



ANTIOXIDANT POTENTIAL IN DOLICHOSE LABLAB, L. (LABLAB PURPUREUS).

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ABSTRACT Lablab purpureus is a major staple food crop, widely distributed to many tropical and subtropical countries. The wild forms of lablab are originated in India or south-East Asia, and it introduced in to Africa from Southeast Asia during eighth century.[R.1]

The present study was to investigate the antioxidant activity of lablab purpureus. The ethanolic extract of seed bean where screened for their enzymatic and nonenzymatic antioxidant activity. The level of enzymatic antioxidant is Glutathione peroxide (GPX), Catalase (CAT), Superoxide dismutase (SOD), Glucose -6-Posphate dehydrogenase (G6PD) and Glucose-S-Transferase (GST) was found. Lablab purpureus good and commendable store of nonenzymatic antioxidants namely reduced glutathione (GSH), Vitamin E and Vitamin C as this increase antioxidant potential of Lablab purpureus. Which provided the antioxidants need in the diet and so Lablab purpureus accomplish high value nutritive and natural store of antioxidants.

KEYWORDS : Antioxidant, Dolichose LabLab purpureus,(L.), Enzymatic, Nonenzymatic.

The common name of Dolichose LabLab purpureus, L. is in hindi walachi seng. Dolichose Lablab, commonly referred to as field bean is a legume, In India it is an important multipurpose legume crops used as pulse, vegetable and forage. Whereas as its dried beans serve as pulse, its tender pod with beans or the fresh beans alone serve as vegetable. Beside India, it is also cultivated as a forage crop in the United State, Hungary, Nigeria and found in Malesia, Indonesia, South Africa, Zimbabwe, Sierra Leone, Toyo, Madagascar, Central and South America. [R.2,3]

Dolichose Lablab is an Annual woody climbing herb which can rich a length of 5m. Leaves are pinnate and generally 3-foliate, Leaflets are acute, entire, 6-12cm by 5-9cm. Flower are white or purplish pink, Fruits are purple or green pods, 6cm long by 2cm wide flattened. Contain 4-5 seed and turn light brown purple when mature. [R.4,5]

The World Health Organization (WHO) estimates that 4 billion people, 80 percent of the world population presently use herbal medicine for some aspect of primary health care. [R.6,7]. Plant show wide range of pharmacological activities including antimicrobial, antioxidant, anticancer, hypolipidemic, cardiovascular, central nervous, respiratory, immunological, anti-inflammatory, analgesic, antipyretic and many other pharmacological effect. [R.8,9,10]

Antioxidant are added to a variety of food to prevent or deter free radical induced Lipid Oxidation. If free radical production rate exceeds the normal capacity of the antioxidant defenses mechanism, substantial tissue injury results [R.11]. Antioxidant sewerage free radical and quench the subsequent reaction, hence protecting the macromolecule and cellular environment from toxicity and degeneration [R.12]. The present study revive to investigate enzymatic and non-enzymatic antioxidant activity for Dolochose Lablab purpureus, L..



Dolochose Lablab purpureus, L

MATERIALANDMETHOD

Collection of the plant material: the beans of L.purpureus sweet purple were collected in end of November 2017, from field area of

Lakani village, district Bhandara, Maharashtra state, India. This bean was known as wallachi seng. The sampal were identified by the herbarium deposited in Department of Botany Hislop collage Nagpur and from Tropical forages, media [R.13].

EXTRACTION OF PLANT MATERIAL :

The fresh bean seed were washed with tap water and then with distilled water, minced into small pieces, the tested material completely shade drying and made to fine powder with homogenizer, About 2.0 gm of powder was taken along with 20 ml ethonol and mashed well in a homogenizer and then used for analysis [R.14].

Analysis of parameter :

Enzymatic antioxidant

Superoxide Dismutase⁸ (SOD) 0.5 ml of homogenate was diluted to 1 ml with water. Then 2.5 ml ethanol and 1.5 ml chloroform, a cooled reagent was added. This mixture was shaken for 1minute at 4°C and then centrifuged. The enzyme activity in the supermanent was determined [R.15,16].

Glucose -6-Phosphate Dehydrogenese¹⁰ (G6PD) the incubation mixture contain 1.0 ml of buffer, 0.1 ml of magnesium chloride, 0.1 ml of NADP⁺, 0.5 ml of phenazinmethasulphate, 0.4 ml of the dye solution and the requisite amount of the enzyme extract. The mixture was allow to stand at room temperature for 10 minute to permit the oxidation of endogenous material. The reaction was initiated by the addition of 0.5 ml of glucose-6phospate. The absorbance was read at 640 nm against water blank at one minute interval for 3-5 min in a UV Spectrophotometer. The activity of enzyme was calculated in unit by multiplying the change in OD/min by the factor 6/17.6, which is the molar extinction co-efficient of the reduced co-enzyme [R.17].

Glutathione Peroxidase⁶ (GPX) 0.2 ml of tris buffer, 0.2 ml of EDTA, 0.1ml of sodiam- azide and 0.5 ml of homogenate were added. To the mixture, 0.2 ml of glutathione followed by 0.1 ml of hydrogen peroxide where added. The content where mixed well and incubated at 35°C for 10minute along with a tube contening all reagent except sample. After 10 minute the reaction was found by the addition of 10%TCA, centrifuged and supermanat where analyzed [R.18,19].

Catalase⁷ (CAT) 0.9 ml of phosphate buffer, 0.1 ml of EDTA, 0.1ml of homogenate and 0.4 ml of hydrogen peroxide wee added. After 60 sec. 2.0 ml of dichromate acetic acid mixture was added. The tube were kept in boiling water bath for 10 minute and the colour developed was read at 620nm [R.20,21].

Glutathione-S-Transferase⁹ (GST) the reaction mixture contained 1.0 ml of phosphate buffer, 0.1ml of CDNB, 0.1ml of homogenate and 0.7 ml of distilled water. The reaction mixture incubated at 37°C for 5minute then the reaction started by addition of 0.1 ml of 30m glutathione. The absorbance change to red at 340 nm. Reaction mixture without the enzyme was used as the blank [R.22].

Non enzymatic antioxidant

Reduced glutathione¹³ the sample where homogenized in phosphate buffer. 0.5 ml was precipitate with 2.0 ml of 5%TCA. 1.0 ml of supermanant was taken for centrifugation and added to it 0.5 ml of Ellman's reagent and 3.0 ml of phosphate buffer. The yellow colour developed at 412nm. A series of slandered treated in a similar manner along with blank containing 3.5 ml of buffer [R.23,24].

Vitamin C¹² 0.5 ml of sample mixed with homogenizer with 1.0 ml of 6%TCA and centrifuge for 20 minute at 3500g. to 0.5 ml of supermanant, 0.5 ml of DNPH reagent mixed well. The tube allow to stand for 3 hour. Then placed in ice cold water and added 2.5 ml of 85% sulfuric acid allow to stand 30minute. Set a slandered containing 10-50g of ascorbic acid and pressed along with a blank containing 0.5 ml of 4%TCA. The colour develop read at 530nm.[R.25,26,27].

Vitamin E¹ this method involve reduction of ferric ion to ferrous ion by alpha tocopherol and the formation of a red coloured complex with 2,2' dipyridyl. Absorbance of the chromophore was measured at 520nm.[R.28].

Table -1; Level of Enzymatic antioxidant in Dolichose Lablab purpureus,(L.).

Species	Superoxide Dismutase ug/mg	Glucose -6-Phosphate Dehydrogenase ug/mg	Glutathione Peroxidase ug/mg	Catalase ug/mg	Glutathione-S-Transferase ug/mg
Lablab purpureus, (L.).	368.70±0.11 20	0.890±0.00 63	986±0.69 71	71.68±0.1 760	21.93±0.03 67

Table -2; Level of Non-Enzymatic antioxidant in Dolichose Lablab purpureus,(L.).

Species	Reduced glutathione ug/mg	Vitamin C ug/mg	Vitamin E ug/mg
Lablab purpureus,(L.).	5.83±0.0341	81±0.1689	73.66±0.0876

DISCUSSION AND CONCLUSION

The antioxidant effect of ethanolic extract of seed of Lablab purpureus was studied that, the enzymatic antioxidant namely, SOD, G6PD, GPX, CAT, GST. The high amount of GPX, contain follow by SOD and CAT. The very small amount of G6PD and GST. The SOD prevent the formation of OH, that emphasize against the defiance mechanism of toxicity of oxygen. The contain show the low level of GST which protect LPO by the mixing the toxic effect with GSH¹⁶. G6PD contribute to the maintenance of cellular redox homeostasis, by reducing the efficient glutathione reductase activity and recycling glutathione. The Dolichose Lablab purpureus contain good store of Catalyst as a significant antioxidant, which protect the plant by scavenging free radical and H₂O₂, GPX. Catalysis. The decomposition of H₂O₂ and ROOH at expensive of reduced glutathione they formed a glutathione disulphid(GSSH).

At non-enzymatic level of Lablab purpureus, increase the vitamin C at their seedling period, which exhibited the high level of residue formation of vitamin C, and low level of vitamin E, and GR, so the high level of GR is present in plant, vitamin is much effective. Vitamin E residue in the lipid bilayer, of cell transfer the phenolic hydrogen to peroxy free radical, in cellular phospholipids minimum level of vitamin E residue is antioxidant response in development of metabolic state. Which show the good scavenging activity. The seed bean of Lablab purpureus is the cheap source of dietary energy in the form of carbohydrate. The Lablab plant contain the high level of Ascorbic acid, which is the main source of metabolic activity. Lablab purpureus has the potential to protect the cell against free radicals mediated oxidative damage. Hence form result the Lablab purpureus is the promising source of nontoxic natural antioxidant, which contain high level of nutritive potential, to added as a functional food for diet. Thus Dolichose Lablab purpureus,L. contain good and commendable store of both enzymatic and non-enzymatic antioxidant.

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REFERENCES

- Murphy AM and Colucci PE. A tropical forage solution to poor quality ruminant diets: A review of Lablab purpureus. Livestock Research for Rural Development 1999; 11(2). <http://www.cipav.org.co/lrrd>.
- Gowda MB, Dolichose bean (Dolichose lablab), University of Agricultural Science, GKVK, Bangalore India, [http://www.lablab.org\(Nov.04.2017\)](http://www.lablab.org(Nov.04.2017)).
- Us National plant germplasm system, taxon: Lablab purpureus(L.) Sweet, <http://npgsweb.arsgrin.gov/gringlobal/taxonomydetail.aspx;104887>
- Duke JA and Ayensu ES Medicinal plant of China, Volume 1and2. Reference Publication Inc. United states of America 1985.
- Wee YC. A guide to medicinal plant. Singapore Science Center Publication: Singapore 1992.
- Davison Hunt I, Ecological ethanobotany: stumbling towards new practices and paradim. MASAJ 2000; 16:1-6.
- World Health Organization (1997). Medicinal plant in China, A selection and commonly used species, WHO Regional Publication. Western Pacific Series No.2, 110-11.
- Al-Snafi AE, Medicinal plant with antioxidant and free radical scavenging effect (part2):plant based review. IOSR Journal of pharmacy 2016;6(7):62-82.
- Al-Snafi AE. Antimicrobial effect of medicinal plants (part 3): plant based review IOSR Journal of Pharmacy 2016;6(10):67-92.
- Al-Snafi AE, Therapeutic properties of medicinal plant: a review of there detoxification capacity and protective effect (part1). Asian Journal of Pharmaceutical Science and Technology 2015;5(4):257-270.
- Rahman MAA and Moon ss. Antioxidant polyphenol glycosides from the plant Drabanemorosa. Bull Korean ChemSoc 2007;28(5):827-31.
- Vichnevshaia, K.D. and Roy, D.N.(2001). Oxidative stress and ant –oxidative dispense with a emphasis on plants antioxidants. Enviro. Rev.,7:31-35.
- Tropical Forages, Dolichose lablab <http://www.tropicalforage.info/key/forage/Media/Html/lablab-purpureus.htm>
- Singh,R.andSankar, C. Screening for antidiabetic activity of the ethanolic extract of Dolichose Lablab Leaves.ph Tech. Med. 2012; 1(5):177-180.
- KakkarPandVishwanathan P.N.(1984). A modified Spectrophotometric assay of superoxide dismutase. Indian JOURNAL OF Biochem. Biophys.21:130-132.
- Brou YC, Adolphe Z, Omar D, Murielle E (2007). Water stress induce overexpression of SOD that contribute to the production of Cowpea plant against oxidative stress Afr. J. Biotechnol 6(17):1982-1986.
- Leopold J.A. and Losoalzo J.(2000). Cyclic strains modulate resistance to oxidant stress by increasing G6PD expression in vascular smooth muscle cell. Am.K. physiol, 279:2477-2485
- Rotruck J.J, Pope A.L., GanterH.E and Swanson A.B.(1973).Selenium: Biochemical role as a component of glutathione Peroxidase. Science, 179:588-590
- Aebi,H (1984). Catalase in vitro. Methods Enzymol 105:121-126.
- Sinha A.K.(1972). Colorimetric assay of catalase. Anal.Biochem.47:389-394.
- Chance B, Maehly AC (1955). Assays of catalases and peroxidase. In: Method in enzymology, Vol II Academic press, New York.
- Habig W.H., Pabst M.J, and Jakoby W.B(1974).Glutathione S Transferase. The first enzymatic step in mercapturic acid formation. J.Biol. Chem.246:7130-7139.
- Oyaizu M, Studies on product of browning reaction prepared from glucose amine. Jpn J Nutr 1986;44:307-315.
- Carlberg I, Mannervik B(1985) Glutathione reductase. Method Enzymol 113:488-495.
- Sadasivam S, Manickam A (1997). Vitamins. In-Sadasivam S,Manickam A (eds) Biochemical method, 2nd edn. New Age International (p) Limited, New Delhi pp185-186.
- Burguieres EP, Mc Cxue Y, Kwon In, Shely K (2006). Effect of vitamin C and follic acid on seed vigour response Bioresour Technol. 95:1393-1404.
- Prieto P, Pineda M and Aguilar M. Spectrophotometric quantitation of antioxidant capacity through the formation of phosphomolybdenum complex specific application to the determination of vitamin C. Anal Biochem 1999;269:337-341
- Sen C., Khanna S. and Roys(2006). Touotrienols: vitamin E beyond tocopherol. LifeScience, 78(18):2088-98.