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Nutritional and antinutritional properties of the leaf of *Ardisia solanacea* Roxb. (Myrsinaceae), a fodder additive

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Abstract

Nutritional and antinutritional properties of the leaf of *Ardisia solanacea* Roxb., a common shrub found in Kuttanad wetlands, Kerala, India, were analysed to find out its utility as a cattle food additive. The results of proximate analysis revealed the presence of high moisture (67%) and ash (25.31%) contents. This plant is a good source of protein (31.25%) and carbohydrate (7.3%). The fat (0.00067%) and crude fiber (6.6%) contents were found in appreciable quantity. The total energy value was estimated at 154.20 kcal/100g. The mineral analysis revealed the presence of abundant calcium (Ca) (76952 mg/kg) and phosphorus (P) (0.1 g/100g) recorded the lowest value. The other elements like sodium (Na) (4347 mg/kg), potassium (K) (12637 mg/kg), magnesium (Mg) (2197 mg/kg), zinc (Zn) (11.2 mg/kg) and copper (Cu) (5.7 mg/kg) were also found in sufficient quantity. The total free amino acid content was found to be 26.95 µg/500 mg and the in vitro protein digestibility of the leaf was 64.29%. The presence of vitamins, ascorbic acid (4.44 mg/g), beta carotene (240 mg/ml) and tocopherol (7.29 µg/g) were also found in appreciable quantities. The antinutritional factors such as phenols (113.42 µg/g), tannins (2.44 µg/g), phytic acid (1562.7 mg/100g), trypsin inhibitor unit (4.03mg/g) and lectins (75 mg/ml) were recorded. The leaf of *A. solanacea* is recommended as a food additive after proper treatments.

Introduction

Ardisia solanacea Roxb. (Family: *Myrsinaceae*) is a glabrous shrub or small tree that reaches a maximum height of 20 feet under natural habitat conditions (Chen and Pipoly, 1996). It shows a fair distribution in several pockets of Kuttanad (9° 17′ to 9° 40′ N latitude and 76° 19′ to 76° 33′ E longitude), which forms a part of the Vembanad wetland system, one of the Ramsar sites in Kerala, India. It is located on the south-western end of Indian peninsula. *A. solanacea* is reported from different parts of the Western Ghats in India and also in Indo-Malesia and West China (Sasidharan, 2004). It is often cultivated in the home gardens of the Kuttanad, and in the wild it is found in association with another species *Ardisia littoralis*. Nearly 500 species of the genus *Ardisia* are recorded throughout the tropical and subtropical parts of the world. They are used as medicinal and ornamental plants, apart from forming a wild fruit resource for the natives. *Ardisia* spp. has several biologically active phytochemicals, including saponins, coumarins and quinines, and is a rich source of biologically potent compounds, such as bergin and ardisin (Kobayashi and de Mejia, 2005). Our recent study on the antioxidant potential of methanolic and aqueous extract of Ardisia solonacea leaf exhibited potential 2,2-diphenylpicrylhydrazyl (DPPH), metal ion, hydroxyl radical and reducing power activities, and established that the methanolic extract of its leaves is a better radical terminator than the aqueous one (Chandran *et al*., 2013).

The antinutritional properties present in plants can cause depression in growth and health of animals when they consume these plants, through a variety of mechanisms including reducing digestibility, binding of various nutrients or damaging the intestinal wall, and thereby lowering digestive efficiency (Mikic *et al*., 2009). The presence of endogenous antinutritional factors within plants limits their use in food and feeding stuff (Aganga and Tshwenyane, 2003). Taking these into consideration, the present

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study focused on evaluating the nutritional and antinutritional properties of *A. solanacea* leaf and its suitability as a cattle food additive. The results of the present investigation together with our previous study on the antioxidant potential of *A. solanacea* extract (Chandran *et al.*, 2013) indicate that the leaf of this plant can be used as a food additive for livestock, after appropriate treatments to remove or reduce some of the antinutritional properties present in it.

Materials and Methods

Identification of A. solanacea

Ardisia solanacea Roxb., a native shrub/small tree belongs to the family *Myrsinaceae*. Its glossy leaves are up to 6 inches long by 2 inches wide. Flowers are 1.5 to 2 cm across, pink or pinkish-white, in axillary, corymb like racemes, shorter than the leaves. Fruit is 7-13 mm in diameter, depressed-round, black with pink juice when ripe, tipped by style base, supported on persistent sepals. The plant prefers partial shade and moist rich soil. The berries of *A. solanacea* have been used in traditional fabric dyeing and yellow is the most typical colour produced.

The taxonomic identification of *A. solanacea* 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, and the voucher specimens of the plant are deposited in the Herbarium of Environmental Resources Research Centre (ERRC), Thiruvananthapuram, Kerala.

Collection and preparation of A. solanacea leaf

The leaves of *A. solanacea* were collected from Kuttanad wetlands. The leaves were washed with distilled water and air dried in shade for a week at room temperature (24 ± 2°C). The dried samples were milled into powder using an electric blender. The powder was stored in an airtight container for further analysis.

Chemicals

All the solvents used for the extraction process were procured from SD Fine Chemicals, Mumbai, India. Sulphuric acid, phosphate buffered saline, petroleum ether, glycine, and the enzymes (typsin, chymotrypsin and peptidase) were procured from Hi-Media Laboratories Pvt. Ltd., Mumbai. Trichloroacetic acid (TCA), ferric chloride, ethylene diaminetetra acetic acid (EDTA), benzoyl-DL arginine paranitroanilide (BAPNA) and ascorbic acid were procured from Sisco Research Laboratories, Mumbai, India. 2, 2-dipyridyl was purchased from Spectrochem Pvt. Limited, Mumbai. Xylene and Folin ciocalteu reagent was purchased from Merck Limited, Mumbai. Catechol was purchased from Rolex Chemical Industries, Mumbai. Sodium carbonate was purchased from Thermo Fisher Scientific India Pvt. Ltd. Mumbai. All the chemicals and reagents used were of analytical grade and were prepared in deionized water.

Nutritional properties

Proximate analysis of leaf

Moisture, ash, crude lipid (ether extract) and crude fiber of the plant were determined following the methods described by the 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 by multiplying N x 6.25. Crude protein included both true protein and non-protein nitrogen. Crude lipid (ether extract) content was determined using soxhlet apparatus. Total carbohydrate was estimated following the protocol of Sadasivam and Manickam (1996).

Determination of energy or calorific value

The total energy value in the leaf of *A. solanacea* in Kcal/100 g was estimated following the method of FAO (2003). The equation for energy determination was

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

Mineral analyses

The method described by AOAC (1990) was followed to estimate sodium (Na), potassium (P) and calcium (Ca) in the leaf of A. solanacea using atomic absorption spectrometer (PerkinElmer, PinAAcle 900H] and magnesium (Mg), copper (Cu), zinc (Zn) and phosphorus (P) in the leaf were measured using Flame Photometry, (Systronics, Flame Photometer 128).

Determination of Ascorbic acid

Ascorbic acid (vitamin c) in the leaf of *A. solanacea* was determined through volumetric method described by Sadasivam and Manickam (1996) and expressed as milligrams per 100 gram of powdered sample.

Determination of Beta carotene

The beta carotene content of the leaf sample was determined following the method of AOAC (1980)

with slight modifications. The sample (10 g) was macerated with 95% ethanol (50 ml) and kept in a waterbath for 20 minutes with periodic shaking. The supernatant was decanted. 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 a 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 separating funnel for re-extraction with 80% ethanol (50 ml). The absorbance of final extract was measured using spectrophotometer (Shimadzu UV 1800) at 436 nm. The concentration of β-carotene was calculated using Beer-Lamberts Law.

Determination of tocopherol

The tocopherol content in the plant leaf was estimated spectrophotometrically following the method of Rosenberg (1992). The sample (2.5 g) was homogenized in sulphuric acid (0.1N) and the volume was finally made up to 50 ml with sulphuric acid slowly, without shaking and was allowed to stand overnight. The mixture was shaken vigorously on the next day and filtered. 1.5 ml each plant extract, standard and water were pipetted out into three centrifuge tubes namely test, standard and blank respectively. 1.5 ml each of ethanol and xylene were added, mixed well and centrifuged. After centrifugation, the xylene layer (1ml) was transferred into another tube and equal amount 2, 2-dipyridyl reagent was added, stoppered and mixed. The optical density was read at 460 nm in a spectrophotometer. Then 0.33 ml (1.2 g in one liter of ethanol) of ferric chloride solution was added to all the tubes including the blank, mixed well and exactly after 15 minutes, the test and the standard were read against the blank at 520 nm.

Determination of total free amino acid

The leaf powder was extracted using ethanol (80%) 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 waterbath 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 solutions as standard and total free amino acid content of the extract was expressed as percentage equivalent of glycine (Sadasivam and Manickam, 1996).

In vitro *protein digestibility*

In vitro protein digestibility (IVPD) was determined using a 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 the sequential digestion of the samples containing protein with a 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 sample 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.

Antinutritional properties

Determination of total phenol

The total phenolic content in the leaf extracted in water was determined following the method described by McDonald *et al*. (2001). Different concentrations of the extracts were mixed with 0.4 ml Folin ciocalteu reagent (diluted 1:10 v/v). After 5 min, 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 minutes at room temperature. Absorbance of sample was measured against the blank at 750 nm using spectrophotometer. A calibration curve was constructed using catechol solutions as standard and the total phenolic content of the extract was expressed in terms of milligrams of catechol per gram of dry weight.

Determination of tannin

Tannin in leaf extract was determined following the protocol of Schanderl (1970). The powdered plant material (0.5 g) was boiled in water (75 ml) for 30 min and was centrifuged at 375.7 g for 20 min. The supernatant was made up to 100 ml in a volumetric flask. To the sample extract (1 ml), 75 ml water, Folin-Denis reagent (5 ml) and sodium carbonate solution (10 ml) were added and diluted to 100 ml with water in a volumetric flask. The absorbance was read at 700 nm after 30 min incubation.

Analysis of phytic acid

The Phytic acid in the leaf sample was determined spectrophotometrically following the method of Wheeler and Ferrel (1971).

Trypsin inhibitory assay

The activity of trypsin inhibitors in the samples 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 inhibitory activity has been defined in terms of trypsin units inhibited per mg protein (Kakade *et al*., 1974).

Hemagglutinin assay

For sample processing, two grams of leaf sample was added to phosphate buffered saline (PBS, 20 ml), shaken vigorously for 1 min and left to stand for 1 h. The sample was then centrifuged at 375.7 g 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 diamine tetra acetic acid. The whole blood was centrifuged at 375.7 g for 10 min. The supernatant and 'buffy coat' of white cells, which formed a layer on top of the packed erythrocytes, was removed using a micropipette. One volume of the RBC was diluted with 4 volumes of PBS centrifuged at 375.7 g for 10 min and discarded the supernatant. The sediment cells were washed with saline three times until the supernatant became colorless. 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 microtiter multi-well plates. The clear supernatant (50 µl) was poured into the wells of the microtiter plate and serially two fold diluted 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. After the incubation period, the titer values were recorded. Agglutination of erythrocytes by lectin (antibody) was indicated by a complete carpet of cells covering the bottom of the well in microtiter plate while non-agglutinated cells formed a compact button or ring at the center of the curved well. The agglutination titer was recorded as the dilution of the lectin at the end point. A haemagglutination unit (H.U.) was defined as the minimum amount of the lectin capable of inducing agglutination (Singh and Saxena, 2013).

Statistical analysis

All analysis were done in triplicate and the data were expressed as mean ±SE

Results and Discussion

Nutritional properties

The proximate compositions of the leaf of *A. solanacea* are presented in Table 1a. The moisture content was 67% and the value was higher than the values obtained for the same for *Moringa oleifera* leaves (Anwar *et al*., 2007; Thurber and Fahey, 2009). The crude protein content (31.25%) of the leaf could be considered high which indicated that the plant is a good source of protein and the present value was significantly higher than the values of the same reported in the leaves of *Moringa oleifera* and *Morus alba* (Deshmukh *et al*., 1993; Ogbe and John, 2011) the common rabbit feed. Similarly, Kulkarni *et al*. (2003) observed the highest value of crude protein (29.6%) in *Emilia sonchifolia*. However, the values of carbohydrate (7.3%), crude lipid (0.00067) and crude fiber (6.6%) recorded in *A. solanacea* leaf were lower and the ash content (25.31%) was higher than the values recorded in the leaves of *M. oleifera* and *M. alba* (Deshmukh *et al*., 1993; Ogbe and John, 2011). A higher carbohydrate content of feed is desirable while its deficiency causes depletion of body tissue (Barker, 1996). Ash content constitutes the total mineral content in the leaf. The energy (total calorific) value (154.20 kcal/100 mg) recorded in the present study was reasonably sufficient for the animals which feed on the leaves. However, this was lower than (305.62 kcal per 100 g) the value reported for the leaves of *M. oleifera* (Oduro *et al*., 2008). The result of the present study was comparable with the proximate analysis and calorific values of Aegle marmelos (Vishwakarma and Dubey, 2011).

The results of the nutritionally valuable mineral contents of *A. solanacea* leaf are presented in Table 1b. The values indicated that the leaf *A. solanacea* is a rich source of macro elements like Ca (76952 mg/kg), Mg (2197 mg/kg), Na (4347 mg/kg) and K (12637 mg/kg) and were a poor source of P (0.1 g/100 g). The phosphorous content is low due to the presence of high calcium in the leaf (Davidson and Stanley, 1975). The micro elements such as Cu (5.7 mg/kg) and Zn (11.2 mg/kg) were also present in the leaf in sufficient quantity. Minerals present in the plants play a major role in regulating many vital physiological processes in the body of animals which feed them such as regulation of enzyme activity, skeletal structures, neuromuscular irritability and clotting of blood (Kalita *et al*., 2007). A deficiency of any one of the essential minerals leads to acute metabolic disorders and compromise the health of the

Table 1. Nutritional and antinutritional properties of *A. solanacea* leaf

a Proximate chemical composition (%) and energy content

Moisture	Carbohydrate	Crude protein (N x 6.25)	Crude lipid (ether extract)	Crude fiber	Ash	Energy value (Kcal/100 g)
67±0.06	7.3±0.09	31.25±0.17	0.00067±0.00001	6.6±0.2	25.31±0.3	154.20±0.04

b. Mineral composition (mg/kg)

Na	K	P (g/100g)	Ca	Mg	Cu	Zn
4347±14.36	12637±54.56	0.1±0.02	76952±83.35	2197±56.44	5.7±0.29	11.2±0.26

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)
4.44±0.04	240±6.03	7.29±0.06	26.95±0.03

d. Concentration of antinutritional factors and IVPD

Phenols (µg/g)	Tannins (µg/g)	Phytic acid (mg/100g)	Trypsin inhibitor unit (TIU) (mg/g)	Haemagglutination unit (mg/ml)	IVPD (%)
113.42±0.01	2.44±0.04	1562.7±16.68	4.03±0.007	75±1.53	64.29±0.07

The values are the mean ± SE of three analyses of each factor

animal (Lopez *et al*., 2002).

The vitamins such as beta carotene (240 mg/ml), ascorbic acid (4.44 mg/g) and tocopherol (7.29 µg/g) were present in appreciable quantities in the leaf of *A. solanacea* (Table 1c). In plant leaves beta carotene serves as potent precursor to vitamin A, which is necessary for many functions in the ruminants including vision, bone growth, immunity and maintenance of epithelial cells in animals which feed them and vitamin A also maintains adequate level of iron in plasma that supply different body tissues including the bone marrow (Thurber and Fahey, 2009). Ascorbic acid (vitamin C) increases iron absorption in the animal body (Anwar *et al.*, 2007). Tocopherol acts as an important antioxidant and all of their biological properties are considered to derive from their ability to prevent oxidation of poly unsaturated lipids (Traber and Atkison, 2007). The total free amino acids in the leaf were 26.95 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). Chandran *et al*. (2014) reported the presence of alanine, glutamic acid and valine in the leaf of *A. solanacea*. The in vitro protein digestibility of the leaf was about 64.29% (Table 1d). The in vitro protein digestibility data provides a suitable and reliable estimation of protein nutritional quality and availability in different feeds and the ingredient with high digestibility is more suited for feed formulations (Ali *et al*., 2009).

Antinutritional properties

The antinutritional properties of the leaf of *A. solanacea* are presented in Table 1d. The total phenol content present in *A. solanacea* was found moderate quantity (113.42 µg/g) and, therefore, the plant has the potential to act as a free radical scavenger (Shahidi and Wanasundara, 1992; Chandran *et al.*, 2013). The leaf also exhibited a low amount of tannin (2.44 µg/g). Tannin, however, brings about their antinutritional activity, especially in non ruminants by binding dietary proteins and digestive enzymes into complexes, which are not readily digestible (Aletor, 1993). It has a large influence on the nutritive potential of many foods and feedstuffs and has been found to interfere with digestion by displaying anti-trypsin and anti-amylase activity. Tannins adversely affect an animal's feed intake, feed digestibility, and efficiency of production (Butler, 1989). They also cause intestinal damage, interference with iron absorption and there is a possibility of tannins producing a carcinogenic effect (Butler, 1989). Tannins have been shown to be beneficial at low concentrations, between 20 and 40 g/kg (Lees, 1992). The benefits include, bloat, prevention enhanced escape of proteins from rumen due to the ability of formation of protein-tannin complex under the neutral pH. These proteins are later released at the acidic pH of the abomasums for subsequent digestion in the small intestine (Perez-Maldonado and Norton,

1996). As a result, nitrogen (N) retention and live weight gain may increase in ruminants fed tannin containing feeds (Nsahlai *et al*., 1999).

The phytic acid content of the leaf of *A. solanacea* (1562.7 mg/100 g) was high when compared to the values reported for the *M. oliefera* and *M. alba* (Deshmukh *et al*., 1993; Ogbe and John, 2011). The lethal standard value for phytate is 2500 mg/100 g (FAO, 1990). Phytic acid acts as a potent chelating agent for divalent cations (Ca, Mg, Fe and Zn) and interferes with bioavailability of minerals (Agbede and Aletor, 2005). Moreover, it has the capacity to bind with starch and proteins while preventing their assimilation through the digestive system (Noureddini and Dang, 2008). Phytic acid also inhibits the action of gastrointestinal tyrosinase, trypsin, pepsin, lipase and amylase (Liener, 1980; Hendricks and Bailey, 1989). The concentration of phytic acid in *A. solanacea* leaf could be reduced to some extent by various processing techniques. They included heat treatment (cooking, baking, autoclaving and extrusion), soaking, germination, dehulling and alkaline treatment. Action of phytate degrading enzymes was found to reduce phytic acid content in food samples and increase absorption of minerals in organisms (Hidvegi and Lasztity, 2003).

Trypsin inhibitor (TI) is a widespread anti-nutrient substance in many plant derived nutritional ingredients. Trypsin inhibitor unit (TIU) value of 4.03 mg/g recorded in the leaf of A. solanacea in the present study was found to be lower than the value reported for the leaf of M. oliefera (30.00 mg/g) (Ogbe and John, 2011). TI inhibits proteolytic enzymes, result in reduced digestion of protein, endogenous loss of amino acid (D'Mello, 2000) and also cause pancreatic hypertrophy and growth depression (Aletor and Fetuga, 1987). It seems that below the 5 mg/g level of dietary TI, most cultured fish are able to compensate it by increasing trypsin production (Francis *et al*., 2001) and the fish carp is capable of tolerating high levels of TI (24.8 mg / g) in their feed (Makkar, 1993).

Phytohaemagglutinins or lectins are glycoproteins of 60,000-100,000 molecular weight that are known for their ability to agglutinate erythrocytes in vitro. The *A. solanacea* leaf extract with different dilutions was screened for possible haemagglutination activity (HA) against sheep erythrocytes. The HA titer of the test sample was found to be 75 mg/ml (Table 1d). Lectins are proteins capable of damaging the intestinal mucosa, they resist digestive breakdown and substantial quantities of ingested lectins may be recovered intact from the faeces of animals fed diets (D'Mello, 2000).

Conclusion

It is evident from the present study that *A. solanacea* leaves possess important nutrients such as proteins, vitamins, carbohydrates, fats, minerals etc. The antinutritional factors present in the plant may not adversely affect the animals feeding on them and this can be further reduced, if it is subjected to suitable thermal processing. Thus, the leaf of this plant can be used as a food additive for livestock.

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