NUTRITIONAL AND ANTI NUTRITIONAL STATUS OF *Acorus calamus* L. RHIZOME

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Abstract
Acorus calamus, a medicinal herb occasionally planted in the home gardens and often found as garden escape in Kuttanad wetlands of Kerala, India and its rhizome was investigated for its nutritional and antinutritional properties. Proximate composition, mineral profiles, vitamins, total free amino acids, in vitro protein digestibility, calorific value and certain anti nutritional factors like phenol, tannin, phytic acid, trypsin inhibitory and haemaglutinin assay were analysed. The results of proximate analysis revealed the presence of high moisture (68.02 %) and ash (17.3 %) and protein (15.62 %) contents. The carbohydrate (37.26 %), crude lipid (0.00057 %), crude fiber (6.6 %) contents and vitamins (ascorbic acid, beta carotene and tocopherol) were found in appreciable quantity. The total energy value was estimated at 121.65 Kcal/100 g and total free amino acid content was 25.71 µg/500mg. Mineral analyses recorded highest value for Potassium (K) and the lowest value for Phosphorus (P). The other elements like Sodium (Na), Calcium (Ca), Magnesium (Mg), Zinc (Zn) and Copper (Cu) were also found in sufficient amount. The rhizome was also found to contain anti-nutritional factors such as tannins (0.32 µg/g), phytic acid (935.43 mg/100g), phenols (113.68 µg/g) and trypsin inhibitor unit (3.02 mg/g). The results of the present investigation revealed the presence of essential nutrients and also certain antinutritional factors in the rhizome of A. calamus.

Keywords: *Acorus calamus*, haemagglutinin, *in vitro* protein digestibility, minerals, proximate composition.

1. INTRODUCTION

Acorus calamus L. (family: Araceae), commonly known as 'Sweet flag', is a semi aquatic, perennial, tuberous and aromatic medicinal herb, with creeping rhizomes and sword shaped leaves. It is found in near-marshy areas, associated with river banks and lakes throughout India and other Central Asian countries, Central Europe and North America (Nadkarni, 2007). Each part of the plant contains volatile oil, which comprises of terpenoids, calamine, calamenol, calamenone, eugenol, camphene, pinene and asaronaldehyde, and also acorafuran, which is a newly described sesquiterpenoid found in calamus oil (Tkachev *et al*., 2006).

The rhizomes were extensively used by Indians, Chinese and American Indians as well as by other cultures (Motley, 1994) and related alternative healthcare systems. The rhizomes are considered to possess anti spasmodic, carminative and antihelmintic, aromatic, expectorant, nauseant, nervine, sedative, stimulant properties, and it is used in the treatment of various ailments like epilepsy, mental disorders, chronic diarrhea, dysentery, bronchial catarrh, intermittent fevers and glandular and abdominal tumors. The rhizomes are used for kidney and liver troubles, rheumatism, sinusitis, eczema and it also possess antioxidant property (Manju *et al*., 2013). In Ayurveda, the powder of this drug is being used to produce therapeutic emesis. The use of paste of the rhizome in children to improve or rectify the speech defect and improving the memory power is in vogue in most of the rural areas of southern India

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(Meena *et al*., 2010). Traditionally, the rhizome is used in the treatment of inflammatory disorders and as an ingredient of various cocktail preparations (Muthuraman and Singh, 2011) in Indian System of Medicine.

The plant constitutes various nutritional and antinutritional components in addition to the therapeutically important secondary metabolites (Mathews *et al*., 1999, Dingman, 2002). They are used in animal feed as the growth promoters and for enhancement of productivity (Tipu *et al.*, 2006). The current interest in search for alternative food and feed ingredients is due to the low production of oil seeds and grains, the restricted use of antimicrobial growth promoters, and the stiff competition between man and livestock industry for the existing stock of food and feed materials (Siddhuraju *et al*., 2000).

Plants which are under consideration for consumption as food preferably should contain an enhanced quantity of the nutritional constituents, essential minerals and vitamins but all the same should have only low quantities of anti-nutritional factors (Armah *et al.*, 2001). The adverse effects of antinutritional factors can be reduced by using appropriate and effective processing techniques and thereby improve the nutritive value of the plant produces (Akande *et al.*, 2010). The present study was aimed at evaluating the nutritional and antinutritional components present in *A. calamus* rhizome.

2. MATERIALS AND METHODS

A. Collection and preparation of plant sample

The rhizomes of *A. calamus* were collected from Kuttanad wetlands (9° 17′ to 9° 40′ N latitude and 76° 19′ to 76° 33′E longitude) and the taxonomic identity of *A. calamus* was confirmed by Dr. T. Shaju, Plant taxonomist, Division of Plant Systematics and Evolutionary Science, Jawaharlal Nehru Tropical Botanic Garden and Research Institute, (JNTBGRI), Palode, Kerala, India, and the voucher specimens of the samples are deposited in the Herbarium of Environmental Resources Research Centre (ERRC), Trivandrum, Kerala. The rhizomes were washed with distilled water and air dried in shade for one week at room temperature. The dried samples were milled into powder using an electric blender. The powder was stored in an airtight container for further analysis.

B. Determination of nutritional factors

a. Proximate analysis

The proximate composition of rhizome samples (moisture, ash, crude lipid (ether extract) and crude fiber) was determined by the method of Association of Official Analytical Chemists (AOAC, 2000). Total nitrogen (N) was measured using macro Kjeldahl apparatus and crude protein content in the sample was calculated using the formaula N x 6.25. Crude lipid (ether extract) content was determined using soxhlet apparatus. Carbohydrates were determined by the protocol described by Sadasivam and Manickam (1996).

b. Mineral analysis

The mineral analysis was performed as per the method of AOAC, 1990. The minerals tested were sodium (Na), zinc (Zn), copper (Cu), magnesium (Mg), phosphorus (P), potassium (K) and calcium (Ca). Na, K and Ca were determined by flame photometry while Mg, Zn, P and Cu were determined using atomic absorption spectroscopy (PerkinElmer, PinAAcle 900H).

c. Analysis of vitamins

Ascorbic acid (vitamin C) content was determined by the volumetric method described by Sadasivam and Manickam, 1996. The ascorbic acid content present in the samples was calculated and expressed as milligrams per 100 gram of powdered samples.

The beta carotene in rhizome sample was determined using the protocol of AOAC (1980). The sample (10 g) was macerated with 95% ethanol (50 ml) and kept in a water bath for 20 minutes with periodic shaking. The supernatant was decanted and the ethanol concentration of the mixture was brought to 85% using distilled water and cooled. The

mixture was transferred into a separating funnel with 25 ml of petroleum ether and cold ethanol was poured over it. The bottom layer was run off into a beaker while the top layer was collected in a 250 ml conical flask. The bottom layer was transferred into the funnel and re-extracted with 10 ml petroleum ether for 5-6 times until the extract became fairly yellow. The entire petroleum ether was collected and transferred into a separating funnel for re extraction with 80% ethanol (50 ml). The absorbance of final extracts was measured using spectrophotometer at 436 nm and the concentration of β-carotene was calculated using Beer-Lamberts Law.

Tocopherol (vitamin E) content in rhizome sample was estimated spectrophotometrically by the method described by Rosenberg (1992). The rhizome sample (2.5 g) was homogenized in sulphuric acid (0.1N) and the volume was finally made up to 50 ml slowly with sulphuric acid, without shaking and was allowed to stand overnight. The mixture was shaken vigorously on the next day, and filtered. 1.5 ml each of plant extract, standard and water were pipetted out into three centrifuge tubes as test, standard and blank respectively. 1.5 ml each ethanol and xylene was added mixed well and centrifuged. After this, the xylene layer (1ml) was transferred into another tube and equal amount 2, 2- dipyridyl reagent was added, stoppered and mixed well. The absorbance was read at 460 nm in a spectrophotometer. Then ferric chloride solution (0.33 ml) was added to all the tubes including the blank, mixed well and after exactly 15 minutes, the test and standard were read against the blank at 520 nm.

d. Analysis of total free amino acids

The rhizome powder was extracted in 80 % ethanol by grinding using acid washed sand. Ninhydrin solution (1 ml) was added to 0.1 ml of extract and made up to 2 ml with distilled water and the tubes were boiled in water bath for 20 min. 5 ml of diluents was added and mixed. Absorbance was measured at 570 nm using a spectrophotometer. A calibration curve was constructed using glycine as standard and total free amino acid content of the extract was expressed as percentage equivalent of glycine (Sadasivam and Manickam, 1996).

e. Determination of *in vitro* protein digestibility

In vitro protein digestibility (IVPD) was determined using the multi-enzyme technique (Hsu *et al.*, 1977). Sample was weighed out (so as to contain 6.25 mg protein per ml), hydrated in 10 ml of distilled water and refrigerated at 5°C for 1h. The samples containing protein and enzymes were all adjusted to pH 8.0 at 37°C. The IVPD was determined by sequential digestion of the samples containing protein with multi-enzyme mixture (trypsin, chymotrypsin, and peptidase) at 37°C followed by protease (type IV from *Streptomyces griseus*) at 55°C. The pH drop of the samples from pH 8 was recorded after 20 min of incubation. The IVPD was calculated according to the regression equation Y= 234.84 – 22.56 X, where Y is the % digestibility and X the pH drop.

f. Determination of energy or calorific value

The total energy value in the rhizome of *A. calamus* in Kcal/100 g was estimated using the protocol described by FAO (2003) and calculated using the formula

Energy value (kcal/100g) = [% crude protein x 4.0] + [% crude fat x 9.0] + [% carbohydrate x 4.0]

B. Anti nutritional analysis

a. Determination of tannins

The antinutritional factor, tannin was determined as per the protocol described by Schanderl, 1970. The powdered rhizome (0.5g) was boiled in water (75 ml) for 30 min and was centrifuged at 2000 rpm for 20 min. The supernatant was made up to 100 ml in a volumetric flask. To the sample extract (1ml), 75 ml water, Folin-Denis reagent (5 ml) and sodium carbonate solution (10 ml) were added, diluted to 100 ml with water in a volumetric flask and kept for 30 min incubation and the absorbance was read at 700 nm.

b. Determination of total phenol

The total phenolic content in the water extract was determined using the Folin- Ciocalteu reagent (McDonald *et al*., 2001). Different concentrations of the extract was mixed with 0.4 ml Folin ciocalteu reagent (diluted 1:10 v/v). After 5 minutes, sodium carbonate solution (4 ml) was added. The final volume of the tubes were made up to 10 ml with distilled water and allowed to stand for 90 min at room temperature. Absorbance of sample was measured against blank at 750 nm using spectrophotometer. A calibration curve was constructed using catechol solutions as standard and total phenolic content of the extract was expressed in terms of milligrams of catechol per gram of dry weight.

c. Analysis of phytic acid

The phytic acid content in the sample was determined spectrophotometrically using the method described by Wheeler and Ferrel, 1971.

d. Trypsin inhibitory assay

The activity of trypsin inhibitors in the sample was determined by using benzoyl-DL-arginine-paranitroanilide (BAPNA) as a substrate. One trypsin inhibitor unit (TIU) has been expressed as an increase of 0.01 absorbance units per 10 ml of reaction mixture at 410 nm. Trypsin inhibitor activity has been defined in terms of trypsin units inhibited per mg protein (Kakade *et. al.,* 1974).

e. Hemagglutinin assay

Sample processing: Two grams of sample was added to 20 ml phosphate buffered saline (PBS), shaken vigorously for 1 min and left to stand for 1 h. The sample was then centrifuged at 2000 rpm for 10 min and the suspension was filtered and used as crude agglutinating extract.

Preparation of erythrocytes: Fresh whole blood (3.5 ml) was collected from the animal using a syringe containing ethylene diaminetetraacetic acid and was centrifuged at 2000 rpm for 10 min. The supernatant and 'buffy coat' of white cells, which formed a layer on top of the packed erythrocytes, were removed using a micropipette. One volume of the RBC was diluted with 4 volumes of PBS centrifuged at 2000 rpm for 10 min and supernatant discarded. The sediment cells were washed with saline three times until the supernatant became colourless. Washed erythrocytes were suspended in PBS at a concentration of 4% v/v. One volume of trypsin solution was added to 100 volume of the diluted cell suspension and incubated at 37 ˚C for 1 h. After centrifugation the trypsinised cells were washed 4-5 times in PBS. The washed cells were re suspended at a concentration of 4% v/v in PBS for agglutination assay.

Haemagglutination was carried out in U-shaped micro titer multi-well plates. Clear supernatant (50 µl) was poured into the depression on a micro titer plate and serially two fold with PBS. To all the wells of each row, 50 µl of the 4 % erythrocyte suspension was added. The plates were incubated for 3 hours at room temperature and after the incubation, the titre values were recorded. Agglutination of erythrocytes by lectin (antibody) was indicated by a complete carpet of cells covering the bottom of the well in microtitre plate while non-agglutinated cells formed a compact button or ring at the center of the curved well. The end point of titration was taken as an even carpet of cells with a ring at the edge. The agglutination titre was recorded as the dilution of the lectin at the end point or the reciprocal of this dilution (Singh and Saxena, 2013).

3. RESULTS AND DISCUSSIONS

A. Nutritional factors

a. Proximate analysis

The results of proximate composition of *A. calamus* (rhizome) are given in Table 1a. The moisture content was high (68.02 %) and it is among the most important and mostly used measurement in the processing, preservation and storage of food (Onwuka, 2005). The level of crude protein (15.62 %) found in rhizome makes it nutritionally a good source of protein. Proteins play a major role in the organoleptic properties of food apart from their nutritional

Table 1. Nutritional and anti-nutritional properties of *A. calamus* rhizome.
The values are the mean ± SE of three analyses of each factor

a. Proximate chemical composition and energy content						
Moisture (%)	Crude protein (%)	Carbohydrate (%)	Crude lipid (%) (ether extract)	Crude fiber (%)	Ash (%)	Energy value (Kcal/ 100 g)
68.02±1.6	15.62±0.67	37.26±1.34	0.00057±0.00002	6.5±1.14	17.3 ±0.45	121.65 ±1.56
b. Mineral composition (mg/kg)						
Na	K	P	Ca	Mg	Cu	Zn
1823±10.23	15078.33±24.08	0.91±0.06	1585.66±13.07	644±3.26	11.59 ±1.61	18.03 ±1.78
c. Vitamins and total free amino acid composition						
Ascorbic acid (mg / g)		β carotene (mg/ml)		Tocopherol (µg/g)		Total free amino acid (µg/500 mg)
3.93±0.08		2.43±0.09		7.35±1.42		25.71±1.26
d. Concentration of IVPD and antinutritional factors						
IVPD (%)	Phenols (µg/g)	Tannins (µg/g)	Phytic acid (mg/100g)			Trypsin inhibitor unit (TIU) (mg/g)
72.15±1.31	113.68±1.36	0.32±0.05	935.43±2.28			3.02±0.16

significance as a source of amino acids (Aremu *et al*., 2006). The values obtained for crude protein in the present study indicated that the rhizome can supply sufficient quantity of amino acids. Chandran et al. 2014, reported the presence of glutamic acid, L-cystine, lysine and serine in the rhizome of *A. calamus.*

The crude lipid content (0.00057 %) obtained was very low. Fat content present in the diets promotes absorption of fat soluble vitamins (Bogert *et al*., 1994). The crude fiber content was 6.5 %. The presence of high fiber contents in the food helps in the absorption of trace elements in the gut, increases digestibility and reduce absorption of cholesterol. Conversely, high level of fibers in the diet can lead to intestinal irritation, which ultimately effects decreased nutrient uptake (Oyenuga and Fetuga, 1975). A diet low in fiber is undesirable and could cause constipation and disease of colon like piles, appendicitis and cancer (Okon, 1983). The fiber content is present only in minimal level in *A. calamus* rhizome and it possesses beneficial effects rather than hazardous consequences.

The carbohydrate content (37.26 %) shows that it is a good source of energy and is also needed for efficient oxidation of fats (Omoyeni and Adeyeye, 2009). Carbohydrate is beneficial since it constitutes a major class of naturally occurring organic compounds that are essential for the maintenance of plant and animal life and also provide raw materials for many industries (Ebun and Alade, 2007). The ash value was found to be 17.3 % which indicated the presence of inorganic components. The ash contents of nuts, seed and tubers should fall in the range 1.5 to 2.5 % in order to be suitable for animal feeds (Pomeranz and Meloan, 1978). The rhizome exhibited a lower calorific value of 121.65 % and was found to be reasonably sufficient for the animals.

b. Mineral composition

The results of nutritionally valuable mineral contents of *A. calamus* rhizome are given in Table 1b. The values indicated that the rhizome of *A. calamus* is a good source of macro elements like K (15078 mg/kg) followed by Na (1823 mg/kg), Ca (1585 mg/kg) and Mg (644 mg/kg). The micro elements such as Cu (11.59

mg/kg) and Zn (18.03 mg/kg) were also present in sufficient quantity. Phosphorus (0.91 g/100 g) is seen in low quantity among all the minerals. The low amount of phosphorous may be attributed to the presence of calcium in the rhizome (Davidson and Stanley, 1975).
Minerals present in plants play a crucial role in regulating essential physiological processes such as regulation of enzyme activity, skeletal structures, neuromuscular irritability and clotting of blood in the body of animals which feed them (Kalita *et al*., 2007). A deficit in any one of the essential minerals leads to chronic metabolic disorders and compromise the health of the organism which feed them (Lopez *et al*., 2002).

c. Vitamins and total free amino acids

The vitamins such as ascorbic acid (3.93 mg / g) beta carotene (2.43 mg/ml) and tocopherol (7.35 µg/g) were found to be in appreciable amount (Table 1c). Beta-carotene, the most potent precursor of vitamin A which is necessary for many vital biological processes in the ruminants including vision, bone growth, immunity and maintenance of epithelial tissue. The animals have the ability to convert beta-carotene into vitamin A within their body (Panday and Tiwari, 2002). Ascorbic acid increases iron absorption in the animal's body (Anwar *et al*., 2007). Vitamin C has anti-infective properties, promotes wound healing, may boost the immune system and may help ward off infections (Wright, 2002). Tocopherol acts as a potent antioxidant in the body and also maintains the storage of vitamin A and iron in the body (Smolin and Grosvenor, 2007). Vitamin A and tocopherol are among the nutrient that assists animals to develop resistance to diseases (Anwar *et al*., 2007). The total free amino acids of the rhizome were found to be 25.71 µg/500 mg (Table 1c). Amino acids are required for the production of enzymes, immunoglobulins, hormones, growth, repair of body tissues and form the structure of red blood cell (Brisibe *et al*., 2009) and they also contribute to the formation of glucose, acting as a buffer when other precursors are in short supply (Swanepoel *et al*., 2010).

d. *In vitro* protein digestibility

The *in vitro* protein digestibility of the rhizome was about 72.15 % (Table 1d). The *in vitro* protein digestibility data provides an accurate estimation of protein availability and nutritional quality in different feeds and the component with high digestibility is well suited for feed formulations (Ali *et al*., 2009).

f. Antinutritional factors

The data of antinutritional factors are given in table 1d. The value of phytic acid detected was 935.43 mg/100 g and the lethal standard value for phytate is 2500 mg/100 g (FAO, 1990). Phytic acids in foods chelates with essential minerals such as iron, calcium, zinc and magnesium in the digestive tract, resulting in mineral deficiencies (Bello *et al*., 2008). The concentration of phytic acid in rhizome can be reduced to some extent by various processing techniques such as heat treatment (cooking, baking, autoclaving and extrusion), soaking, germination, dehulling, alkaline treatment. The action of phytate degrading enzymes can also reduce phytic acid content in food and increase bioavailability of minerals (Hidvegi and Lasztity, 2003).
The amount of tannin was found to be in appreciable amount (0.32 µg/g). So it may not cause an adverse effect on the animals. Tannins exhibit their antinutritional ability largely by precipitating dietary proteins, digestive proteins and digestive enzymes to form indigestible complexes (Omoyeni and Adeyeye, 2009). Tannin sours the mucus secretions and contracts the membranes so that the secretions from the cells are restricted. Excessive use of herbs containing high quantity of tannins is not recommended (Reed, 1995). Tannins have been shown to be beneficial at low concentrations, between 20 and 40 g/kg. The benefits include bloat prevention (Lees, 1992), enhanced rumen escape protein due to the ability of formation of protein tannin complex under the neutral pH conditions but later release these proteins at the acidic pH of the abomasums for subsequent digestion and in the small intestine (Perez Maldonado and Norton, 1996). As a result, nitrogen retention

and live weight gain may increase in ruminants fed tannin containing feeds (Nsahlai *et al*., 1999).

The presence of phenolic compounds in high quantities will inhibit iron absorption, reduce protein and carbohydrate digestibility (Glahn *et al*., 2002; Emine Nur Herken *et al*., 2005). The phenol content in *A. calamus* was in appreciable amount (113.68 µg/g) which shows its ability to act as a potent antioxidant (Shahidi and Wanasundara, 1992; Manju *et al*., 2013).

Trypsin inhibitors, which are anti nutritional factors for monogastric animals, do not exert adverse effects in ruminants because they are degraded in the supplement rumen (Kumar, 2003). In the present study, the value obtained was found to be very low (3.02 mg / g). It was found that below 5 mg /g level of dietary TI, majority of cultured fish are able to neutralize it by increasing trypsin production (Francis *et al*., 2001) and the carp have the ability to tolerate high levels of TI (24.8 mg/g) in their feed (Makkar and Becker, 1999).

Phytohaemagglutinins or lectins are glycoproteins of 60,000-100,000 molecular weights that agglutinate erythrocytes *in vitro*. The *A. calamus* rhizome extract (300 mg/ml) were serially diluted and screened for possible haemagglutination activity against sheep erythrocytes. There was no detectable agglutination. Phytohaemagglutinins are not only capable of damaging the intestinal mucosa and reduces the permeability of the mucosa, subsequently affecting the reabsorption of nutrients but also damages the immune system (Seifert, 1996). It also resists digestive breakdown and substantial quantities of ingested lectins may be recovered intact from the faeces of animals fed diets (Aletor and Fetuga, 1987).

4. CONCLUSIONS

It is evident from the results that *A. calamus* rhizome possess essentials nutritive contents including proteins, vitamins, carbohydrates, fats and minerals. The antinutritional contents of the rhizome were low which may not cause any harmful effects in animals and it can be further reduced by subjecting it to suitable thermal processing.

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