Research Paper

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

Botany

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

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ABSTRACT

In India, aloe vera has been referred to as "kumari' in Ayurvedic treatments where it was popularly used to treat the sore eyes, abrasions, wounds and antioxidants properties. Different fractions of Aloe vera as well as unfractionated whole gel have antioxidant effects. Glutathione reductase activity, superoxide dismutase, catalase enzymes and a phenolic antioxidant (ascorbic acid and β-carotene) were found to be present in Aloe vera gel, which may be responsible for these antioxidant effects.

Introduction:

Aloe vera belongs to the family liliacae and is mainly cultivated for its thick fleshy leaves from which the yellow resinous latex or yellow sap or anthraquinones (the bitter yellow liquid between the leaf rind and gel) exudes and can be used as a laxative or purgative. Nearly there are about 150 species in *Aloe vera* and these species belong to the succulent family like any other lilies or onions. In India, *Aloe vera* has been referred to as "kumari' in Ayurvedic treatments where it was popularly used to treat the sore eyes, abrasions, wounds and antioxidants properties.[1]

The three structural components of the *Aloe vera* pulp are the cell walls, the degenerated organelles and the viscous liquid contained within the cells. These three components of the inner leaf pulp have been shown to be distinctive from each other both in terms of morphology and sugar composition.[2] The raw pulp of *Aloe vera* contains approximately 98.5% water, while the mucilage or gel consists of about 99.5% water.[3] The remaining 0.5–1% solid material consists of a range of compounds including water-soluble and fat-soluble vitamins, minerals, enzymes, polysaccharides, phenolic compounds and organic acids.[4] It has been hypothesized that this heterogenous composition of the *Aloe vera* pulp may contribute to the diverse pharmacological and therapeutic activities which have been observed for aloe gel products.[5]

Technically, the term 'pulp' or 'parenchyma tissue' refers to the intact fleshy inner part of the leaf including the cell walls and organelles, while 'gel' or 'mucilage' refers to the viscous clear liquid within the parenchyma cells.[6] It has been reported by several authors that different fractions of *Aloe vera* as well as unfractionated whole gel have antioxidant effects. Glutathione reductase activity, superoxide dismutase enzymes and a phenolic antioxidant were found to be present in *Aloe vera* leaf gel, which may be responsible for these antioxidant effects. It was shown in two cell-free *in vitro* systems and by incubation with inflamed colorectal mucosal biopsies that *Aloe vera* gel has a dose dependent antioxidant effect. The cell free techniques used in this study assessed the scavenging of both superoxide and reactive oxygen species and evaluate antioxidant and antioxidants enzymes of *Aloe vera* leaf gel (AVLG).

Material and methods:

The study was to evaluate the "**Effect of graded levels of fertilizer application on antioxidants dynamics of A*loe 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–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 homogenised with a hand held blender. Each leaf produced approximately 120 ml of gel. The homogenised gel was lyophilised at 22ºC 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 sensitivity 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.[7]

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.[8]

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.[9]

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.[10]

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 antioxidation ability of the *Aloe vera* leaf gel (**AVLG**) fractions. An absorbance at 470 nm wavelength was recorded by using colorimeter.[11,12]

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$_{(1:1:1)}$ (One 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	38–42	39.89±1.55	47	41–56	48.13±4.48	10.56	P<0.0001
2.	GSH-R **(Unit/mg protein/ml)**		13.04–14.03	13.46±0.41		14.20–14.90	14.55±0.23	15.19	P<0.0001
3.	Catalase **(Unit/mg protein/ml)**		09.60–10.10	09.80±0.16		14.10–14.90	14.49±0.259	96.02	P<0.0001
4.	Ascorbic acid **(mg/ml)**		0.41–0.45	0.43±0.01		0.41–0.44	0.43±0.011	1.30	P<0.197 (NS)
5.	Total β–carotene **(mg/ml)**		0.031–0.035	0.033±0.001		0.040–0.044	0.042±0.001	34.33	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$_{(NPK)}$ (One 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	39–43	40.62±1.48	50	42–58	49.90±5.07	11.44	P<0.0001
2.	GSH-R **(Unit/mg protein/ml)**		13.08–14.60	13.78±0.51		15.02–15.09	15.05±0.024	17.72	P<0.0001
3.	Catalase **(Unit/mg protein/ml)**		9.70–10.30	10.00±0.20		14.80–15.40	15.10±0.20	21.01	P<0.0001
4.	Ascorbic acid **(mg/ml)**		0.44–0.47	0.45±0.012		0.43–0.46	0.44±0.010	2.64	P<0.001
5.	Total β–carotene **(mg/ml)**		0.032–0.036	0.034±0.001		0.042–0.046	0.044±0.001	33.06	P<0.0001

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

Results and Discussion:
Aloe vera is a cactus like plant with green, dagger-shaped leaves that are fleshy, tapering, spiny, marginated and filled with a clear viscous gel.[13] It is also known as 'lily of the desert' the plant of immortality and the medicine plant with qualities to serve as alternate medicine. *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.[14]

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According to Faryabi and Ghazanchi[15], increasing N and P fertilizers application rates increased leaf fresh weight and gel content. In another experiment in was reported that application of higher rates of nitrogen fertilizer enhanced the number of leaves, leaf weight, leaf diameter and leaf length of *Aloe vera*. [16] Mirza et al.[17] also found that the highest leaf yield, number of leaves, leaf fresh weight and leaf area index of *Aloe vera* were obtained when 50% organic manure was mixed with 50% soil. The effect of mineral and organic fertilizers on *Aloe vera* growth and development is mostly unknown, so this experiment was conducted with the aim of evaluating the effect of different organic wastes on this important medicinal plant. Results of laboratory analysis of organic wastes applied in this experiment showed that farm yard manure had the highest N content. Regarding the role of N in plants vegetative growth, high N content in farm yard manure would probably increase the number of leaves, leaf weight and diameter and leaf chlorophyll content which increase antioxidants levels. In this study exposed of *Aloe vera* with fertilizer and farm yard manure was increase significant (P<0.0001) amount of Superoxide dismutase, Catalase, Glutathione reductase and β–carotene (T–2) one year plants when compared with (T–5) control one year plants (**Table: 1**).

The study of the antioxidant and antioxidant enzymes revealed that activities of these molecules were affected by foliar application of fertilizer and it was especially dependent on the applied nitrogen concentrations. Plants have antioxidant defense systems comprised of enzymatic and non-enzymatic components, which control reactive oxygen species balance within the cell. As part of this system, antioxidant enzymes are key elements in the plant defense mechanisms. The activities of antioxidant enzymes play a crucial role in scavenging reactive oxygen species and therefore their stimulation could elevate the ability of stress tolerance and delay the senescence.[18–19] 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.[20] In this study exposed of farm yard manure in black soil with fertilizer were increase significantly (P<0.0001) in the amount of Superoxide dismutase, Catalase, Glutathione reductase and β–carotene (T–4) one year plants when compared with (T–6) control one year plants. Ascorbic acid was increase significantly (P<0.001) in (T–4) one year plants when compared with (T–6) control one year plants (**Table: 2)**.

Conclusion:

The study of the antioxidant and antioxidant enzymes revealed that activities of these molecules were affected by foliar application of farm yard manure and 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 and are known to be implicated in plant differentiation and in the response against environmental stress.

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