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Isolation and Determination of Protein Breakdown Activiti from Moth Bean Vigna Aconitifolia

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ABSTRACT

Moth bean (Vigna aconitifolia) is an orphan legume of Vigna genus, exhibiting wide adaptability and has the potential to grow well in arid and semi-arid areas, predominantly across different eco-geographical regions of Asia, particularly the Indian subcontinent. The inherent adaptive attributes of this crop have made it more tolerant towards a diverse array of abiotic and biotic stresses that commonly restrain yield among other Vigna species. Additionally, the legume is recognized for its superior nutritional quality owing to its high protein content as well as amino acid, mineral and vitamin profile and is utilized as both food and fodder. Moth bean can play a vital role in sustaining food grain production, enhancing nutritional security as well as provide a source of income to resource-poor farmers amid rise in global temperatures and frequent drought occurrences, particularly in rain-fed cropping systems which accounts for about 80% of the world's cultivated land. Vigna aconitifolia contains protein breakdown activities ie protease activiti and it also have protein breakdown enzyme" Trypsine " which is determined by colorimetrically and report the amount of Trypsine present in the given Moth bean (Vigna aconitifolia).

KEYWORDS: Proteins, Amino Acids, Enzyme, Moth bean, Activity and Proteins break down .

INTRODUCTION

Protease is a general term for a class of enzyme that hydrolysis protein peptide linkage. Proteins are decomposed into small molecules such as amino acids and polypeptide under the action of Protease. Therefore the human body food absorption and digestion physiological activities peptide activation, etc. are all closely related to protease, involving all aspects of life. According to the nomenclature committee of the international union of Biochemistry and molecular biology, Protease are classified in enzyme class 3, the hydrolyse and the subclass 3.4, the peptidase. The term " peptidase " is recommended by the nomenclature committee of international union of Biochemistry and molecular biology to be used as synonymous with "peptide hydrolase " for any of the enzyme that hydrolysis peptide bonds.

Peptidase are generally categorized in to two major groups based on their site of action, that is exopeptidase and exopeptidase .

Exopeptidase are those Protease that Cleave the amino or carboxy terminal of the substrate, where endopeptidase cleave peptide bonds distant from the termini of the substrate.

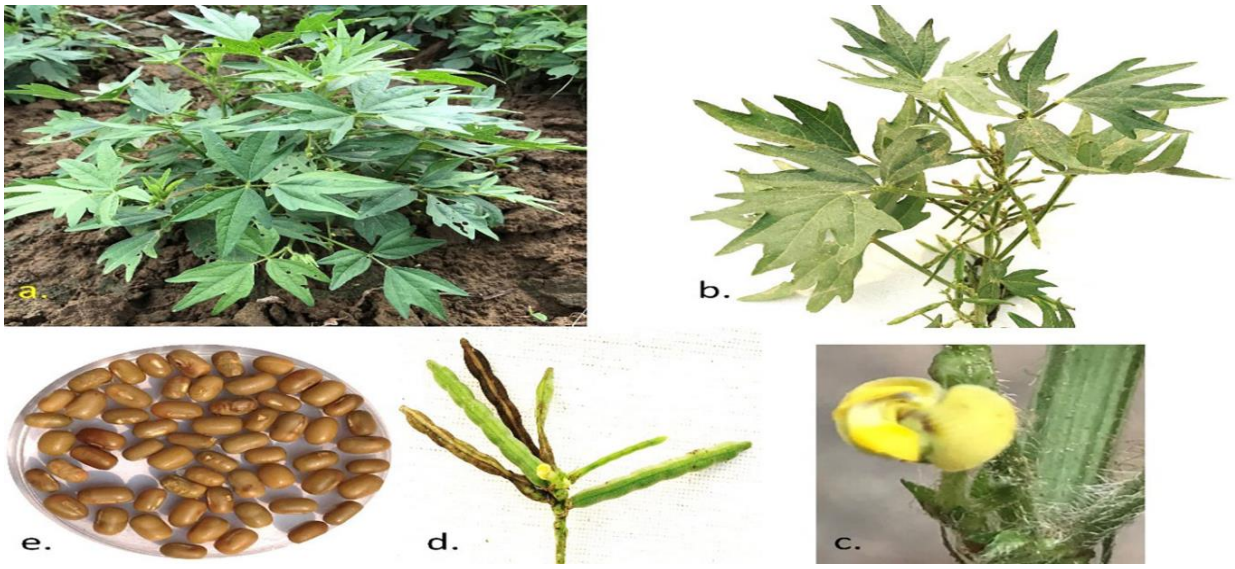
Protease are classified into four types

1 Cysteine Protease
2 Aspertate Protease
3 Metalloprotease
4 Serine protease

MATERIAL AND METHOD

ISOLATION METHOD

Vigna aconitifolia seeds were purchased from general shop and used that as source of our protease inhibitor.



MOTH BEAN (Vigna aconitifolia plant)

MOTH BEAN (Vigna aconitifolia seeds)

Defatting method : 150g of Vigna aconitifolia seeds was weighed and griended to fine powder and stirring continuously for 1 hours using 30% chilled Acetone on magnetic sterer . The obtained mixture was filtering using suction filters and obtained powder was dried at room temperature and stored at 4ºc.

Extraction method : 10% of extract to 150ml of 100mM Phosphate buffer pH 7.6 add weighed 16 g of defatted powder stirred continously for 3 hours on magnetic Stirrer at 4ºc . Then centrifuge for 10 minutes at 10000rpm . Measure the obtained supernatant and discard the obtained pellet.

pH fractionation : 80ml of extract 9f crude was taken whose pH was adjusted to 5 using 1N acetic acid ,the whole reaction was performed at 4ºc the solution after the pH was adjusted it was kept in refrigerator for 10minutes, 4ºc for about 10 minutes . Collect the supernatant and resuspend the pellet using minimal amount of Phosphate buffer and centrifuge the suspended pellets for 10 minutes at 10000 rpm 4ºc. Collect the clear solution for further assay . With the Collected supernatant, it'd pH was brought down to 7.6 with 25% ammonia, measure the supernatant. The clear solution was used for protease inhibition activity and it'd protein content.

CHEMICAL REAGENTS

1 1 N- alpha Benzoy-l DL-Arginine -p- Nitroanilidine
2 Trypsine, Bromelain and Rutoude Trihydrate tablets
3 Sodium phosphate buffer pH 7.6
4 Tris buffer pH 8.2
5 1 mM HCl buffer

1. Standard curve for protein (Bovine serum Albumin) by FC method

Principle:

The principle involved in this method is determining the protein concentration by calculating the reactivity of the peptide nitrogen with the copper ions under alkaline conditions followed by reduction reaction of Folinciocaltealy phosphomolybdic phosphotungestic acid to Heteropolymolybdenum blue by copper catalysis oxidation of aromatic acids (tryptophan and tyrosine)

Chemical Reagent

1. Copper Reagent A
2. Copper Reagent B
3. Alkaline Copper Reagent
4. FC Reagent
5 Protein Standard solution

Procedures

1. Pipette out 0.0,0.2,0.4,0.6,0.8 and 1,0 ml of standard protein solution (200ug/ml) into different test tubes.
2. Make Up to 1ml using distilled water
3. Add 5ml of Alkaline copper Reagent to all tubes . Mix the solution well and allow the tubes to stand for 10 minutes
4. The add 0.6ml FC reagent to each tube and thoroughly mix the content of each tube

5. After incubation gor 30 minutes read absorbance at 660nm against the suitable blank solution.

2. Standard curve for Trypsine

Principle

N- alpha Benzoy-l DL-Arginine -p- Nitroanilidin hydrochloride is a synthetic substrate with a dye bound to an amine acids . Trypsine cleaves the peptide bonds between the carboxy group of arginine and release p- notroanine . Therefore BAPNA the colour less solution upon treatment with Trypsine realses out p- nittoaniline which is Yellow in colour. The yellow colour is direct evidence that Trypsine hydrolysis the peptide bonds in substrate to produce a chromogenic product.

Reagents

1. Tris HCl buffer pH8.2
2. 0.1 molar Phosphate buffer pH 7.6
3. Trypsine solution
4. BAPNA substrate
5. 30% acetic acid
6. Extract solution

Procedure

1. pipette out 0.0,0.2,0.4,0.6,0.8 and 0.1ml Alliquotes of Trypsine in to different test tubes.
2. Make Up to 1.0ml using 0.1M phosphate buffer pH 7.6
3. Start the reaction with 3.5ml of substrate BAPNA and incubate for 10minutes at 37ºC
4. Arrest the reaction with 1.0ml of 30% acetic acid
5. Read the Absorbance at 410nm against a suitable blank.

3. Standard curve for protease inhibitor

Principle

N- alpha Benzoy-l DL-Arginine -p- Nitroanilidin hydrochloride is a synthetic substrate with a dye bound to an amine acids . Trypsine cleaves the peptide bonds between the carboxy group of arginine and release p- notroanine . Therefore BAPNA the colour less solution upon treatment with Trypsine realses out p- nittoaniline which is Yellow in colour. The yellow colour is direct evidence that Trypsine hydrolysis the peptide bonds in substrate to produce a chromogenic product.

Determination of Trypsine inhibitor activity

Trypsine inhibitor activity is determined by incubating different Alliquotes of inhibitor extract with 50ug of Trypsine for 10 minutes and redifual activity were determined buy using synthetic BAPNA.

Reagents

1. Tris HCl buffer pH8.2
2. 0.1 molar Phosphate buffer pH 7.6
3. Trypsine solution
4. BAPNA substrate
5. 30% acetic acid

6 Extract solution
s

Procedure

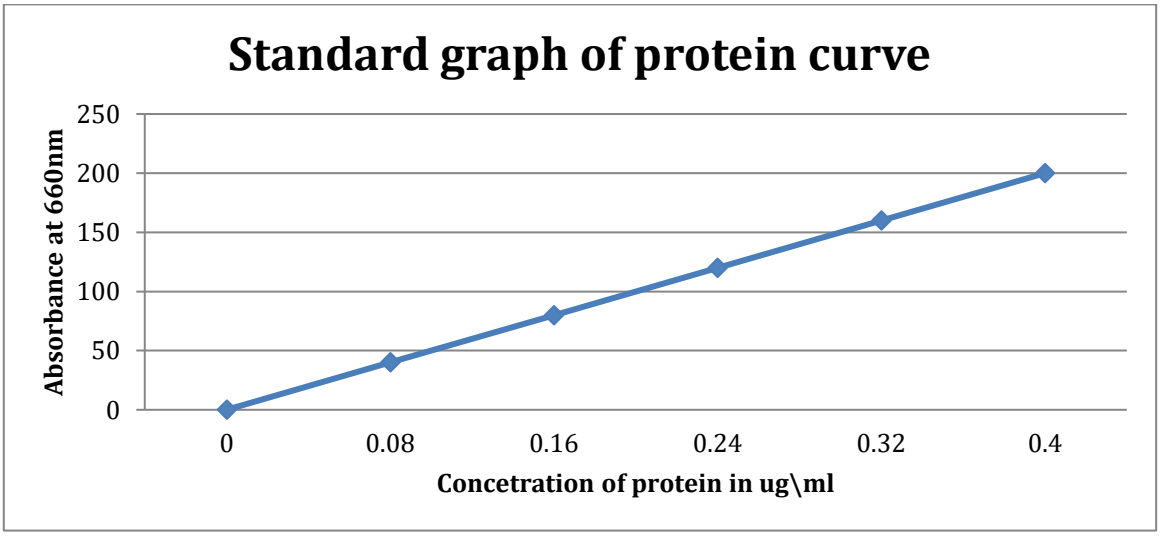
1. pipette out 0.0,0.2,0.4,0.6,0.8 and 1.0 ml of Alliquotes of dilute extract into different test tube .
2. Make Up to 1ml using 0.1 M phosphate buffer .
3. Add 1.0ml of Trypsine to each tube and stand gor 10 minutes at 37ºC
4. Add 3.5 ml of substrate BAPNA for each tube and incubate at 37ºC for about 10 minutes .
5. Then add 1.0ml of 30% of acetic acid to all the tubes .

6 Read absorbance at 410nm against a suitable blank.

RESULT AND DISCUSSIONS

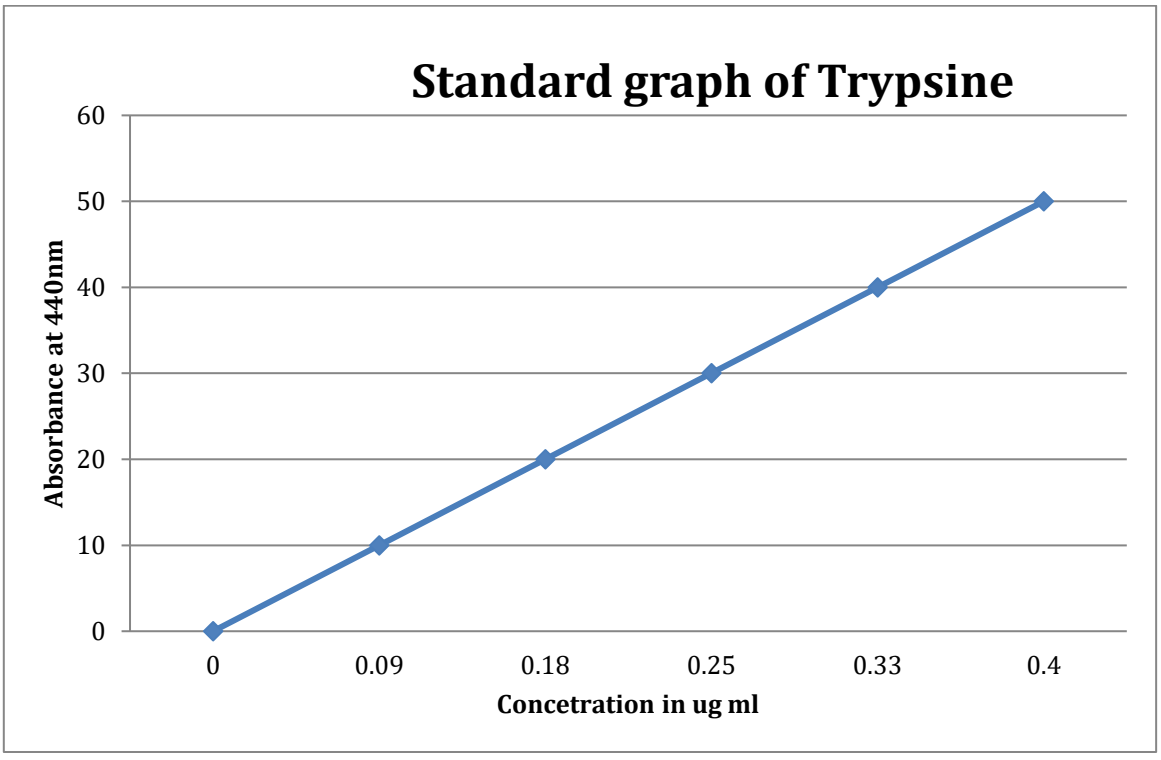
1. Standard curve for protein (Bovine serum Albumin) by FC method

Test tube no	Valume of BSA	Valume of distilled water	Amount of BSA in ug	Valume of Alkaline copper Reagent	Inculcate at room temperature for 10min	Valume of FC reagent	Inculcate 10minutes at room temperature	Absorbance
1	0.0	1.0	00	5		0.6		0.0
2	0.2	0.8	40	5	-	0.6	-	0.08
3	0.4	0.6	80	5	-	0.6	-	0.16
4	0.6	0.4	120	5	-	0.6	-	0.24
5	0.8	0.2	160	5	-	0.6	-	0.32
6	1.0	0.0	200	5	-	0.6	-	0.40



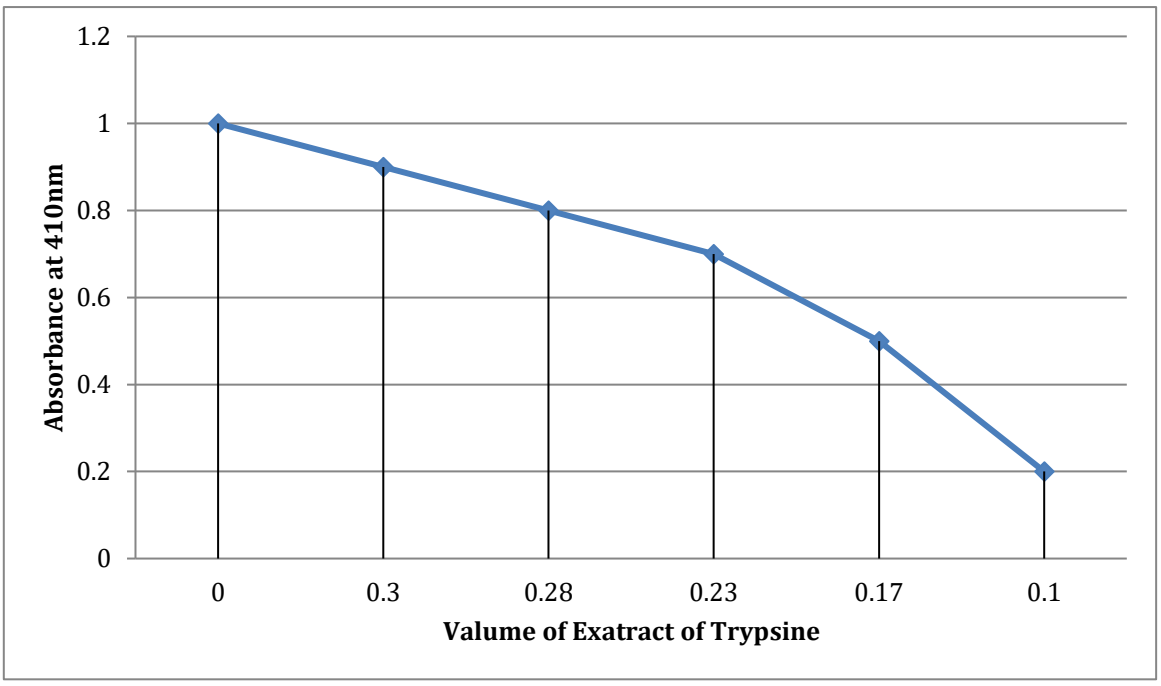
2 Standard curve for Trypsine

Test tube Number	Valume of Trypsine in ml	Valume of phosphate buffer in ml	Amount of Trypsinein ug	Valume of substrate in ml	Incubate at 37ºC for room temperature	Valume of 30% acetic acid in ml	Absorbance at 410nm
1	0.0	1.0	00	3.5	-	1.0	0.0
2	0.2	0.8	10	3.5	-	1.0	0.09
3	0.4	0.6	20	3.5	-	1.0	0.18
4	0.6	0.4	30	3.5	-	1.0	0.25
5	0.8	0.2	50	3.5	-	1.0	0.33
6	1.0	0.0	60	3.5	-	1.0	0.40



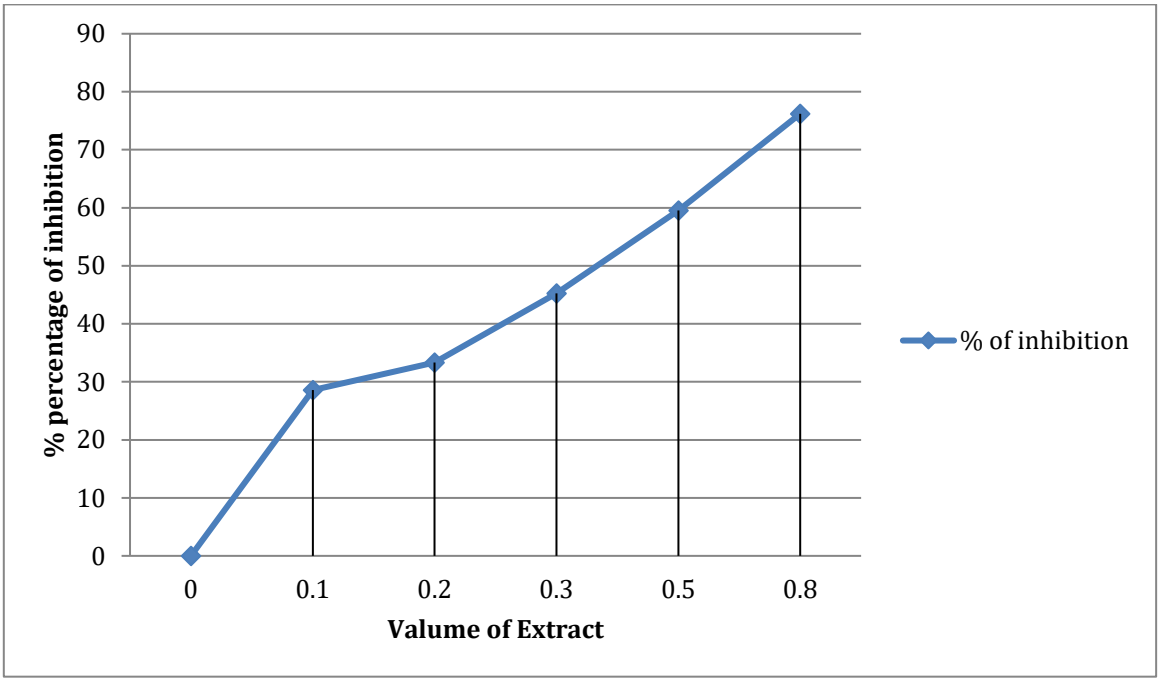
3 Standard curve for protease inhibitor

Test tube Number	Valume of Extract in ml	Volume of phosphate buffer in ml	Volume of Trypsine in ml	Incubate at 37ºC for 10minutes	Volume of substrate in ml	Incubate at 37ºC for 10minutes	Valume of 30% acetic acid in ml	Absorbance at 410nm
1	1.0	1.0	1.0	-	3.5	-	1.0	0.0
2	0.1	0.9	0.9	-	3.5	-	1.0	0.30
3	0.2	0.8	0.8	-	3.5	-	1.0	0.28
4	0.3	0.7	0.7	-	3.5	-	1.0	0.23
5	0.5	0.5	0.5	-	3.5	-	1.0	0.17
6	0.8	0.2	0.2	-	3.5	-	1.0	0.10
7	1.0	0.0	0.0	-	3.5	-	1.0	0.05



4 Percentage of inhibition activity

Valume of Extract in ml	Amount of protein in ug	Absorbance at 410nm	% activity	% inhibition
Control 0.0	0.0	0.42	100	0.0
0.1	25.76	0.30	71.43	28.57
0.2	51.52	0.28	66.66	33.34
0.3	77.28	0.23	54.76	45.24
0.5	128.8	0.17	40.47	59.53
0.8	206.08	0.10	23.80	76.20
1.0	257.6	0.08	21.42	78.58



CONCLUSION

Vigna aconitifolia is very useful habit for treating various types of diseases. Various studies have demonstrated that V.aconitifolia possess antioxidant, antiinflammatory, neurodegenerative activity. The chemical constituents such as phenolics, trypsin inhibitors, protease inhibitors are responsible for this activities. Review of the literature concluded that Vigna aconitifolia is considered to be a useful herbal medicinal plant.

REFERENCE

1 Springer US, Sathe SK, Venkatachalam M. Fractionation and biochemical characterization of moth bean (Vigna

2 aconitifolia) proteins. LWT-Food Science and Technology. 2007; 40(4):600-610.
3 Adsule RN. Moth bean (Vigna aconitifolia (Jacq.) Marechal). In Food and Feed from Legumes and Oilseeds, 1996, 203-205.
4 Brink M, Jansen PCM. Vigna aconitifolia (Jacq.) Maréchal. [Internet] Record from PROTA4U. Brink, M. & Belay, G. (Editors). PROTA (Plant Resources of Tropical Africa / Ressources végétales de l'Afrique tropicale), Wageningen, Netherlands. <"Archived copy". Archived from the original on November 8, 2013. Retrieved October 20, 2013.>. Accessed 15 November 2013-200.
5 https://en.wikipedia.org/wiki/Vigna_aconitifolia#Human_consumption.
6 Millerd A. Biochemistry of legume seed proteins, Annual Review of Plant Physiology. View at Publisher View at Google Scholar. 1975; 26(1):53-72.
7 Hammerstrand GE, Black LT, Glover JD. Trypsin inhibitor in soy products: modification of standard analytical procedures. Cereal Chemistry. 1981; 15:215-218.22.
8 Awah FM, Uzoegwu PN, Oyugi JO, et al. Free radical scavenging activity and immunomodulatory effect of Stachytarpheta angustifolia leaf extract. Food Chemistry. 2010; 119(4):1409-1416.doi:10.1016/j.foodchem.2009.09.020. [Cross Ref]
9 Perumal Siddhuraju and Klaus Becker, The antioxidant and free radical scavenging activities of processed cowpea (Vigna unguiculata (L.) Walp). Seed extracts aagriculture, ecosystems &environment. 1988;24(4):453-458. References and further reading may be available for this article.
10 Franks PW, Wong MY, Luan J, Mitchell J, Hennings S, Wareham NJ. Non-esterified fatty acid levels and physical inactivity: the relative importance of low habitual energy expenditure and cardio-respiratory fitness, British Journal of Nutrition, View at Publisher • View at Google Scholar • View at Scopus. 2002; 88(3):307-313.
11 Schandrel SH. Method in Food Analysis. New York, NY, USA: Academic Press, 1970.
12 Wilcox JR, Premachandra GS, Young KA, Raboy V. Isolation of high seed inorganic P, low-phytate soybean mutants. Crop Science. 2000; 40(6):1601-1605. doi: 10.2135/cropsci2000.4061601x.[Cross Ref]