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RESEARCH ARTICLE

ANTIOXIDANT AND BIOCHEMICAL ANALYSIS OF CEIBA PENTANDRA (KAPOK) SEEDS

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ABSTRACT

An unconventional Seeds of *Ceiba pentandra* (Kapok) were analyzed for enzymatic antioxidants, biochemical complexes and protein functionality to assess its potential usage as an alternative source of protein. Buffer extract of seeds had scrutinized for enzymatic antioxidants like Superoxide dismutase, Catalase, peroxidase as well as Glutathione Peroxidase and found to be ample. Total Carbohydrates and Proteins were determined by Phenol Sulphuric acid method and Lowry method, results obtained with the above procedures were 6.5% and 58.5% correspondingly and also the Proteolytic activity was estimated with trypsin as µg of tyrosine liberated in 30 minutes at pH 7.4 and temperature 37^0 C per ml of enzyme was found to be 275µg/ml. Seed storage protein profiles of *Ceiba pentandra* seeds studied by Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE),total soluble proteins were resolved on 10% SDS polyacrylamide gels. A total of 13 major protein bands were noticed in seed sample ranging in molecular weight from 31.40 KD to 15.71 KD.

INTRODUCTION

Protein-energy malnutrition (PEM) was also referred to as protein-calorie malnutrition. It was the leading cause of death for children in developing countries, due to the insufficient consumption of protein and energy (measured by calories) to satisfy the body's nutritional needs. PEM was serious problems in many developing countries; most commonly affected were children between the ages of 6 months and 5 years (W.H.O, 2000). Excessive free radicle production and lipid peroxidation had been shown as significant contributors to the process of atherosclerosis, ischemic heart disease, diabetes, carcinogenesis, neurodegenerative disorder, rheumatic disorders, aging etc. in humans (Tiwari, 2001, 2004). Various phytochemicals present in plants help in providing protection against cancer, cardiovascular diseases, dementia, cataract, macular degeneration, ageing and various other disorders associated with increased oxidative stress. These phytochemicals act as antioxidants which intercept free radicals and protect the cells from the oxidative damage (Nuttall et al., 1999). There have been a substantial number of studies that have used sodium dodecylsulphate-polyacrylamide gel electrophoresis (SDS-PAGE) to profile seed storage proteins in legumes (Ladizinsky 1975, 1979; Ladizinsky and Hymowitz 1979; Ahmad and Slinkard 1992; Fotso *et al.,*1994; Ahmad *et al.,*1996; Limongelli *et al.,*1996; Ahmad 1997; El-Shanshoury 1997; Krochko and Bewley 2000; Valizadeh 2001; Mallick and Sawhney 2002; Asghar *et al.,*2003). Seed storage protein profiling based on SDS-PAGE can be employed for various purposes, such as varietal identification, biosystematics analysis, determination of phylogenetic relationship between different species, generating pertinent information to complement evaluation and passport data (Sammour 1991). Like pulses, oil seeds were rich in protein and in addition they contain a high level of fat. Hence they were not only good sources of protein but were concentrated source of energy. Kapok seeds (*Ceiba pentandra*) seeds were high in protein content and could be classified as oil seeds. Kapok (*Ceiba pentandra*, family Bombacaceae) trees, in India, it was distributed from Rajasthan, and south ward into sarakallu and adjacent area of chittoor district, Andhra Pradesh. India. Kapok fruits, while still hanging on the tree, the pods will eventually split into 5 parts from base to apex at maturity and release seeds covered with long silky hairs (Lotschert & Beese 1991). The soft, silky, yellowish white lint was an outgrowth from the inner wall of the woody pod which also covers the seeds inside. It is totally different from the cotton tree where the lint/cotton is formed from the outer covering of the seed with 10-35mm long, kapok fibers were found to be too fine and slippery to be

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spun into thread. They were also lustrous, as a result of waxy coating, thus suitable for insulating material. The fiber contents make up to 64 % of cellulose. They were very elastic and have been used for stuffing material such as in cushions, pillows, mattresses, life jackets and life belts (Vaughan 1970).

MATERIALS AND METHODS

Chemicals

Chemicals and reagents used for antioxidant estimations and SDS-PAGE were purchased from Merck. All additional chemicals used were of analytical grade. Altogether the experiments were performed at room temperature unless otherwise stated.

Collection of seeds

Kapok fruits were very striking with pointed capsule shape, which looks like cocoa pod (Figure.1). The woody pods hang down in bunches like small cucumber. They were green at first, and then turn brown and blackish when ripe**.** Mature Dried fruits of *Ceiba pentandra* were obtained from in and around Andhra University area, Visakhapatnam, India, and were explored for enzymatic antioxidants, carbohydrates, proteins, protease and protein profiling.

Preparation of seed extract

Two grams of *Ceiba pentandra* seeds were soaked overnight in 0.06M sodium phosphate buffer, pH 6.8.The sample was grounded using mortar and pestle with 10 ml of same buffer and filtered through four layers of cheese cloth to remove fiber debris. The filtrate of the sample was centrifuged at 5500 rpm for 30min at 5^0C.The supernatant was then vacuum filtered again with Whatman No.42 filter paper. Finally, the last filtrate of sample was stored at 4^0C.

Assay of superoxide dismutase

The assay of superoxide dismutase was carried out based on the reduction of Nitroblue tetrazolium (NBT) (Beuchamp and Fedovich B C 1976). To 0.5 ml of seed extract, 1ml 125mM of Sodium Carbonate, 0.4 ml of 24µM NBT and 0.2 ml of 0.1mM EDTA were added. The reaction was initiated by adding 0.4 ml of 1mM Hydroxylamine hydrochloride. Zero time absorbance was taken at 560 nm using spectrophotometer, followed by recording the absorbance after 5 min at 25˚C.The control was simultaneously run without seed extract. Units of SOD were expressed as amount of enzyme required for inhibiting the reduction of NBT by 50%.The specific activity was expressed in terms of Units per milligram of protein.

Assay of Catalase

Catalase activity was determined by the titrimetric method. (Chance and Maehly. 1995). To 1ml seed extract, 5 ml of 300 µM phosphate buffer (pH 6.8) containing 100 µM hydrogen peroxide (H_2O_2) was added and left at 25°C for 1 min. The reaction was stopped by adding 10 ml of 2% sulphuric acid, and residual H_2O_2 was titrated with potassium permanganate (0.01N) till pink colour was obtained. Enzyme activity was estimated by calculating the decomposition of µM H_2O_2 per min per mg protein.

Assay of Peroxidase

To 3.5 ml of phosphate buffer (pH 6.5), 0.2 ml of seed extract and 0.1 ml of O- dianisidine solution were added. The reaction was initiated by adding 0.2 ml of 0.2 mM H_2O_2 and the absorbance was read at every 30 sec intervals upto 3 min. The peroxidase activity was calculated using an extinction coefficient of oxidized O-dianisidine and the enzyme activity was expressed as units per mg of protein. (Malik C P and singh M B 1980).

Assay of Glutathione Peroxidase

To 0.2 ml each of 0.8mM EDTA, 10mM sodium azide, 1mM GSH, 2.5 mM H_2O_2, 0.32M phosphate buffer (pH 7.0) and seed extract were mixed and incubated at 37^0C for 10 min. The reaction was arrested by the addition of 0.5 ml of 10% TCA and the tubes were centrifuged at 5000 rpm for 5 min. To 0.5 ml of supernatant, 3.0 ml of 0.33 mM phosphate solution and 1.0 ml 0.6 mM DTNB reagent were added and the colour developed was read at 420 nm immediately. Graded amount of standards were also treated similarly. Glutathione peroxidase activity was expressed as µg of Glutathione utilized per mg of protein. (Rotruck J T *et al* 1973).

Estimation of Carbohydrates

Total Carbohydrates were estimated by Phenol Sulphuric acid method. (Dubois *et al*.1956).

Estimation of Proteins

Total protein concentration in the seed samples was determined by Lowry method using BSA as the standard. (Lowry *et al*., 1951).

Assay of Protease

A100µl of aliquot of the titrate was incubated with 900 µl of 2% casein in NaOH-KH_2PO_4 standardization buffer (0.1M, pH: 7) at 37^0C for 30 min. The reaction was stopped by the addition of 1.5 ml of 10% trichloroacetic acid. After 15 min, the mixture was filtrated and the protein concentration in 0.5 ml of filtrate was measured by method of Lowry *et. al.* One unit (U) of protease activity was equivalent to µg of tyrosine liberated per ml of enzyme under the assay conditions.

SDS Polyacrylamide Gel Electrophoresis

SDS-PAGE was performed to study the protein profile of *Ceiba Pentandra* seed according to the method of Laemmli (1970) on 10% (W/V) polyacrylamide gel in tris-Hcl buffer, pH 8.3 containing 0.1% SDS. Sample incubation buffer also contain 5% (V/V) β-mercaptoethanol. About 10µl of sample containing 5-20 µg of protein was loaded in each well and electrophoresis was carried out for about 3h.The gel was stained with 0.2% (W/V) coomassie brilliant blue R-250 and destained in 5% methanol, 7% acetic acid solution. Different marker proteins along with their molecular weights (adapted from technical bulletin of Bio-Rad (2000) prestained SDS-

PAGE standards for Tris-HCl gel) given in parenthesis: Lysozyme (19,809 Da), Trypsin (20,100 Da), and BSA (84,796 Da) were used for calibration. It is important to note that molecular weights values of some of these preparations are not matched with the reported values due to the formation of dye protein complex as reported by the manufacturer (Bio-Rad, 2000).

RESULTS AND DISCUSSION

Antioxidant compounds in food play important role in disease prevention and health promotion. The screening of seed extracts and natural products for antioxidant and antimicrobial activity had revealed the potential of higher seeds as a source of new agents to serve the processing of natural products (Mokbel and suganuma, 2006). Use of natural antioxidants, as food additives for inactivating free radicles receives a lot of attention nowadays, not only for their scavenging properties, but also because they were natural, non-synthetic products and their appreciation by consumers were very favorable. Synthetic antioxidants had not proved very useful as compared to plant derived natural antioxidants because the advantage of using natural antioxidants was that they might provide more useful flavonoids and other antioxidant compounds not present in standard oral synthetic antioxidants (vievkanathan et al., 2003). Looking to all this, the present investigation was an important step in developing new plant based antioxidant food supplement. The four enzymatic antioxidants assayed in *Ceiba pentandra* seeds namely Superoxide dismutase, catalase, peroxidase and Glutathione Peroxidase were perceived as 1.574±0.469 units/mg protein, 67.193±1.128 μMH_2O_2 decomposed/min/mg protein, 0.493±0.036 units/mg protein and 56.5±1.30 units/mg protein respectively all these values were the average of three determinations and were expressed as mean ± S.D.

Dietary fat, carbohydrate and protein were the primary energy-containing macronutrients consumed on a routine basis by humans. Crude protein value of 58.5% was observed for *Ceiba pentandra* seeds. This value was obviously much higher than most legumes/grains consumed in India. It was an essential macromolecule without which our bodies would be unable to repair, regulate, or protect itself. Essential body processes such as water balancing; nutrient transport and muscle contractions require protein to function (Robert et al., 2006). Some beans grown in other regions of the world once mentioned as underutilized but currently receiving research attention (Carmona-Garcia *et al.,* 2007, Coelho *et al.,* 2007, Rajeev *et al.,* 2008). Similar high protein content was reported for some faba beans (*Vicia faba* L.) grown in Egypt and Canada (El-Sayed *et al.,* 1986, Sosulski and McCurdy, 1987). The notably high level of protein in this little known seed underscores its importance as a potential protein source. Carbohydrate constitutes a major class of naturally occurring instant energy giving biomolecule and were essential for the maintenance of plant and animal life and also provide raw materials for many industries. The total Carbohydrate content of seeds observed as 6.45%. The protein digesting enzyme, Protease play a part in helping to soften the fruit tissues as the fruit ripes, making it even more attractive to animals that might disperse the seeds. So perhaps the activities of these protease enzymes will increase during the ripening process. Applications of plant proteases, such as Bromelain from the stems and fruits of

Figure.1:Kapok fruit pods, seeds imbedded in lint and seeds respectively.

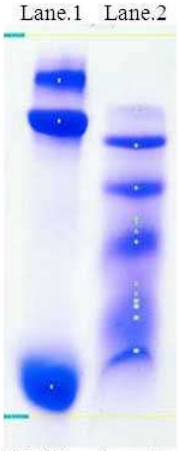

Figure.2:SDS–PAGE of marker proteins (lane.1),seed sample of *Ceiba pendandra* (lane.2) performed according to the method of Leammli (1970) on 10% polyacrylamide gel. Tracking dye, bromophenol blue position is also marked as well pos 58 and dye end 1054.Marker proteins used were: 1.BSA; 2. Lysozyme; 3.Trypsin. Fractioned proteins in each preparation are number accordingly.

QUERIES			
Band	Position	Mol. Wt.in KD	Rf
1	254	31.40	0.197
2	346	22.94	0.289
3	457	21.21	0.401
4	539	20.02	0.483
5	544	19.95	0.488
6	571	19.57	0.515
7	597	19.21	0.541
8	706	17.79	0.651
9	734	17.44	0.679
10	752	17.22	0.697
11	766	17.05	0.711
12	796	16.69	0.741
13	882	15.71	0.827

Table.1:Relative Mobility (Rm) and molecular weight (MW) values of different proteins present in seed extract of *Ceiba pentandra*.

pineapples, include uses in the pharmaceutical industry as a blood anti-coagulant, and in the prevention of proteinaceous hazes in chill-proof beers. The Proteolytic activity of the *Ceiba pentandra* seed was estimated with trypsin as µg of tyrosine liberated in 30 minutes at pH 7.4 and temperature 37^0 C per ml of enzyme was 275µg/ml. Electrophoretic pattern of

Table.2: Preliminary phytochemical screening of Ceiba pentandra seeds

Chemical Group/Enzyme	Observation
Superoxide dismutase	1.574±0.469 units/mg protein
Catalase	67.193±1.128 µM H_2O_2 decomposed/min/mg protein
Peroxidase	0.493±0.036 units/mg protein
Glutathione Peroxidase	56.5±1.30 units/mg protein
Carbohydrates	6.45%.
Proteins	58.5%
Protease	275µg/ml

Mean±SD (n=3) for enzymatic antioxidants

protein sample of *Ceiba pentandra* seeds showed presence of a number of fractionated proteins ranging from low to high molecular weights. The Molecular weights of these proteins were determined using Gel documentation and listed in Table 1. A total of 13 major protein bands were noticed in seed sample (Figure.2, Lane.2) ranging in molecular weights from 31.40 KD to 15.71 KD, having the R_f Range between "0.192 to 0.827". These proteins represent the storage, structural and biologically active proteins including enzymes associated with the hydrolysis of stored food (amylase, maltose, Protease, carbohydrase and lipase), germination, aerobic/anaerobic respiration, lectins and enzyme inhibitors (Fukushima, 1991; Mandal and Mandal, 2000).Besides, proteins required for seed protection i.e., defense against micro-organisms and insects may also constitute the total protein content of the seed (Kelly *et al.,* 1998). All the above results were mentioned in Table: 2.

Conclusion

From this it was conclude that *Ceiba Pentandra* seeds were good source of proteins besides as antioxidants. These were currently underutilized/ unexplored in most regions of the world, stood nutritionally promising and could solve the problem of protein malnutrition which was a major public health concern in the developing world. Toxicological studies, fatty acids analysis and evaluating it in a large number of subjects should be carried out in order to be certain for it uses as food supplements.

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