RESEARCH PAPER | Botany | Volume : 6 | Issue : 3 | March 2016 | ISSN - 2249-555X | IF : 3.919 | IC Value : 74.50

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

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

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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, 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. Antioxidants are the substances in Aloe vera which can 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.*

Introduction:
Plants, vegetables and herbs used in the folk and traditional medicine have been accepted currently as one of the main source of drug discovery and development.[1] 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.[2] Aloe gel has demonstrated wound healing[3], anti-inflammatory[4], antiviral[5], spermicidal[6], gastroprotective[7] and immune stimulating[8] properties. Plant derived natural products such as flavonoids, terpenoids and steroids *etc* have received considerable attention in recent years due to their diverse pharmacological properties including antioxidant and antitumor activity.[9, 10] Antioxidants play an important role in inhibiting and scavenging free radicals, thus providing protection against infection and degenerative diseases. From this viewpoint, the present study was carried out to evaluate the antioxidant status of ethanol extract of one year *Aloe vera* plants.

Material and methods:
A study undertaken to evaluated 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 one year *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

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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 small 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.[11]

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.[12]

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 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.[13]

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.[14]

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.[15, 16]

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$_{(1:1:1)}$ (One 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	38–42	39.89±1.55	46	40–55	47.80±5.33	8.62	P<0.0001
2.	GSH-R **(Unit/mg protein/ml)**		13.04–14.03	13.46±0.41		14.08–14.50	14.23±0.14	11.8	P<0.0001
3.	Catalase **(Unit/mg protein/ml)**		9.60–10.10	9.70±0.16		10.10–11.50	10.83±0.44	13.3	P<0.0001
4.	Ascorbic acid **(mg/ml)**		0.41–0.45	0.43±0.01		0.41–0.44	0.42±0.01	1.3	0.19 **(NS)**
5.	Total´ –carotene **(mg/ml)**		0.031–0.035	0.033±0.001		0.034–0.037	0.035±0.001	9.13	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$_{(1:1:1)}$ (One 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	39–43	40.62±1.48	45	42–58	50.49±4.94	12.42	P<0.0001
2.	GSH-R **(Unit/mg protein/ml)**		13.08–14.60	13.78±0.51		14.30–14.80	14.55±0.17	9.61	P<0.0001
3.	Catalase **(Unit/mg protein/ml)**		9.70–10.30	10.00±0.20		11.60–12.40	12.02±0.24	41.77	P<0.0001
4.	Ascorbic acid **(mg/ml)**		0.44–0.47	0.45±0.012		0.43–0.46	0.44±0.011	2.94	P<0.001
5.	Total´ –carotene (mg/ml)		0.032–0.036	0.034±0.001		0.036–0.039	0.037±0.001	12.61	P<0.0001

Note: P<0.0001(Extremely statistically significant), P<0.001 (Very statistically significant).

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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 ´ - carotene) existed in different parts of the plant.[17] 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.[18]

Farm yard manure did affect all measured qualitative characteristics while previous research indicated that nutrient solution with the highest level of nitrogen increased *Aloe vera* vegetative growth. Therefore, without any negative effect on qualitative indices we are able to produce the highest level of vegetative growth in *Aloe vera*. In other hand we may be possible to optimize for higher yields of target organs as mentioned previously.[19]

The major groups of phytochemicals that have been suggested as a natural source of antioxidants may contribute to the total antioxidant activity of plant materials including polyphenols, carotenoid and traditional antioxidant vitamins such as vitamin C and E. Antioxidant is any substance that when present at low concentration compared to those of an oxidisable substrate significantly delays or prevents oxidation of that substrate.[20] In fact, many authors have reported a direct relationship between total phenolic content and antioxidant activity in numerous seeds, fruits and vegetables.[21] Previous study reported that antioxidant activity of plant material is very well correlated with the content of phenolic compounds.[22] Contribution of phenolic compounds is one of the mechanisms of the overall antioxidant activities. This mainly due to their redox properties involve in the plant material. Generally, the mechanisms of phenolic compounds for antioxidant activity are neutralizing lipid free radicals and preventing decomposition of hydroperoxides into free radicals.[23] The study clearly indicates that it is vital to measure the antioxidant activity using various radicals and oxidation systems and to take both phenolic content and antioxidant activity into account while evaluating the antioxidant potential of plant extracts. The results obtained in this work have considerable value with respect to the antioxidant activities of the selected parts of the *Aloe vera*. The author previously reported that *D. salina* (algae) accumulated large amount of carotenoids, ´ -tocopherol and ascorbic acid. Enhanced the activities of the antioxidant enzymes when grown under high light intensity, in media containing high salt concentration and/or limiting nitrogen.[24]

In this study exposed of *Aloe vera* from high Sun light field caused significant increase in the amount of carotenoids, as compared with unexposed cells. The results revealed that significant higher growth of *Aloe vera* leave antioxidants (superoxide dismutase, glutathione reductase, catalase and total ´ -carotene) in (T–1) one year ($P<0.0001$) plants when compared to (T–5) control one year plants (**Table: 1**). Thus, a positive relationship between carotenoids content and relief from high Sun light field was observed. Also, the results showed that in Sun light field exposed cells, the carotenoids biosynthesis is shifted toward prevents formation of peroxidation processes in liposomes much better than ´ -carotene.[25] Furthermore, these results are evidence for a mechanism for protecting the cells against irradiation damage. The *Aloe vera* has 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 & ´ -tocopherol) and hydrophilic antioxidant (ascorbic acid & glutathione) and antioxidant enzymes likes superoxide dismutase (SOD), catalsae (CAT), glutathione reductase.[26–29]

In this study exposed of farm yard manure in black soil caused significant ($P<0.0001$) increase in the amount of Superoxide dismutase, Catalase, Glutathione reductase and total ´ –carotene (T–3) one year plants as compared with (T–6) control one year plants. Significant ($P<0.001$) increased ascorbic acid was found in (T–3) one year plants as compared with (T–6) control one year plants (**Table: 2**). Evidence suggests that drought causes oxidative damage through generation of oxygen radicals or inhibition of antioxidant systems in plant.[30–32] Drought related physiological changes such as a decrease in leaf water result in limited CO_2 availability to the channeling of reducing equivalents to the production of active oxygen species rather CO_2 fixation. Among the four major active oxygen species [superoxide radical (O^{-2}), hydrogen peroxide (H_2O_2), hydroxyl radical ($OH^{\cdot}$) and singlet oxygen (O_2)] are most active toxic and destructive. Hydrogen peroxide can be produced by either dismutation of O^{-2} by SOD or photorespiration and catalase hydrolysed H_2O_2 to water molecule.[33]

Conclusion:

The study of the antioxidant and antioxidant enzymes revealed that activities of these molecules were affected by foliar application of farm yard manure. 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.

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