Research Paper Life Science

Antioxidant Activity of Two Year Old Aloe Vera Plants Extract After Applying of Organic Manure

Rakesh Mehta	Department of Botany, M. G. M. Science College, Itarsi (M. P.), India.
*** Saroj Mawase**	Department of Botany, M. G. M. Science College, Itarsi (M. P.), India. * corresponding author
Zea–ul–Hasan	Department of Botany, Sofea College, Bhopal (M.P.), India.

ABSTRACT

The Aloe vera plant has been known and used for centuries for its health, beauty, medicinal and skin care properties. Aloe vera is a stemless or very short-stemmed succulent plant growing to 60–100 cm (24–39 inch) spreading by offsets. The leaves are thick and fleshy, green to grey-green, with some varieties showing white flecks on their upper and lower stem surfaces like other Aloe species. Aloe vera forms a symbiosis that allows the plant better access to mineral nutrients in soil with applied of farm yard manure. Aloe also neutralizes oxidative stress. Antioxidants present in Aloe vera prevent or slow oxidative damage to cells. These agents are able to remove the deleterious effects of free radicals within cells. Solvent extraction methods are widely used for extracting antioxidants in food and other sources.

KEYWORDS Superoxide dismutase, glutathione reductase, catalase, ascorbic acid, total β-carotene.

Introduction:

The ***Aloe vera*** plant has been known and used for centuries for its health, beauty, medicinal and skin care properties. The name ***Aloe vera*** is derived from the Arabic word "Alloeh" meaning "shining bitter substance" while "vera" in Latin means "true." 2000 years ago, the Greek scientists regarded ***Aloe vera*** as the universal panacea. The Egyptians called Aloe "the plant of immortality." Today, ***Aloe vera*** plant has been used for various purposes.[1, 2]

Aloe also neutralizes oxidative stress. First, aloe naturally contains a variety of antioxidants including vitamins, phenolic compounds and peroxidases that directly quench free radicals on the skin and in the body. In fact, a recent investigation of the antioxidant potential of an ***Aloe vera*** extract found that it exhibited a radical scavenging activity of 72%, compared with only 65% for alpha-tocopherol.[3] Second, in addition to containing its own stores of antioxidants; ***Aloe vera*** gel may also activate the body's endogenous antioxidant enzyme system. Research in mice found internal administration of the gel elevates liver levels of three out of five cellular antioxidant enzyme families: glutathione family, superoxide dismutase (SOD) family and catalase family.[4] Antioxidants such as carotenoids, phenols, flavonoids, vitamins and dietary glutathiones are capable of acting as free radical scavengers, peroxide decomposers, singlet and triplet oxygen quenchers, enzyme inhibitors and synergists.[5] The antioxidant activity of phenolic compounds are mainly due to their redox properties, which can play an important role in adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen or decomposing peroxides and have health functional properties that may protect humans from various diseases. [6–7]

The phenomenon is described as oxidative stress and complex protective mechanisms have been evolved by plants (and other organisms) to mitigate and repair the damage initiated by free radicals. The primary constituents of these protective mechanisms include enzymes such as superoxide dismutase (SOD), catalases and glutathione reductase and free radical scavengers such as carotenoids, ascorbate, tocopherols, and reduced glutathione (GSH) respectively.[8] From this viewpoint, the present study was carried out to evaluate the antioxidant status of ethanol extract of two year old ***Aloe vera*** plants.

Material and methods:

The study was to evaluate the "**Effect of graded levels of fertilizer application on antioxidants dynamics of *Aloe vera***". The study was conducted in the Department of Botany, Sofea College, Bhopal (M.P.), India (23°16´ 0´ ´ North, 77°24´ 0´ ´ East). Experimental ***Aloe vera*** plants were completely cultivated in four pots from 2010 to 2014.

Field of proposed works:

The present study is performed to evaluate the antioxidants effect of aqueous extract of two year old ***Aloe vera*** plants.

(i) Climate:

The climate of the area is semi-arid subtropical monsoon type with an average annual rainfall received during the monsoon month (June–September). The mean maximum and minimum temperature ranged from 29–44°C and 9–23°C respectively. The area is characterized by hot summer and mild winter. The mean date of commencement of monsoon is around June–19 whereas the mean date of withdrawal of monsoon is September–21.

(ii) Preparation of experimental soils:

A field experiment is laid out at **Sofea College, Bhopal (M.P.)**. The experiment is conducted in fixed pots for cropping of ***Aloe vera***. The soil for experimental is sandy coastal and heavy black cotton soils.

(iii) Pot's soil preparation (Treatment):

T–1. Sandy coastal soil**:** Golden sand**:** Farm yard manure (1**:**1**:**1), (4 Pots).

T–3. Heavy black cotton soil**:** Golden sand**:** Farm yard manure (1**:**1**:**1), (4 Pots).

T–5. Control (Sandy coastal soil**:** Golden

sand – (1**:**1), (4 Pots).

T–6. Control (Heavy black cotton soil**:**

Golden sand – 1**:**1), (4 Pots).

(iv) Planting time:

Suckers should be planted in July–August (2010) during monsoon season to get better field survival and subsequent growth of the plants.

Sample collection for investigation of antioxidants (*Aloe vera*):
The thick fleshy leaves are ready for harvest from the first after planting. It is carried out in the morning. Leaves were weighed and expressed in mg per leaf. Leaf extracts were prepared from 2 g fresh weight. Fresh clean whole ***Aloe vera*** leaves were cut and the outer green rind was discarded. The tip and basal portions of ***Aloe vera*** leaves are trimmed off and washed in clean water to remove soil and other dirty materials. Finally the leaves were soaked in clean distilled water. After removing the rinds from the leaves, the inner gel was collected. The mucilaginous inner pulp was minced and thoroughly homogenised with a hand held blender. Each leaf produced approximately 120 ml of gel. The homogenised gel was lyophilised at 22°C and the resultant lyophilised material was stored frozen until further extraction.

Preparation of *Aloe vera* leaf extract:
Freshly collected lyophilised material of ***Aloe vera*** homogenized with 800 ml PBS (phosphate-buffered saline: 0.06 mM sodium phosphate buffer containing 0.15 M NaCl, pH 7.4) in a warring blender, extracted with PBS, at room temperature overnight, filtered through cloth and then centrifuged at 6000 rpm for 30 min. The precipitate was discarded and the clear yellow supernatant (45 ml) was named ***Aloe vera*** leaf gel (AVLG).

Chemicals – All chemicals and reagents used in the study were of analytical grade and mostly purchased from Sigma chemicals, (India). Temperatures during the experiment were 25±3°C during the day. Investigations for antioxidants performed for various parameters by using their methodology, described principle in following manner:

Assay of Superoxide dismutase:
The rate of autoxidation of epinephrine or sensivity of autoxidation which inhibited by superoxide dismutase. The avaibility of superoxide dismutase enzyme, capable to remove superoxide radicals from reactant mixture by catalyzing its dismutation of O^{2-} to H_2O_2. Supeoxide ione (O^{2-}) generated by xanthin oxidase reaction which oxidized epinephrine to adrenochrome. The production of adrenochrome increased with increasing concentration of epinephrine which measured by using colorimeter at 480 nm.[9]

Assay of glutathione reductase:
Glutathione reductase catalyses the reduction of glutathione (GSSG) in the presence of NADPH which is oxidised to $NADP^+$. The decrease in absorbance at 340 nm is measured (by using colorimeter) and it is directly proportional to the glutathione reductase activity in sample.[10]

Assay of catalase:
The method based on the fact that dichromate in acitic acid is reduced to chromic acid when heated in the presence of hydrogen peroxide with the formation of perchromate (acid) as an unstable intermediate. The chromic acetate thus produced is measured colorimetrically at 570 nm. The reaction stopped at a particular time by the adding dichromate acetic acid mixture and the remaining hydrogen peroxide is determined by measuring chromic acetate colorimetrically after heating the reaction mixture.[11]

Assay of ascorbic acid:
Ascorbic acid was giving coloured complex with presence of dye 2, 4-dichlorophenol indophenols in an alcoholic acidic medium (n-amyl alcohol, m-Phosphoric acid) which measured at wavelength of 546 nm by using colorimeter.[12]

Assay of total β-carotene:
Oxidation of linoleic acid occur when react with oxygenated water. The oxidative losses of ´ -carotene were used to assess the antioxidation ability of the ***Aloe vera*** leaf gel (AVLG) fractions. An absorbance at 470 nm wavelength was recorded by using colorimeter.[13,14]

Observation:
Table: 1. Comparative study of antioxidants values of *Aloe vera* between (T–5) Control (Sandy coastal soil: Golden sand$_{(1:1)}$ and (T–1) Sandy coastal soil: Golden sand: Farm yard manure$_{(1:1:1)}$ (Two year plant).

S. No.	Aloe vera gel extract parameters	(T–5) Control (Sandy coastal soil: Golden sand$_{(1:1)}$			(T–1) Sandy coastal soil: Golden sand: Farm yard manure$_{(1:1:1)}$			t–test	P–value
		No. of leaves	Antioxidant value		No. of leaves	Antioxidant value			
			Range	Mean±SD		Range	Mean±SD		
1.	SOD **(Unit/mg protein/ml)**	36	39–43	41.33±1.47	46	42–56	48.76±4.40	9.69	P<0.0001
2.	GSH-R **(Unit/mg protein/ml)**		14.10–14.70	14.41±0.19		15.09 – 15.60	15.39±0.19	22.49	P<0.0001
3.	Catalase (Unit/mg protein/ml)		10.20–11.10	10.68±0.30		11.50 – 12.10	11.82±0.21	20.37	P<0.0001
4.	Ascorbic acid **(mg/ml)**		0.47– 0.48	0.45±0.014		0.41 – 0.46	0.43±0.16	4.70	P<0.0001
5.	Total´ –carotene **(mg/ml)**		0.032–0.036	0.034±0.038		0.036 – 0.039	0.038±0.001	13.53	P<0.0001

Note: P<0.0001(Extremely statistically significant).
SOD; Superoxide dismutase, GSH–R; Glutathione reductase.

Table: 2. Comparative study of antioxidants values of *Aloe vera* between (T–6) Control (Heavy black cotton soil: Golden sand$_{(1:1)}$ and (T–3) Heavy black cotton soil: Golden sand: Farm yard manure$_{(1:1:1)}$ (Two year plant).

S. No.	Aloe vera gel extract parameters	(T–6) Control (Heavy black cotton soil: Golden sand$_{(1:1)}$			(T–3) Heavy black cotton soil: Golden sand: Farm yard manure$_{(1:1:1)}$			t–test	P–value
		No. of leaves	Antioxidant value		No. of leaves	Antioxidant value			
			Range	Mean±SD		Range	Mean±SD		
1.	SOD **(Unit/mg protein/ml)**	42	41–44	42.50±1.31	45	44–59	50.60±4.76	10.64	P<0.0001
2.	GSH-R (Unit/mg protein/ml)		14.20–14.90	14.52±0.23		14.90–15.30	15.09±0.15	13.54	P<0.0001
3.	Catalase **(Unit/mg protein/ml)**		10.10–11.90	11.02±0.54		12.30–13.10	12.69±0.25	18.95	P<0.0001
4.	Ascorbic acid **(mg/ml)**		0.45–0.43	0.46±0.01		0.44–0.47	0.45±0.01	3.93	P<0.001
5.	Total´ –carotene **(mg/ml)**		0.034–0.047	0.038±0.005		0.038–0.041	0.039±0.001	1.72	P<0.05

Note: P<0.0001(Extremely statistically significant), P<0.001 (Very statistically significant), P<0.05 (Quite statistically significant).
SOD; Superoxide dismutase, GSH–R; Glutathione reductase.

Results and Discussion:

Aloe vera is as old civilization and throughout history and it has been used as a popular folk medicine. It is present in the arid regions of India and is believed to be most effective as important antioxidants properties.[15] Treatment of ***Aloe vera*** with farm yard manure restored the antioxidants parameters towards normal levels.[16] Yield depends to a large degree on the content of photo synthetically active pigments. Authors of numerous papers showed a close correlation between the level of these pigments and nitrogen content in leaves determined by the dose and time of fertilization.[17–20] Organic materials are an essential factor for keeping fertility in the soil-plant system.[21] Organic agriculture facilitates the recycling of nutrients and minimizes the negative effects caused by the different agricultural activities.[22–23] The results revealed that significant higher growth of ***Aloe vera*** leave antioxidants in (T–1) two year (P<0.0001) when compared to (T–5) two year plants (**Table: 1**). The ***Aloe vera*** have developed defense system against photo oxidative damage by antioxidative mechanisms to detoxify and eliminate these highly reactive oxygen species. These antioxidant defense system includes hydrophobic (carotenoids and´ -tocopherol) and hydrophilic antioxidant (ascorbic acid and glutathione) and antioxidant enzymes likes superoxide dismutase (SOD), catalsae (CAT), glutathione reductase (GSH-R).[24–25] The antioxidant activity of ***Aloe vera*** was 33.64-37.33%, which is similar to the one reported by Narsih ***et al***.[26]

Atmospheric oxygen has been recognized for more than 100 years as the agent responsible for the deterioration of organic materials exposed to air. The parallel role of oxygen, a molecule essential form many forms of life, as a destructive (toxic) agent for living tissues has been discovered much more recently. It is apparent that many environmental stresses exert at least part of their effect by causing oxidative damage. [27] Consequently, the antioxidant defense system of plants has been attracting considerable interest.[28] Characterization of mutants and transgenic plants with altered expression of antioxidant is a potentially powerful approach to understanding the functioning of the antioxidant system and its role in protecting plants against stress, and significant progress is now being made in this area. The formation of reactive oxygen species is prevented by an antioxidant system: low molecular mass antioxidants (ascorbic acid, glutathione, and tocopherols), enzymes regenerating the reduced forms of antioxidants and reactive oxygen species interacting enzymes such as superoxide dismutase and catalases.[29] In this study exposed of farm yard manure in black soil caused significant (P<0.0001) increase in the amount of Superoxide dismutase, Catalase and Glutathione reductase (T–3) two year plants as compared with (T–6) control two year plants (**Table: 2**). Ascorbic acid was increased (P<0.001) very statistically significantly in study group (T–3) when compared with (T–6) control two year plants. Total´ -carotene was increased (P<0.05) significantly in study group (T–3) when compared with (T–6) control two year plants. Treatment of ***Aloe vera*** with farm yard manure restored the antioxidants parameters towards normal levels and decreased the levels of lipid peroxidation and increased the levels of reduced glutathione and other antioxidant enzymes (SOD, CAT and GSH-R).[16]

Conclusion:

It can be concluded that the study of the antioxidants and antioxidant enzymes revealed that activities of these molecules were affected by foliar application of farm yard manure and it was especially improve symbiotic system for nitrogen fixation in ***Aloe vera*** plants. Plants have antioxidant defense systems comprised of enzymatic and non-enzymatic components, which control reactive oxygen species balance within the cell.

References:

1. Amar Surjushe, Resham Vasani, and D. G. Saple. (2008). ***Aloe vera***: A short review. ***Indian J Dermatol***. 53(4); 163–166.
2. Marshall J. M. (1990). Aloe vera gel: What is the evidence? ***Pharma Jr***. 24; 360–362.
3. Hu Y. et al. (2003). Evaluation of antioxidant potential of ***Aloe vera*** (***Aloe barbadensis miller***) extracts. ***J Agric Food Chem***. 51 (26); 7788–7791.
4. Singh R. P. et al. (2000). Chemomodulatory action of ***Aloe vera*** on the profiles of enzymes associated with carcinogen metabolism and antioxidant status regulation in mice. ***Phytomedicine***. 7; 209–213.
5. Larson R. A. (1988). The antioxidants of higher plants. ***Phytochemistry***. 4; 969–978.
6. Heinonen I. M., Meyer A. S., Frankel E. N. (1998). Antioxidant activity of berry phenolics on human low-density lipoprotein and liposome oxidation. ***Journal of Agricultrual Food Chemistry***. 46 (10); 4107–4112.
7. Rice-Evans C. A. and Miller N. J. (1998). Structure antioxidant activity relationships of flavonoids and isoflavonoids. In C. A. Rice-Evans, & C. Packer (Eds.), Flavonoids in health and disease. New York, NY: Marcel Dekker Press. pp. 199–219.
8. Halliwell B. and Gutteridge J. M. C. (1985). Doxorubicin dependent lipid peroxidation at low partial pressures of O_2. ***Free Radicals in Biology and Medicine***. 1 (1); 43–49.
9. Misra H. P. and Fridovich I. (1972). The role of superoxide anion in the autooxidation of epinephrine and a simple assay for SOD. ***J Biol Chem***. 247; 3170–3175.
10. Bergmayer H. U. (1963). Methods of Enzymatic Analysis. New York Academic Press. pp875–879.
11. Sinha K. A. (1972). Colorimetric assay of catalase. ***Analytical Biochemistry***. 47; 389–394.
12. Chinoy J. J. (1962). Formation and utilization of ascorbic acid in the shoot apex of Wheat. As factor of growth and development. ***Ind. J. Plant Physiol***. 5; 172–201.

13. Chevolleau S., A. Debal, E. Ucciani. (1992). ***Rev. Fr. Corps Gras***. 39 (1-2); 3–5.
14. A. Moure, D. Franco, J. Sineiro, H. Dominguez, M. J. Nunez, J. M. Lema. (2000). Evaluation of extract from ***Gevuina avelana Halls*** as antioxidants. ***J. Agr. Food Chem.*** 48; 3890–3895.
15. A. D. Klein, N. S. penneys. (1988). ***Aloe vera. Journal of the American Academy of Dermatology.*** 18; 714 – 719.
16. Naveenna, Bharath B. K. and Selva subramaniam. (2011). Antitumor activity of ***Aloe vera*** against ***ehrlich ascitis*** carcinoma (EAC) in swiss albino mice. ***International Journal of Pharma and Bio Sciences***. Vol-2 (02); 400–409.
17. Baghour M., Ruiz J. M., Romero L. (2000). Metabolism and efficiency in nitrogen utilization during senescence in pepper plants: Response to nitrogenous fertilization. ***J Plant Nutr***. 23; 91–101.
18. Biczak R., Gurgul E., Herman B. (1998). The effect of NPK fertilization on yield and content of chlorophyll, sugars and ascorbic acid in celery. ***Folia Hort***. 10; 23–34.
19. Smith D. L. (1999). Field evaluation of the chlorophyll meter to predict yield and nitrogen concentration of switchgrass. ***J Plant Nutr***. 22; 1001–1010.
20. Swiader J. M., Moore A. (2002). SPAD-chlorophyll response to nitrogen fertilization and evaluation of nitrogen status in dryland and irrigated pumpkins. ***J Plant Nutr***. 25; 1089–1100.
21. Lopez-Mosquera M. E., E. Fernandez-Lemaa, R. Villaresa, R. Corralb, B. Alonsob and C. Blanco. **(**2011). Composting fish waste and seaweed to produce a fertilizer for use in organic agriculture**.** ***Procedia Environmental Sciences.*** 9; 113–117.
22. Oraghi Ardebili Z., A. R. Ladan Moghadam, N. Oraghi Ardebili and A. R. Pashaie. **(**2012). The induced physiological changes by foliar application of amino acids in ***Aloe Vera*** L. plants**.** ***Plant Omics.*** 5; 279–284.
23. Ladan Moghadam A. R., Z. Oraghi Ardebili and F. Saidi. **(**2012). Vermicompost induced changes in growth and development of ***Lilium Asiatic*** hybrid var. Navona. ***African Journal of Agricultural Research.*** 7; 2609–2621.
24. Pareek O. P., Sharma B. D., Nath V., Singh R. S., Bhargawa R. (1999). Effect of nitrogen and phosphorus fertilizers and organic manure on growth and yield of Indian aloe (***Aloe barbadensis*** Mill.). ***Ann Arid Zone***. 38; 85–86.
25. Tawaraja K., Turjaman M., Ekamawanti H. A. (2007). Effect of arbuscular mycorrhizal colonization on nitrogen and phosphorus uptake and growth of ***Aloe vera*** L. ***Hort sci.*** 42; 1737–1739.
26. Smirnoff N. (1993). The role of active oxygen in the response of plants to water deficit and desiccation. ***New Phytol***. 125; 27–58.
27. Smirnoff N. (1995). Antioxidant systems and plant response to the environment. In: Environment and plant Metabolism (Smirnoff, N. ed.). Bios Scientific Publishers. PP. 217–243.
28. Alscher R. G., Donahue J. I., Cramer C. L. (1997). Reactive oxygen species and antioxidants: relationships in green cells. ***Physiol. Plant.*** 100; 224–233.
29. Gout E., Boisson A. M., Aubert S., Douce R. and Bligny R. (2001). Origin of the cytoplasmic pH changes during anaerobic stress in higher plant cells. Carbon-13 and phosphorus-31 nuclear magnetic resonance studies. ***Plant Physiology.*** 125; 912–925.