Volume-5, Issue-3, March - 2016 • ISSN No 2277 - 8160 IF : 3.62 | IC Value 70.36

Research Paper Botany

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

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ABSTRACT *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. Aloe vera is a perennial succulent belonging to the Liliaceae family and is called the healing plant or the silent healer. 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. The primary constituents of these protective mechanisms include enzymes such as superoxide dismutase (SOD), catalases, glutathione reductase and free radical scavengers such as carotenoids, ascorbic acid etc. The present study was carried out to evaluate the antioxidant status of ethanol extract of two year old Aloe vera plants.*

KEYWORDS : **Superoxide dismutase, glutathione reductase, catalase, ascorbic acid and total β-carotene**

Introduction:

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. There is a growing interest in the pharmacological evaluation of various plants used in Indian traditional system of medicine. *Aloe vera* (*Sottrukattalai*, Tamil) is a perennial succulent belonging to the Liliaceae family and is called the healing plant or the silent healer. *Aloe vera* contains 75 potentially active constituents: vitamins, enzymes, minerals, sugars, lignin, saponins, salicylic acids and amino acids.[1]

Antioxidants play an important role in inhibiting and scavenging free radicals, thus providing protection against infection and degenerative diseases. 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 percent, compared with only 65 percent for alpha-tocopherol.[2] Second, in addition to containing its own stores of antioxidants; *Aloe vera* gel may also activate the body's endogenous antioxidant enzyme systems. Research in mice found internal administration of the gel elevates liver levels of three out of five cellular antioxidant enzyme families: the glutathione family, the superoxide dismutase (SOD) family and the catalase family.[3] 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.[4] 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. [5–6]

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.[7] 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–2. Sandy coastal soil**:**Golden sand**:**Farm yard manure (1**:**1**:**1) + Fertilizers$_{(NPK),}$ (4 Pots).
T–4. Heavy black cotton soil**:**Golden sand**:** Farm yard manure (1**:**1**:**1)+ Fertilizers$_{(NPK),}$ (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 homogenized with a hand held blender. Each leaf produced approximately 120 ml of gel. The homogenized gel was lyophilized at 22°C and the resultant lyophilized material was stored frozen until further extraction.

Preparation of *Aloe vera* leaf extract:
Freshly collected lyophilized 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 removing 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.[8]

Assay of glutathione reductase:
Glutathione reductase catalyses the reduction of glutathione (GSSG) in the presence of NADPH which is oxidized 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.[9]

Assay of catalase:
The method based on the fact that dichromate in acetic 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 calorimetrically 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 calorimetrically after heating the reaction mixture.[10]

Assay of ascorbic acid:
Ascorbic acid was giving colored 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.[11]

Assay of total β-carotene:
Oxidation of linoleic acid occur when react with oxygenated water. The oxidative losses of β-carotene were used to assess the anti-oxidative ability of the *Aloe vera* leaf gel (**AVLG**) fractions. An absorbance at 470 nm wavelength was recorded by using colorimeter.[12,13]

Observation:

Table: 1. Comparative study of antioxidants values of *Aloe vera* between (T–5) Control (Sandy coastal soil: Golden sand$_{(1:1)}$ and (T–2) 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–2) Sandy coastal soil: Golden sand: Farm yard manure$_{(1:1:1)}$ + Fertilizers$_{(NPK)}$			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	47	43–59	46.66±4.56	9.25	P<0.0001
2.	GSH-R **(Unit/mg protein/ml)**		14.10–14.70	14.41±0.19		15.10–15.80	15.42±0.23	20.88	P<0.0001
3.	Catalase **(Unit/mg protein/ml)**		10.20–11.10	10.68±0.30		15.30–15.90	15.59±0.20	89.99	P<0.0001
4.	Ascorbic acid **(mg/ml)**		0.47–0.48	0.45±0.01		0.43–0.47	0.45±0.014	0.00	1.00 $_{(NS)}$
5.	Total β–carotene **(mg/ml)**		0.032–0.036	0.034±0.001		0.043–0.047	0.045±0.001	34.97	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–4) Heavy black cotton soil: Golden sand: Farm yard manure$_{(1:1:1)}$ + Fertilizers$_{(NPK)}$ (Two year plant).

S. No.	Aloe vera gel extract parameters	(T–6) Control (Heavy black cotton soil: Golden sand$_{(1:1)}$			(T–4) Heavy black cotton soil: Golden sand: Farm yard manure$_{(1:1:1)}$ + Fertilizers$_{(NPK)}$			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	50	44–59	51.38±4.72	11.81	P<0.0001
2.	GSH-R **(Unit/mg protein/ml)**		14.20–14.90	14.52±0.23		15.20–15.70	15.46±0.17	22.33	P<0.0001
3.	Catalase **(Unit/mg protein/ml)**		10.10–11.90	11.02±0.54		15.80–16.70	16.25±0.29	59.52	P<0.0001
4.	Ascorbic acid **(mg/ml)**		0.45–0.48	0.47±0.011		0.43–0.49	0.46±0.02	1.04	0.3 $_{(NS)}$
5.	Total β–carotene **(mg/ml)**		0.034–0.047	0.038±0.005		0.044–0.048	0.046±0.001	10.29	P<0.0001

Note: P<0.0001(Extremely statistically significant).

SOD; Superoxide dismutase, GSH–R; Glutathione reductase.

Results and Discussion:

The *Aloe vera* plant contains many various bioactive compounds including antioxidants (SOD, glutathione reductase, catalase, ascorbic acid and β- carotene) were existed in different parts of the plant.[14–15] Antioxidant is a bioactive molecule which neutralizes harmful Reactive Oxygen Species (ROS), Reactive Nitrogen Species (RNS), and Reactive Chlorine Species (RCS) that cause damage to living cells.[16]

Our studied showed significant higher growth of *Aloe vera* leaves and numbers of leaves in (T–2) two year and (T–4) two year plants when compared to control (T–5) and (T–6) two plants respectively (**Table–1 & 2**). The lowest number of leaves was observed in the control ((T–5 & T–6). All treatment increased the number of leaves compared with the control. If *Aloe vera* has cultivated in soil with poor nutrition, it is difficult for *Aloe vera* to grow in this system without nutrient amendments. Previous research [17] indicated that nutrient solution with the highest level of nitrogen increased *Aloe* vegetative growth. Organic materials are an essential factor for keeping fertility in the soil-plant system.[18] Phosphorus has many important functions in plants and medicinal plants, the primary one being the storage and transfer of energy through the plant. Adenosine diphosphate (ADP) and adenosine triphosphate (ATP) are high-energy phosphate compounds that control most processes in plants including photosynthesis, respiration, protein and nucleic acid synthesis, and nutrient transport through the plant's cells.[19] Boroomand et al. (2011a) found that application of 100 kg P_2O_5 ha^{-1} increased leaf number and leaf length in *Aloe Vera*.[20] Boroomand et al. (2011b) reported significantly higher plant height of Basil at 150 kg P_2O_5 ha^{-1} (78.96 cm in 2009) which was superior over all other P levels. One of the earliest and most pronounced responses to phosphorus deficiency is reduction in shoot growth, specifically reduction in leaf number and leaf size.[21]

In this study exposed of farm yard manure and fertilizer (NPK) for *Aloe vera* plants were increase significantly (P<0.0001) amount of Superoxide dismutase, Catalase, Glutathione reductase and β–carotene in (T–2) and (T–4) two year plants when compared with control (T–5) and (T–6) two year plants (**Table: 1 and 2**). 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 are 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.[22] Antioxidants enzymes are implicated in a variety of physiological processes including ethylene biogenesis, cell development, membrane integrity, response to injury, disease resistance. Antioxidants enzymes have multiple roles in different aspects of plant metabolism and are known to be implicated in plant differentiation and in the response against environmental stress.[23]

Conclusion:

It can be concluded that activities of antioxidant and antioxidant enzymes molecules were affected by foliar application of farm yard manure and treatment with fertilizer. Antioxidants enzymes are implicated in a variety of physiological processes including cell development, membrane integrity, response to injury, disease resistance. Antioxidants enzymes have multiple roles in different aspects of plant metabolism. After treatment of farm yard manure and fertilizer antioxidants and antioxidant enzyme levels were increased which is responsible for reduce oxidative stress.

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