^{b}	0.1423 ± 0.0001^{b}	3.17 ± 0.04^{d}
Residual fraction	40.94 ± 1.74^{a}	0.1386 ± 0.014^{b}	0.17 ± 0.03^{a}

Each value is expressed as mean ± standard error (n = 3). Means with the different letters differ significantly ($P < 0.05$).

was confirmed by Choudhary and Bandyopdhyay (1998). Sodium 1.39% (13.9 µg mg^{-1}), potassium 4.83% (48.3 µg mg^{-1}), calcium 0.81% (8.1 µg mg^{-1}) and phosphorus 0.40% (4.0 µg/mg) on the basis of dry weight were reported by Banerjee and Matai (1990). The present study showed low Na (0.895±0.011 µg mg^{-1}) and K (26.023±0.845 µg mg^{-1}) concentration but in both cases K concentration is higher. These variations in the mineral concentrations may be due to difference in soil composition and difference in method of analysis.

Table 5. Mineral concentration of hot water extract and fractions of *A. longifolia* (µg/mg).

Name of the sample	Iron (µg mg^{-1})	Cobalt (µg mg^{-1})	Copper (µg mg^{-1})	Zinc (µg mg^{-1})	Sodium (µg mg^{-1})	Potassium (µg mg^{-1})	Calcium (µg mg^{-1})	Magnesium (µg mg^{-1})	Phosphorus (µg mg^{-1})
Leaf (powder)	0.408±0.007^{a}	0.874±0.008^{a}	0.685±0.011^{a}	0.106±0.013^{a}	0.895±0.011^{a}	26.023±0.845^{c}	11.707±0.461^{a}	2.43±0.053^{b}	5.143±0.400^{a}
Hot water extract	0.431±0.003^{b}	0.877±0.003^{a}	0.723±0.005^{b}	0.194±0.002^{c}	1.302±0.004^{b}	50.233±0.326^{e}	30.000±0.577^{c}	1.670±0.050^{ab}	6.470±0.021^{b}
Methanolic fraction	0.422±0.003^{ab}	0.865±0.004^{a}	0.743±0.002^{b}	0.207±0.006^{c}	7.267±0.003^{e}	21.046±0.213^{b}	23.500±0.570^{b}	1.000±0.309^{a}	5.290±0.092^{a}
Aqueous fraction	0.412±0.007^{a}	0.874±0.003^{a}	0.665±0.003^{a}	0.157±0.003^{b}	2.395±0.004^{d}	31.814±0.359^{d}	33.000±0.577^{d}	10.330±0.315^{c}	7.650±0.048^{c}
Residual fraction	0.801±0.005^{c}	1.638±0.013^{b}	1.328±0.026^{c}	1.279±0.004^{d}	1.623±0.004^{c}	1.272±0.002^{a}	33.340±0.487^{d}	18.890±0.509^{d}	17.650±0.303^{d}

Each value is expressed as mean ± standard error (n = 6). Means with the different letters in a column differ significantly (P < 0.05).

Ca (11.707±0.461) and P (5.143±0.400) concentration in dried leaf was almost similar with the findings of Banerjee and Matai (1990). Considering all the data found in this experiment, the present study illustrated that the pH of methanolic, residual and aqueous fractions are near to the physiological pH and hence not having any adverse effect on natural buffering system of the animal body. All the fractions contain sufficient concentration of phenolic compounds and can be a potential source of antioxidants which are responsible for scavenging of free radicals and can be used as a valuable nutraceutical in conditions where free radicals are produced in greater amount as in haemolytic anemia. Higher concentration of zinc and cobalt in residual fraction reveals its better tissue repairing capacity as compared to other two fractions. Apart from its qualitative aspects in agriculture and nutrition, further studies on feed quality such as palatability and digestibility of this plant should be carried out in order to utilize this plant as health – promoting feed source during drought period of the year in augmenting and maintaining better productivity of livestocks.

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