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BIOCHEMICAL AND BIOLOGICAL EVALUATION OF AN UNCONVENTIONAL LEGUME, *Canavalia maritima* OF COASTAL SAND DUNES OF INDIA

[EVALUACIÓN BIOQUÍMICA Y BIOLÓGICA DE LA LEGUMINOSA *Canavalia maritima* COLECTADA EN DUNAS COSTERAS DE LA INDIA]

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SUMMARY

Canavalia maritima [Synonym: *Canavalia rosea* (Sw.) DC. and.*Canavalia lineata* (Thunb.) DC.] is a common mat-forming wild legume frequent on the coastal sand dunes of tropics. Seeds of *C. maritima* collected from the dunes were analyzed for proximate composition, minerals, protein fractions, amino acids, fatty acids and Anti-Nutritional Factors (ANFs) and were compared with *Canavalia ensiformis* (jack bean) and *Canavalia gladiata* (sword bean). Growth and nitrogen balance studies was undertaken to determine protein and food efficiency ratios, true digestibility, biological value and net protein utilization, net protein retention and protein retention efficiency. The seeds of *C. maritima* consisted of 34.1% proteins, 55.5% carbohydrates and 1590 kJ 100^{-1} g DM. The protein content of *C. maritima* was higher than other *Canavalia* spp. and other wild legumes of India. Potassium was the major mineral followed by phosphorus, calcium and sodium (974, 158, 86 and 48 mg 100 g^{-1} respectively). Among the true proteins (29.3%), globulins (18.7%) were the highest. The essential amino acids (EAA) threonine, valine, cystine + methionine, isoleucine, leucine, phenylalanine + tyrosine and lysine were higher than that of FAO/WHO pattern, rice and soybean. These EAA, except for valine and methionine surpassed the whole egg protein. Polyunsaturated/saturated ratio was 3.23 and consisted of an essential fatty acid, linoleic acid (11.5%). The major ANF was phytohemagglutinins (lectins). Tannins and trypsin inhibitor activity was absent and total phenolics (1370 mg 100^{-1}) was insignificant. All the biological indices analyzed for proteins were significantly different (p<0.05, t-test) and inferior to casein. It was concluded that, although *C. maritima* seeds possess high proteins and EAAs, their quality was low. Before these seeds could be used as food component or protein supplement, proper processing methods (e.g. roasting, soaking, cooking, fermentation, ensiling) and safety studies are warranted to eliminate or reduce ANFs.

Key words: *Canavalia maritima,* beach bean, wild legumes, nutritive value, amino acids, antinutritional factors, protein quality.

RESUMEN

Canavalia maritima [Sinónimo: *Canavalia rosea* (Sw.) DC. y *Canavalia lineata* (Thunb.) DC.] es una leguminosa Silvestre comun en las dunas costeras de los trópicos. Semillas de *C. maritima* colectadas en las dunas fueron analizadas para conocer su composición proximal, mineral, fracciones proteínicas, amino ácidos, ácidos grasos y factores antinutricionales (ANFs) y los valores comparados con *Canavalia ensiformis* y *Canavalia gladiata*. Se llevaron a cabo estudios de crecimiento y balance de nitrógeno para la eficiencia de utilización de la proteína y el alimento, digestibilidad verdadera, valor biológico, utilización neta de proteína, retención neta de proteína y eficiencia de retención de la proteína. Las semillas de *C. maritima* contienen 34.1% de proteínas, 55.5% de carbohidratos y 1590 kJ 100^{-1} g energía. El contenido de proteína de *C. maritima* fue mayor al de otras especies de *Canavalia* spp. y otras leguminosas silvestres de la India. Los principales minerales fueron Potasio, fósforo, calcio y sodio (974, 158, 86 y 48 mg 100 g^{-1} respectivamente). Entre las proteínas verdaderas (29.3%), la principal fracción fueron las globulinas (18.7%). Los contenido de amino ácidos esenciales (EAA) treonina, valina, cistina + metionina, isoleucina, leucina, fenilalanina + tirosina y lisina fueron superiores al patrón recomendado por FAO/WHO, el arroz y la soya. Estos EAA, excepto por valina y metionina son superiores a los contenido en la proteína total del huevo. La proporción Poli insaturados/saturados fue 3.23 y consistió principalmente del ácido graso esencial, ácido linoleico (11.5%). Los principales ANFs fueron las fitohemaglutininas (lectinas). No se detectaron taninos e inhibidores de tripsina y los fenoles totales (1370 mg 100^{-1}) fueron mínimos. Todos los índices biológicos estudiados en las proteínas fueron diferentes (p<0.05, t-test) e inferiores a los de la caseína. Se concluyó que

aún cuando las semillas de *C. maritima* poseen son altas en proteína y EAAs, su calidad es baja. Antes de emplear estas semillas en alimentos o suplementos proteínicos, es necesario desarrollar métodos de procesamiento para eliminar o reducir los ANFS.

Palabras clave: *Canavalia maritima*; leguminosas silvestres, valor nutricio, calidad de proteína, amino ácidos, factores antinutricionales.

INTRODUCTION

Marginalization of pulse production in India has resulted in the import of pulses to the extent of 0.5 million tones annually (Ali and Kumar, 2003). Coastal dwellers are under constant threat of protein energy malnutrition mainly due to unfavourable climatic conditions that offer them fewer chances for sea ventures. Underutilized pulses could play an important role in minimizing the import and broadening of protein source to meet optimal nutritional requirements. The genus *Canavalia* comprises 48 species distributed throughout the tropics. *Canavalia maritima* (beach bean) is an underutilized neglected legume gremplasm endemic to coastal sand dunes of the west coast of India. It is a herbaceous vine with woody stem near the base and several branches radiating outward forming a ground cover (15-30 cm height) with evergreen trifoliate elliptic leaflets (3.5-7.5 cm) rounded at their apices, pink to purple flower racemes occur throughout the year followed by woody pods with 1-5 seeds. It has a high drought and salt tolerance, resisting erosion by wind light surf. In the Karnataka coastline (12°52' N, 70°49' E to 14°51' N, 74°7' E) the frequency of occurrence and abundance of *Canavalia maritima* are 44.4 and 19.2% respectively (Arun *et al*. 1999). Deep burial of seeds leads to increased degree of enforced dormancy leading to seed bank formation. The burial of seed at 2-10 cm depth was found to be ideal for germination (Arun *et al*. 2001).

Seeds of beach bean are consumed by both humans and animals and are an important source of dietary protein in West Africa and Nigeria, where it is widely cultivated (Abbey and Ibeh, 1987). The beach bean is also one of the major sand dune legumes of west coast of India (Arun *et al*., 1999, 2003). Although chemical composition and nutritive value of several *Canavalia* spp. are investigated (e.g. Abbey and Ibeh, 1987; Belmar *et al.,* 1999; Bressani *et al*., 1987; Bressani and Sosa, 1990; Rajaram and Janardhanan, 1992; Mohan and Janardhanan, 1994; Akpapunam and Sefa-Dedeh, 1997; Ekanayake *et al*., 2000a; Arinathan *et al*., 2003; Arun *et al*., 2003), only meagre information is available on protein quality and biological evaluation (Bressani *et al*., 1987; Bressani and Sosa, 1990; Ekanayake *et al*., 2000b). In the current study, attempts have been made to analyze proximate and mineral composition, protein fraction, amino acid and fatty acid profiles of *C. maritima*. Raw seeds of this unconventional legume were also screened for certain antinutritional factors and protein quality through animal feeding trials. Biochemical features and antinutritional factors of *C. maritima* were compared with jack bean (*Canavalia ensiformis*) and sword bean (*Canavalia gladiata*) of different geographical locations.

MATERIALS AND METHODS

Seed samples

Dried pods of beach bean, *Canavalia maritima* Thouars were obtained from coastal sand dunes of Kaup (13º14'00" N, 74º44'30" E), west coast of India during summer (February-March, 2003). The seeds were separated after eliminating the debris and damaged seeds. They were sun dried. Seed dimensions, mean weights of seed, seed coat and cotyledons were determined. Seeds were dehulled, milled (Wiley mill, 30 mesh) and stored in airtight glass containers.

Proximate analysis

Moisture was assessed on subjecting the seed flour to 100ºC in an incubator to attain constant weight and the difference in initial and final weight of flour was expressed as percentage moisture. Total nitrogen and the crude protein content (N × 6.25) was determined by micro-Kjeldahl method (Humphries, 1956). Crude lipid, crude fiber and ash contents were detected on employing AOAC methods (AOAC, 1990). Crude carbohydrate was calculated as outlined by Müller and Tobin (1980):

Total crude carbohydrates (%) = 100 – (Crude protein + Crude lipid + Crude fiber + Ash)

Gross energy was calculated based on formula given in Ekanayake *et al*. (1999):

Gross energy (kJ/100 g DM) = (Crude protein × 16.7) + (Crude lipid × 37.7) + (Crude carbohydrates × 16.7)

Mineral composition

Seed flour was digested with concentrated nitric acid, sulfuric acid and perchloric acid (10:0.5:2, v/v) and mineral constituents (sodium, potassium, calcium, magnesium, iron, copper, zinc and manganese) were determined by atomic absorption spectrophotometer

(GBC 902, Australia) as per the method outlined in AOAC (1990). Phosphorus was determined by ascorbic acid method on acid digestion followed by neutralization (phenolphthalein indicator and combined reagent) (APHA, 1995). The absorbance was read at 880 nm with KH_2PO_4 as standard (Bausch & Lomb Spectronic 21, Germany).

Protein isolates

True proteins of seed flour were extracted (Basha *et al*. (1976). To save the prolamin fraction, ethanol treatment was omitted. Proteins were purified by precipitation with 10% trichloroacetic acid (TCA) and estimated (Lowry *et al*., 1951). Albumin and globulin fractions were separated based on Murray (1979). Rest of the pellet was treated with 80% ethanol (1:10 w/v) overnight, centrifuged (20,000 g, 20 min) (Remi C-24; Mumbai, India), prolamins containing supernatant was air-dried, dissolved in 0.1 N NaOH (1:10 w/v), centrifuged (20,000 g, 20 min) and supernatant thus obtained was designated as glutelins. The protein fractions were precipitated with 10% TCA and redissolved in 0.2 N NaOH and estimated by Lowry *et al*. (1951).

Protein separation

Seed powder (100 µg) were dissolved (100 µl) in buffer (60 mM tris-HCl, pH 6.8, 10% w/v; glycerol, 2% w/v; SDS and 10% v/v mercaptoethanol). Samples were boiled for 2 min at 100˚C, cooled and 2 µl 50% (w/v) bromophenol blue solution was added (Miersch *et al*., 1998). Protein separation was carried out using one-dimensional SDS-PAGE with a 5% (w/v) stacking gel and 13.5% (w/v) separating gel (Laemmli, 1970) using mini vertical slab gel electrophoresis unit (BROVIGA; Balaji Scientific Services, Chennai, India). Equal amounts of proteins were loaded on to each lane of gel and run for 3 hr at 70 volts and the gels were stained with Coomassie Brilliant Blue R-250 (Sigma).

Amino acid analysis

Method described by Hofmann *et al*. (1997, 2003) was followed to assay amino acids. Seed flours (15 mg) were hydrolyzed with 15 ml 6 N HCl for 4 hr at 145ºC, cooled and HCl was eliminated in a rotoevaporator (RE121, Büchi Laboratoriumstechnik AG, Switzerland) combined with a diaphragm vacuum pump (MC2C, Vacuubrand GmbH, Germany). Internal standard trans-4-(Aminomethyl)-cyclohexanecarboxylic acid (Aldrich, 85765-3; purity, 97%) was added to each sample for quantitative analysis of amino acids. Derivatization step consisted of esterification with trifluoroacetylation (Brand *et al*. 1994). Samples were dried using CH_2Cl_2. Later, 12 ml fresh acidified isopropanol (acetyl chloride, 3 ml + 2-propanol, 12 ml) was added and the mixture was heated at 110ºC for 1 hr. Samples were cooled and filtered through glass fiber paper and the reagent was eliminated with a gentle stream of helium at 45ºC followed by combined evaporation with aliquots of CH_2Cl_2. Dried residue was acetylated with 300 µl trifluoroacetic anhydride overnight at room temperature. Amino acids were determined using a Gas Chromatography-Combustion-Isotope Ratio Mass Spectrometer (GC-C-IRMS/MS).

The GC-C-IRMS/MS measurements were carried out with a Hewlett-Packard 58590 II gas chromatograph, connected via a split with a combustion interface to the IRMS system (GC-C-II to MAT 252, Finnigan MAT; Germany) for the isotopic determination of nitrogen and via a transfer line with a mass spectrometer (GCQ, Finnigan MAT; Germany) for qualitative analysis and quantification of the amino acids. The capillary column of GC was a 50 m × 0.32 mm i.d. × 0.5 µm BPX5 (SGE), operating with the carrier gas flow of 1.5 ml min^{-1} with following temperature and pressure: initial 50°C (1 min), increased to 100°C at 10°C min^{-1} (10 min), increased to 175°C at 3°C min^{-1} (10 min), increased to 250°C min^{-1} (10 min); head pressure, 13 psi (90 kpa).

The essential amino acid (EAA) score was detected by the formula:

$$\text{EAA score} = \frac{\text{Milligrams of EAA in 100 mg test protein}}{\text{Milligrams of EAA in 100 mg FAO/WHO (1991) reference pattern}} \times 100$$

Fatty acid analysis

Method outlined by Garces and Mancha (1993) was followed to determine Fatty Acid Methyl Esters (FAMEs) of the seed flour. Seed flours weighing 50 mg with internal standards: American Oil Chemists Society 1 (AOCS) (palmitic acid, 6%; stearic acid, 3%; oleic acid, 35%; linoleic acid, 50%; arachidic acid, 3%; linolenic acid, 3%) and AOCS 2 (palmitic acid, 7%; stearic acid, 5%; oleic acid, 18%; linoleic acid, 36%; arachidic acid, 34%) (Merck, Germany) were transferred to tubes with teflon-lined caps and methylated (methanol, benzene, DMP, H_2SO_4; 37:20:5:2 v/v). A 2.1 ml mixture and heptane up to a total volume of 5 ml were added to the sample and placed in a water bath at 80ºC for 2 hr. Tubes were cooled and shaken to separate two phases. One µl upper layer containing the FAMEs was injected to gas liquid chromatograph (GLC) (Sigma Instruments, Baroda, India) in a glass column (Silar, 10%) packed

with 5% ethylene glycol succinate on Supelcoport 80/100 isothermically at 200ºC. Conditions for the analysis include: carrier gas, N_2; injector temperature, 225ºC; FID detector temperature, 265ºC and oven temperature, 200ºC; flow rate: N_2, 35 ml min^{-1}, H_2, 30 ml min^{-1}, O_2, 75 ml min^{-1}. The polyunsaturated and saturated fatty acid ratio was calculated as follows:

$$\text{P/S ratio} = \frac{\text{Sum of saturated fatty acids}}{\text{Sum of polyunsaturated fatty acids}}$$

Antinutritional analysis

Total phenolics of the seed flours were estimated by extracting twice with 50% methanol in a water bath at 95ºC for 10 min (Rosset *et al*., 1982). The pooled extract was made up to 10 ml, 0.5 ml extract was mixed 0.5 ml distilled water and treated with 5 ml Na_2CO_3 (in 0.1 N NaOH). After 10 min, 0.5 ml Folin-Ciocalteu's phenol reagent (Merck) (diluted 1:2 with distilled water) was added and absorbance read at 725 nm. Tannic acid served as standard. Tannins were assayed by radial diffusion method using bovine serum albumin for precipitation (Hagerman, 1987).

Trypsin inhibition activity (TIA) of the seed flour was determined based on enzymatic assay (Kakade *et al*., 1974). One gram of freshly ground and processed seed flour was extracted with 50 ml 0.01N NaOH and the suspension was made up to 2 ml with distilled water, 2 ml trypsin solution (4 mg in 200 ml 0.001 M HCl) was added to each test tube and kept in water bath at 37ºC. To each tube, 5 ml BAPNA solution (40 mg *N*-a-Benzoyl-DL-Arginine *p*-nitroanilide hydrochloride (Aldrich, 85711-4; purity, ≥99%) in 1 ml dimethyl sulfoxide diluted to 100 ml with *tris* buffer at 37ºC) was added, after 10 min the reaction was terminated by adding 1 ml acetic acid (30%), mixed thoroughly, filtered and the absorbance of filtrate was measure at 410 nm against reagent blank (1 ml 30% acetic acid containing 2 ml each trypsin and distilled water + 5 ml BAPNA solution).

Phytohemagglutinating activity was carried out using trypsin-treated rabbit erythrocyte suspension (Hankins *et al*., 1980). Alsever's solution (60 mM glucose, 40 mM citric acid and 70 mM NaCl) was used as an anticoagulant. The pH was adjusted to 6.1 with 1N HCl and the solution was autoclaved prior to use. Three ml blood was collected from six-month-old rabbit (New Zealand White), by ear vein puncturing (Gordon, 1981) directly into a graduated tube containing one ml Alsever's solution by mixing. The solution containing erythrocytes was centrifuged at 1,000 g for 5 min at 4ºC. Erythrocytes were rinsed thrice with phosphate buffered saline (PBS: 10 mM sodium phosphate buffer, pH 7.2 containing 150 mM NaCl), centrifuged at 1,000 g for 5 min at 4ºC, treated with 50 µg ml^{-1} trypsin [*N*-Benzoyl-L-tyrosine ethyl ester (BTEE), 0.04 units/mg solid] (Aldrich, 85658-4; purity, 98%) for one hr at room temperature and centrifuged. Erythrocytes were again rinsed thrice with excess PBS and centrifuged. Trypsin treated erythrocytes were suspended in PBS to make 2% (v/v) cell suspension. For agglutination assay, two-fold serial dilutions of 50 µl crude lectin solution with 0.3 M NaCl was incubated with 50 µl erythrocyte suspension in a microtitre plate for 30 min at 30ºC and was examined for agglutination under a microscope (Hankins *et al*., 1980).

Biological evaluation of protein quality

Male Wistar rats aged 21 days weighing 30±5 g were obtained for growth experiments. They were randomly divided into three groups each consisting of five rats in polypropylene metabolic cages housed in a temperature-controlled room (22±1°C) with 50% relative humidity and 12 hr photoperiod. Food and water were served *ad libitum*. Diet of rats consists of casein, protein-free (basal diet), and raw seed flour (test diet). Casein and raw seed flour diets were formulated to contain 10% protein at the expense of corn starch. The group of rat fed with casein as an active source of protein served as control. The diets were prepared and stored in airtight containers a week prior to the experiment.

Protein efficiency ratio (PER) and net protein ratio (NPR) were carried out according to Pellet and Young (1980) for 28 days. Food consumption and body weight of rats were assessed at weekly and 10-day interval. The PER, corrected PER, food efficiency ratio (FER) at 4 weeks and NPR for 10 days was calculated as follows:

$$\text{PER} = \frac{\text{Weight gain of the test animal (g)}}{\text{Protein consumed (g)}}$$

$$\text{Corrected PER} = \text{PER} \times \frac{2.5}{\text{Determined PER for reference casein}},$$

where, 2.5 as standard value for casein

$$\text{FER} = \frac{\text{Weight gain of the test animal (g)}}{\text{Food consumed (g)}}$$

$$NPR = \frac{\text{Weight gain of the test animal (g) + Weight loss of the protein free test animal (g)}}{\text{Weight of test protein consumed (g)}}$$

The Protein Retention Efficiency (PRE) was calculated according to Bender and Doel (1957):

$$PRE = NPR \times 16$$

Nitrogen balance studies were carried out as outlined by Chick *et al.* (1935). Fifteen adult male albino rats (weighing 60-68 g) were distributed into three batches in polypropylene metabolic cages. One batch of rat was fed with protein-free diet (basal diet), second and third batch with raw seed and casein diet respectively. Food and water were given *ad libitum*. The experiment was carried out for 14 days, nine days as acclimatization period and remaining five days as collection period. On each day urine and faeces were collected and pooled separately. The nitrogen of urine and faeces were estimated by micro-Kjeldahl method (AOAC, 1990). True digestibility (TD) and biological value (BV) were calculated as follows:

$$TD = \frac{N_i - (NF_1 - NF_2)}{N_i} \times 100$$

$$BV = \frac{N_i - (NF_1 - NF_2) - (NU_1 - NU_2)}{N_i - (NF_1 - NF_2)} \times 100$$

where,
N_i = Nitrogen intake of animal fed test diet;
NF_1 = Nitrogen excreted in faeces of animals fed test diet;
NF_2 = Nitrogen excreted in faeces of animal fed protein-free diet (basal diet);
NU_1 = Nitrogen excreted in urine of animals fed test diet;
NU_2 = Nitrogen excreted in urine of animals fed protein-free diet (basal diet).

Net protein utilization (NPU) was calculated (Platt *et al.*, 1961):

$$NPU = \frac{BV \times TD}{100}$$

Statistical analysis

The t-test was employed to ascertain the difference between casein and raw seed diets in growth and nitrogen balance studies (Stat Soft Inc. 1995).

RESULTS AND DISCUSSION

Nutritional evaluation

All the physical characteristics of *C. maritima* seeds analyzed are lesser than that of *C. ensiformis* and *C. gladiata* resulting in low dry matter (Bressani *et al*., 1987; Akpapunam and Sefa-Dedeh, 1997; Ekanayake *et al*., 1999) (Table 1). Seeds of *Canavalia* spp. are as large as many food legumes including faba beans (Bressani *et al*., 1987). Crude protein (34.1%) of *C. maritima* fall within the range of *C. ensiformis* and superior to *C. gladiata* (Table 2). Crude protein is also higher than other wild legumes: *Atylosia scarbaeoides* (17.3%), *Erythrina indica* (21.5%), *Lablab purpureus* (24.8%), *Neonotonia wightii* (15.1%), *Rhynchosia filipes* (16.9%), *Sesbania bispinosa* (31.1%), *Tamarindus indica* (14.0%), *Vigna trilobata* (20.2%) and *V. unguiculata* 15.9%) (Arinathan *et al*., 2003; Pugalenthi *et al*., 2004). Moisture (9.28%), crude lipid (1.85%), ash (3.50%) and crude carbohydrates (50.5%) fall within the range of *C. ensiformis* and *C. gladiata*. Crude fiber of *C. maritima* is lower than *C. ensiformis* and fall within the range of *C. gladiata.* The energy value of *C. maritima* is lower than *Canavalia* spp. (Table 2), but higher than commonly cultivated pulses (Kuzayali *et al*., 1966). Among the minerals (Table 3), manganese (2.04 mg 100 g^{-1}) exceeded the *Canavalia* spp. Iron (4.54 100 g^{-1}) and zinc (13.1 100 g^{-1}) is higher than *C. ensiformis* and *C. gladiata* respectively. Sodium (48.0 mg 100 g^{-1}) and potassium (974 mg 100 g^{-1}) fall within the range, while calcium (86.2 mg 100 g^{-1}), phosphorus (158 mg 100 g^{-1}), magnesium (23.1mg 100 g^{-1}) and copper (0.28 mg 100 g^{-1}) are lower than *Canavalia* spp. Legumes are relatively found to be rich in calcium and iron (Walker and Kochhar, 1982), but in *C. maritima* they are found to be lesser than beans, peas, beef, fish and rice (Paul and Southgate, 1978).

Separation of seed proteins by SDS-PAGE resulted in 14 fractions (91, 71, 63, 56, 43, 39, 34, 29, 26, 25, 22, 16, 11 and 6 kDa). True protein of *C. maritima* is much higher than *C. ensiformis* and *C. gladiata* (29.3 vs. 24.2-28.2, 20.8-21.3 g $100g^{-1}$) (Table 4). As that of other legumes, globulins (18.7%) of *C. maritima* formed the major storage protein surpassing *Canavalia* spp. (Table 4). In legumes, legumin and vicilin are known as the principal storage globulins (Jansman, 1996). Albumins (7.46 mg 100 g^{-1}) of *C. maritima* were comparable to that of *C. ensiformis* (7.80-8.60 mg 100 g^{-1}) and higher than *C. gladiata* (5.10-5.60 mg 100 g^{-1}). The prolamins of *C. maritima* (0.28 mg 100 g^{-1}) are lower than glutelins (2.86 mg 100 g^{-1}), which exceeded the prolamins of *C. gladiata* and *C. ensiformis*. The amino acid composition among the true protein fractions are different, globulin being relatively poor in sulphur-amino acids, while albumins

are rich in sulphur-amino acids and EAA such as lysine (Baudoin and Maquet, 1999). The EAAs, threonine, valine, cystine + methionine, isoleucine, leucine, tyrosine + phenylalanine and lysine exceeded FAO/WHO pattern (1991), rice (Livsmedelsverk, 1998) and soybean (Bau *et al*., 1994) (Table 5). All the amino acids of *C. maritima* are superior except for isoleucine + leucine and arginine of *C.* ensiformis, so also methionine and arginine of *C. gladiata* (Table 5). All the EAAs surpassed FAO/WHO pattern (1991), while threonine, leucine, phenylalanine and lysine are higher and valine is comparable to whole egg protein (FAO, 1970). Ecological conditions markedly influence the total nitrogen of seeds and thereby affect the relative proportion of EAA particularly lysine, methionine and cystine (Baudoin and Maquet, 1999). The P/S ratio of *C. maritima* (3.22) was higher than *C. ensiformis* (2.43) and *C. gladiata* (0.71-0.78) (Table 6) and was on par with many wild tropical legumes (Ezeagu *et al*., 1998). The unsaturated fatty acid, oleic acid (63%) of *C. maritima* exceeded than *C. ensiformis* (36.8) and *C. gladiata* (22.5-23.1%) elevating the P/S ratio. The essential fatty acid, linoleic acid is lesser than *C. ensiformis* and fall within the range of *C. gladiata*, while linolenic acid was not detected in our study. Tannins and trypsin inhibitors are absent unlike *C. ensiformis* and *C. gladiata* (Table 7). Smartt (1985) and Acamovic (1987) reported the absence of trypsin inhibitors in jack beans. Total phenolics of *C. maritima* were higher than *C. ensiformis* and *C. gladiata* (1370 vs. 123-730, 64-710 mg $100g^{-1}$). *Canavalia maritima* seeds exhibited strong hemagglutinating activity compared to *C. ensiformis* and *C. gladiata*.

Table 1. Physical characteristic of seeds of *Canavalia maritima* (n=20; mean±SD) and other *Canavalia* spp.

Seed features	*Canavalia maritima*	Canavalia *ensiformis**	*Canavalia gladiata***
Seed weight (g)	0.42±0.1	1.78-1.84	3.14-4.87
Cotyledon weight (g)	0.29±0.1	1.64	0.48-3.88
Seed coat weight (g)	0.13±0.01	0.2	0.65-0.99
Seed length (cm)	1.30±0.1	1.88	ND
Seed width (cm)	0.86±0.1	1.32	ND
Seed thickness (cm)	0.76±0.1	1.09	ND
Hilum length (cm)	0.55±0.02	1.12	ND

ND, Not Determined
*, Source: Akpapunam and Sefa-Dedeh, 1997; Bressani *et al*., 1987;
**, Source: Bressani *et al*., 1987; Ekanayake *et al*., 1999

Table 2. Proximate compositions of raw seed flours of *Canavalia maritima* (n=5; mean±SD) and other *Canavalia* spp. on dry weight basis

Component	*Canavalia maritima*	*Canavalia ensiformis**	*Canavalia gladiata***
Moisture (%)	9.28±0.1	6.80-13.5	7.58-12.2
Crude protein (g 100 g^{-1})	34.1±0.4	22.8-35.3	12.9-28.9
Crude lipid (g 100 g^{-1})	1.65±0.1	1.60-8.80	1.40-9.90
Crude fiber (g 100 g^{-1})	2.26±0.4	4.70-11.4	2.05-12.8
Ash (g 100 g^{-1})	3.50±0.1	2.30-4.64	3.19-4.15
Crude carbohydrates (g 100 g^{-1})	50.5±0.5	45.8-65.4	45.1-68.5
Energy value (kJ 100 g^{-1})	1590±8	1630-1710	1690-1750

*, Source: Akpapunam and Sefa-Dedeh, 1997; Arora, 1995; Bressani *et al*., 1983, 1987; D'Mello *et al*., 1985, 1988; D'Mello and Walker, 1991; Ellis and Belmar, 1985; Herrera, 1983; Kessler *et al*., 1990; Laviada, 1983; Mohan and Janardhanan, 1994; Molina *et al*., 1974, 1977; Novus, 1994; Ologhobo *et al*., 1993; Rajaram and Janardhanan, 1992; Revilleza *et al*., 1990; Rodrigues and Torne, 1991; Udedibie *et al*., 1994
**, Source: Arinathan *et al.*, 2003; Bressani *et al*., 1987; Mohan and Janardhanan, 1994; Rajaram and Janardhanan, 1992

Table 3. Mineral compositions of seed flours of *Canavalia maritima* (n=5; mean±SD) and other *Canavalia* spp. on dry weight basis (mg $100g^{-1}$)

Minerals	*Canavalia maritima*	*Canavalia ensiformis**	*Canavalia gladiata***
Sodium	48.0±1	5.80-710	0.26-83.9
Potassium	974±6	450-2860	790-2280
Calcium	86.2±4	100-360	150-310
Phosphorus	158±2	263-600	262-441
Magnesium	23.1±0.1	50.0-144	65.2-172
Iron	4.54±1.8	Trace-3.51	Trace-45.2
Copper	0.28±0.1	0.33-10.0	0.36-1.67
Zinc	13.1±1.2	1.10-43.0	1.37-3.70
Manganese	2.04±0.2	0.22-0.87	0.23-0.95

*, Source: Arora, 1995; Bressani *et al*., 1987; D'Mello *et al*., 1988; Kessler *et al*., 1990; Mohan and Janardhanan, 1994; Novus, 1994; Rajaram and Janardhanan, 1992; Rodrigues and Torne, 1991; Udedibie *et al*., 1994
**, Arinathan *et al*., 2003; Bressani *et al*., 1987; Ekanayake *et al*., 1999; Mohan and Janardhanan, 1994; Rajaram and Janardhanan, 1992

Table 4. True protein fractions of raw seed flour of *Canavalia maritima* (n=5; mean±SD) and other *Canavalia* spp. on dry weight basis (mg $100g^{-1}$)

Protein fractions	*Canavalia maritima*	*Canavalia ensiformis**	*Canavalia gladiata**
True protein	29.3±0.2	24.2-28.2	20.8-21.3
Albumins	7.46±0.3	7.80-8.60	5.10-5.60
Globulins	18.7±0.3	13.0-14.6	12.5-13.0
Prolamins	0.28±0.03	0.63-0.91	0.91-0.98
Glutelins	2.86±0.1	1.84-1.96	1.81-2.06

*, Source: Mohan and Janardhanan, 1994; Rajaram and Janardhanan, 1992;

Table 5. Amino acid compositions of acid hydrolyzed seed flour of *Canavalia maritima* and other *Canavalia* spp. (mg 100 mg^{-1} protein)

Amino acid	*Canavalia maritima*		*Canavalia ensiformis**	*Canavalia gladiata***	FAO/ WHO Pattern[b]	Whole egg protein[c]
	Quantity	EAA score[a]	Quantity	Quantity		
Glutamic acid	18		2.4-16	6.3-17		13
Aspartic acid	23		2.3-14	6.0-16		9.6
Serine	5.0		1.1-3.9	2.2-4.9		7.6
Threonine	5.2	152.9	1.0-4.3	2.4-4.2	3.4	5.1
Proline	4.5		0.8-4.3	2.5-4.4		4.2
Alanine	5.2		0.1-4.7	2.5-4.6		5.9
Glycine	4.4		0.9-4.3	2.2-4.6		3.3
Valine	6.8	194.3	1.1-5.3	3.1-5	3.5	6.9
Cystine	6.1	320[d]	Trace-0.3	Trace-0.8	2.5[d]	5.9
Methionine	1.9		Trace-0.6	Trace-3.8		3.4
Isoleucine	5.4	192.9	2.5-16	8.6-13.8	2.8	6.3
Leucine	10.3	156.1			6.6	8.8
Tyrosine	4.0	190.5[e]	0.8-3.2	2.2-4	6.3[e]	4.2
Phenylalanine	8.0		1.1-4.7	2.2-4.6		5.7
Tryptophan	ND		0.3-0.5	0.4-1.2	1.1	1.7
Lysine	13	224.1	1.3-6.8	4.3-6.1	5.8	7
Histidine	ND		0.6-3.2	2.6-3.8	1.9	2.4
Arginine	3.0		1.1-3.8	3.4-5.1		6.1

[a] Essential amino acid score (FAO/WHO, 1991), [b] FAO/WHO pattern (FAO/WHO, 1991), [c] Whole egg protein (FAO, 1970), [d]Methionine+Cystine, [e]Phenylalanine+Tyrosine, ND, Not detected, *, Source: Arora, 1995; Bressani *et al*., 1987; D'Mello *et al*., 1985, 1988; D'Mello and Walker, 1991; Kessler *et al*., 1990; Mohan and Janardhanan, 1994; Novus, 1994; Rajaram and Janardhanan, 1992, **, Source: Bressani *et al*., 1987; Ekanayake *et al*. 1999; Mohan and Janardhanan, 1994; Rajaram and Janardhanan, 1992

Table 6. Fatty acid compositions of seed flours of *Canavalia maritima* (n=3, mean) and other *Canavalia* spp. (g 100 g^{-1} lipid)

Fatty acid	*Canavalia maritima*	*Canavalia ensiformis**	*Canavalia gladiata***
Saturated fatty acids			
Palmitic acid ($C_{16:0}$)	2.18	21.8	47.2-47.3
Stearic acid ($C_{18:0}$)	20.9	7.37	9.23-11
Polyunsaturated fatty acids			
Palmitoleic acid ($C_{16:1}$)	ND	9.44	ND
Oleic acid ($C_{18:1}$)	63	36.8	22.5-23.1
Linoleic acid ($C_{18:2}$)	11.5	18.0	10.7-14.0
Linolenic acid ($C_{18:3}$)	ND	6.62	6.56-8.49
Sum of saturated fatty acids	23.1	29.2	56.4-58.3
Sum of polysaturated fatty acids	74.5	70.9	39.8-45.6
P/S ratio[a]	3.22	2.43	0.71-0.78

ND, Not detected
[a]Ratio of polyunsaturated/saturated fatty acids
*, Source: Mohan and Janardhanan, 1994
**, Source: Mohan and Janardhanan, 1994; Spoladore and Teixeria, 1987

Table 7. Antinutritional components of seed flour of *Canavalia maritima* (n=5; mean ±SD) and other *Canavalia* spp. (mg 100 g^{-1} on dry weight basis)

Component	*Canavalia maritime*	*Canavalia ensiformis**	*Canavalia gladiata***
Total phenolics	1370±0.04	123-730	64-710
Tannins	NP	54-70	23-60
L-DOPA	ND	246-2630	213-3010
Trypsin inhibition activity	NP	ND	ND
Phytohemagglutinin activity	+++	+	++

NP, Not present
ND, Not determined
+, ++, +++, Extent of Red blood cells clumping
*, Source: Mohan and Janardhanan, 1994

Biological evaluation

The quality of a protein cannot alone be assessed by biochemical studies, hence, it has to be substantially supported by animal feeding trials. Scanty information is available on biological evaluation studies on *Canavalia* spp (Belmar *et al.*, 1999; Bressani *et al.,* 1987; Bressani and Sosa, 1990; Ekanayake *et al.,* 2000b*)*. Animal experiments aids to understand possible effects exerted by antiphysiological factors on the test animal. Protein quality of a legume is affected by antiphysiological factors that interact with the intestinal tract (e.g. protease inhibitors, phytate, lectins, tannins and saponins) and thereby reducing the amino acid absorption and protein digestibility (Liener, 1994). In our study, the animals were fed with casein, raw seed flour and protein-free diet (basal diet) (Table 8). Food intake drastically affects the growth rate and animal performance (Forbes, 1986). In the current study, the food intake of raw seeds was less than casein, indicating reduced preference of raw meal (Table 9).

Table 8. Composition of basal diet (g/100 g) of experimental albino rats

Ingredient	Content
Corn starch	80
Corn oil	10
Non-nutritive cellulose	5
Salt mixture[a]	4
Vitamin mixture[b]	1

[a]Salt mixture: $CaCO_3$, 78.6 g; $Ca_3C_{12}H_{10}O_{14}{\cdot}4H_2O$, 308.3 g; $CaHPO_4{\cdot}2H_2O$, 112.8 g; K_2HPO_4, 218.8 g; KCl, 124.7 g; NaCl, 77.1 g; $MgSO_4$, 38.3 g; $MgCO_3$, 35.2 g; $Fe(C_6H_{17}N_3O_7)$, 15.3 g; $MnSO_4{\cdot}H_2O$, 0.201 g; $CuSO_4{\cdot}5H_2O$, 0.078 g; KI, 0.041 g; $AlNH_4(SO_4)_2{\cdot}12H_2O$, 0.507 g.
[b]Vitamin mixture: Vitamin A, 1000 IU; Vitamin D, 100 IU; Vitamin E, 10 IU; Vitamin K, 0.5 mg; Thiamine, 0.5 mg; riboflavin, 1 mg; Pyrodoxine, 0.4 mg; Pantothenic acid, 4 mg; Niacin, 4 mg; Choline, 200 mg; Inositol, 25 mg; Para-aminobenzoinc acid, 10 mg; Vitamin B_{12}, 2 µg; Biotin, 0.02 mg; Folic acid, 0.2 mg; added cellulose to make up to 1 g.

Gain in body weight is significantly lower than casein diet, which might have resulted due to low protein intake and assimilation. Due to low food and protein intake, the FER and PER drastically reduced. A decrease in weight (4 g per rat) was registered on feeding whole *C. ensiformis* meal by Bressani and Sosa (1990). The corrected PER (based on values 2.5 for casein) is also calculated. The NPR was very low and resulted in low PRE when compared to casein fed animals (NPR, 0.99 vs. 2.66; PRE, 15.84 vs. 42.6 1) (Table 9).

In addition to the growth studies, nitrogen balance studies are also conducted. The TD, BV and NPU of raw seed flour and casein are 42.26 vs. 90.8, 37.55 vs. 88.94 and 16.88 vs. 80.76 respectively and are significantly different ($p<0.05$) (Table 9). Ekanayake *et al*. (2000b) observed low NPU (13.8) on feeding the rats with whole *C. gladiata* seeds. They advocated the removal of seed coat to improve the nutritional quality.

Table 9. Food intake, protein intake, gain in body weight, food efficiency ratio, net protein retention (NPR), protein retention efficiency (PRE), true digestibility (TD), biological value (BV) and net protein utilization (NPU) of casein and raw seed flours of *Canavalia maritima* fed to albino rats (n=5; mean±SD)*

Assay	Casein	*Canavalia maritima*
Growth studies		
Food intake for 28 d (g)	143.42±3.42[a]	93.1±7.36[b]
Protein intake for 28 d (g)	14.33.±0.34[a]	9.31±0.74[b]
Gain in body weight for 28 d (g)	33.79±1.6[a]	0.97±0.32[b]
FER	0.24±0.01[a]	0.01±0.004[b]
PER	2.36±0.09[a]	0.1±0.04[b]
Corrected PER**	2.5±0[a]	0.11±0.04[b]
Gain in weight for 10 d (g)	9.77±0.16[a]	0.33±0.01[b]
Weight loss for 10 d (g)	3.1±0.36	3.1±0.36
Protein consumed for 10 d (g)	4.88±0.03[a]	3.46±0.05[b]
NPR	2.66±0.1[a]	0.99±0.12[b]
PRE	42.61±1.58[a]	15.84±1.85[b]
Nitrogen balance studies		
TD (%)	90.8±0.58[a]	42.26±0.33[b]
BV (%)	88.94±0.33[a]	37.55±1.66[b]
NPU (%)	80.76±0.77[a]	16.88±0.58[b]

*, Figures across the columns with different letters are significantly different ($P<0.05$, t-test)
**, Based on values of 2.5 as standard for casein

Antinutritional factors

The raw *Canavalia* encompasses several antinutritional factors and toxic components. The *C. ensiformis* possesses concanavalin (con A) that agglutinates the RBC of chicken, guinea pigs, rabbits, sheep, rats and most of the human blood types (Liener, 1979) and mitogenic in resting lymphocytes (Hankins and Shannon, 1978). Similarly, our study indicated that *C. maritima* consists of con A-like lectins or hemagglutinins. D-mannose or D-glucose specific con A, when injected into the intestinal loops binds to the lower half of absorptive villi, reducing the area of small intestine available for the uptake of nutrients. Tannins and lectins are known to inhibit the digestive enzymes (Jambunathan and Singh, 1981) and are undesirable from the nutritional point of view even at the low level (Arinathan *et al*., 2003). Hemagglutinins are localized in the cytoplasm of cotyledons and embryonic cells (Gupta, 1987). Ekanayake *et al*. (2000b) reported that con A is known to trigger over production of mucus, which resulted in high fecal output in rats fed with raw seeds. Canavanine, structural analogue of arginine, causes poor growth performance of chick on being fed with autoclaved or cooked *C. ensiformis* (Escobar *et al*., 1983; D'Mello *et al*. 1985), but Belmar and Morris (1994a, 1994b), Leon *et al*. (1991) and Michelangeli and Vargas (1994) reported that canavanine was not the only cause of poor performance of chicks fed on diet containing *C. ensiformis*. Canavanine is known to induce reduction in protein and glycoprotein synthesis, inhibition of alkaline phosphate activity and RNA synthesis (Rosenthal, 1977). L-canaline, another antinutritional factor of *C. ensiformis* is produced from canavanine by hydrolytic cleavage (Rosenthal, 1972a and 1972b; Rosenthal and Bell, 1979). Canaline (analogue of ornithine) toxicity is exhibited in animals

by the inhibition of pyridoxyl phosphate-containing enzymes, by competition with ornithine in the arginine urea cycle or by forming a complex with pyridoxal phosphate cofactor (Acamovic, 1987). It is known that jack bean is a commercial source of urease and does not exert any toxic effect in the uriolytic and uricolytic animals (see Belmar *et al*., 1999). Saponins can exert its effect by erythrocyte hemolysis reduction of blood and liver cholesterol, depression of growth rate, inhibition of smooth muscle activity, enzyme inhibition and reduction in nutrient absorption (Cheeke, 1971). Canatoxin, a neurotoxic protein though present in jack bean is not considered as an antinutritional factor. Other antinutritional factors *viz*., polyphenols and trypsin inhibitors gets destroyed by moist heat (Babar *et al*. 1988). Low NPU in animals is also due to polyamines, which is known to inhibit the amino acid transport (Ekanayake *et al*., 2000b). Several methods (heat processing, ensiling, fermentation and addition of chemicals) have been employed to lower the antinutritional factors in jack bean (see Belmar *et al*., 1999). The con A and canavanine is reported as the main ANFs in jack bean seeds that cause poor animal performances (Jayne-Williams, 1973; Michelangeli and Vargas, 1994).

CONCLUSIONS

Investigations on the nutritional and antinutritional qualities of seeds of *Canavalia maritima* are scanty. The nutritional evaluation of *C. maritima* seeds in the current study proved to be an attractive protein source and EAAs in southwest coast of India. The EAAs are more than the recommended FAO/WHO standards. Among the ANFs studied, hemagglutinins are found to be most potent and hence, reflected on feeding experimental rats with the raw seeds. The declined food intake may be due low palatability and toxic principles and hence, future research should be directed to understand biochemistry and physiology of antinutritional principles of seeds of *C. maritima*. This is the first detailed investigation on protein quality evaluation of raw seeds of *C. maritima* providing a baseline data for future exploration. Further exploration of the ANFs associated with seeds, their toxic effects and elimination have to be achieved to employ *C. maritima* seeds as a potential protein source.

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