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STUDIES ON HYDROGEN PEROXIDE AND SUPEROXIDE RADICAL SCAVENGING ACTIVITIES OF *ALOE VERA* L. (*IN VIVO* & *IN VITRO* REGENERATED) WHOLE LEAF AND INNER GEL EXTRACTS

Neelofar Khanam*[1,2] and G. K. Sharma[3]

[1]Department of Medical Laboratory Sciences, College of Paramedical Sciences, SCPM Medical College, Lucknow Road, Haripur, Gonda - 271003, Uttar Pradesh, India.
[2]School of Biotechnology, IFTM University, Moradabad-244001, Uttar Pradesh, India.
[3]Division of Biotechnology, Department of Botany, Hindu College, Moradabad-244001, Uttar Pradesh, India.

***Corresponding Author: Neelofar Khanam**

Department of Medical Laboratory Sciences, College of Paramedical Sciences, SCPM Medical College, Lucknow Road, Haripur, Gonda - 271003, Uttar Pradesh, India.

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ABSTRACT

As oxygen is an indispensable part of aerobic life. The production of oxygen free radicals is a natural consequence of aerobic metabolism. However, under certain circumstances, it can seriously affect our well being through the formation of reactive oxygen species, representing both free radicals and non-free radical species. Any free radical involving oxygen can be referred to as Reactive Oxygen Species or ROS. Reactive oxygen species (ROS) are necessary for various physiological functions but an imbalance in favour of reactive oxygen species results in oxidative stress (OS). Recently there has been an upsurge of interest in the therapeutic potential medicinal plants as antioxidants in reducing oxidative stress-induced tissue injury and damages rather than looking for synthetic ones. Several naturally occurring antioxidants are there to scavenge free radicals and active oxygen species by propagating a reaction cycle. In current study hydrogen peroxide and super oxide free radical scavenging activities of *in vivo* grown and *in vitro* propagated *Aloe vera* L. whole leaf and only gel extracts were tested. final results were showing that among the various extracts (prepared with the solvents of different polarities such as aqueous, methanol, ethyl acetate, chloroform and n-hexane) of *in vivo* and *in vitro* regenerated *Aloe vera* L. whole leaf and only gel, methanol extracts with *in vitro* regenerated *Aloe vera* L. leaves (only gel extract followed by whole leaf extract) were showing the highest free radical scavenging potentials.

KEYWORDS: *Aloe vera* L., free radicals, Reactive oxygen species (ROS), hydrogen peroxide and super oxide radical.

INTRODUCTION

Free radicals are classified as reactive oxygen species (ROS) and reactive nitrogen species (RNS). ROS are oxygen centered free radicals, which include radicals such as superoxide anion, hydroxyl radical and alkoxyl and non-radicals hydrogen peroxide and singlet oxygen. RNS are nitrogen centered radicals, which include nitric oxide and peroxynirite.[1]

Molecular oxygen is required by living organisms and biological systems to survive and is depended upon heavily, whereas, any free radical involving oxygen (ROS) causes damage to other molecules by extracting electrons from them in order to attain stability. ROS are ions, atoms or molecules that have the ability to oxidize reduced molecules. ROS are various forms of activated oxygen, including free radicals such as superoxide anion radicals ($^{\bullet}O_2^-$) and hydroxyl radicals ($OH^{\bullet}$), as well as non-free radicals (H_2O_2) and singlet oxygen.[2]

Most atoms and molecules remain reasonably stable when placed in contact with living cells. However, free radicals are group of particles that are considered to be less benign. Free radicals are unstable, highly reactive molecules characterised by the presence of unpaired electrons in their outermost shells around the nucleus [3]. The production of oxygen free radicals is a natural consequence of aerobic metabolism, with these molecules being constantly generated in the body by normal metabolic processes.[4]

There are many medicinal plants using traditionally as the source of protective agents against oxidative stress. Stress is one of the major contributory factors that stimulate numerous intracellular pathways leading to the increased free radical generation causing oxidative

damage. Oxidative process is one of the most important routes for producing free radicals in foods, drugs and even in living systems. Free radicals are highly reactive moieties playing an important role in health and disease. Oxidative stress depicts the existence of products as free radicals and reactive oxygen species (ROS), which are formed under normal physiological conditions but become deleterious when not being eliminated by the endogenous systems. In fact, oxidative stress results from an imbalance between the generation of reactive oxygen species and endogenous antioxidant systems. ROS are major sources of primary catalysts that initiate oxidation *in vivo* and *in vitro* and create oxidative stress which results in numerous diseases and disorders such as cancer, cardiovascular disease, neural disorders, Alzheimer's disease, mild congnitive impairment, Parkinsons disease, alcohol induced liver disease, ulcerative colitis, ageing, atherosclerosis.[5]

The most effective path to eliminate and diminish the action of free radicals which cause the oxidative stress is antioxidative defence mechanisms. Antioxidants are those substances which possess free radical chain reaction breaking properties. The screening studies for antioxidant properties of medicinal and food plants have been performed increasingly for the last few decades in hope of finding an efficient remedy for several present-day diseases and means to delay aging symptoms.[6]

Plants have been known to contain components of phytomedicine since times immemorial.[7] Man is able to obtain from them a wondrous assortment of industrial chemicals.[8] Plant based natural constituents can be derived from any part of the plant like bark, leaves, flowers, roots, fruits, seeds, etc. that is any part of the plant may contain active components. These plant-derived substances have recently become of great interest owing to their versatile applications.[7] Medicinal plants are the richest bioresource of drugs of traditional systems of medicine, modern medicines, nutraceuticals, food supplements, pharmaceutical intermediates and chemical entities for synthetic drugs.[8]

Several methodologies, based on free radical capture or formation suppression, are used to measure the antioxidant capacity of biological material and model compounds. The most commonly used for their ease, speed and sensitivity are those involving chromogen compounds of a radical nature to simulate radical oxygen and nitrogen species. The most widely used assays are based on the scavenging of radical. The presence of antioxidant species leads to the disappearance of these radical chromogens which can be followed by spectrophotometric methods.[9]

In current study we have identified the hydrogen peroxide and super oxide free radical scavenging activities, of different extracts of *in vivo* and *in vitro* regenerated *Aloe vera* L. whole leaf and only gel.

MATERIALS AND METHODS

Preparation of crude extract

Leaves of the *Aloe vera* L. were collected from the already *in vitro* propagated and properly acclimatized 9-12 months old plants. *In vitro* propagation was the previous phase of our study to produce quality plant material to meet industrial requirement. Simultaneously leaves from 9-12 months old *in vivo* grown *Aloe vera* L. plants were also collected. Freshly collected.

Aloe vera L. leaves were washed with distilled water, followed by disinfecting with ethanol 70%. Later, in case of whole leaf crude extract preparation, leaves were chopped into the small pieces and were exposed to 50ºC for 3 days to get dried. After complete drying, leaf parts were powdered using electric grinder, simultaneously in case of only gel crude extract preparation, upper green skin/rind of leaves was removed and latex was cut into small pieces and both types of leaf materials were homogenized separately. The homogenized materials were extracted with ethanol (95%). The ethanol from the extracted leaf materials was evaporated at 65^{o}C temperature in water bath. The solvent was completely removed and dried to get powder. All the powdered plant materials including whole leaf and only gel were used for the preparation of aqueous and solvent extracts.

Aqueous extract

Extracts were prepared using the modified method of Case.[10] 1:3 (w/v) ratios were used for the powdered leaf material and distilled water for extract preparation. The pulverized leaf material was used to prepare an infusion in hot (95ºC) distilled water. The infusion was left overnight under refrigeration (4ºC) to prevent any possible contamination. After 24 h the extracts were kept in rotary shaker at 100 rpm for 1 h and filtered with Whatman No.1 filter paper and subsequently subjected to lyophilization at – 47.5ºC. The frozen extract was then freeze dried to a powder, weighed, transferred into separate vial and preserved at 4ºC for future analysis.

Solvent extracts

As in case of aqueous extract here also 1:3 (w/v) ratios were used for the powdered leaf material and different solvents for extract preparation. The pulverized leaves material was mixed with sufficient quantity of solvents viz., hexane, ethyl acetate, methanol and chloroform. It was kept in rotary shaker at 100 rpm overnight and filtered with Whatman No.1 filter paper and subsequently subjected to lyophilization at – 47.5ºC. The dried extracts thus obtained was weighed, transferred into separate vials and preserved at 4ºC for future analysis.

Determination of hydrogen peroxide scavenging abilities

The ability of the leaf extracts to scavenge hydrogen peroxide was assessed by the method of Ruch *et al*.[11] A solution of H_2O_2 (40mM) was prepared in phosphate buffer. Leaf extracts at the concentration of 10mg/10µl

were added to H_2O_2 solution (0.6ml) and the total volume was made up to 3ml. The absorbance of the reaction mixture was recorded at 230nm in a spectrophotometer. A blank solution containing phosphate buffer, without H_2O_2 was prepared. The extent of H_2O_2 scavenging of the plant extracts was calculated as:

$$\%\ scavenging\ of\ hydrogen\ peroxide = \frac{(A_0 - A_1) \times 100}{A_0}$$

where,
A_0 - Absorbance of control, A_1 - Absorbance in the presence of plant extract.

Determination of super oxide radical scavenging abilities

The superoxide scavenging ability of the extracts was assessed by the method of Winterbourn *et al.*[12] This assay is based on the inhibition of the production of nitroblue tetrazolium formazan of the superoxide ion by the plant extracts and is measured spectrophotometrically at 560nm.

Superoxide anions were generated in samples that contained in 3.0ml, 0.02ml of the leaf extracts (20mg), 0.2ml of EDTA, 0.1ml of NBT, 0.05ml of riboflavin and 2.64ml of phosphate buffer. The control tubes were also set up where DMSO was added instead of the plant extracts. All the tubes were vortexed and the initial optical density was measured at 560nm in a spectrophotometer. The tubes were illuminated using a fluorescent lamp for 30 minutes. The absorbance was measured again at 560nm. The difference in absorbance before and after illumination was indicative of superoxide anion scavenging activity.

Statistical analysis

All the analysis were carried out in triplicates and expressed as mean ± SD. Analysis of variance (ANOVA) were performed using the one-way analysis of variance. Significant differences between means were determined by Duncan's multiple range tests. P values less than 0.05 were considered statistically significant.

RESULTS AND DISCUSSION

Reactive oxygen species (ROS), from both endogenous and exogenous sources, may be involved in the etiologies of such diverse human diseases as arteriosclerosis, ischemic injury, cancer, and neurodegenerative diseases, as well as in processes like inflammation and ageing. There is evidence that indigenous antioxidants may be useful in preventing the deleterious consequences of oxidative stress and there is increasing interest in the protective biochemical functions of natural antioxidants contained in spices, herbs, and medicinal plants.[5]

As in present study *Aloe vera* L. (*in vivo* and *in vitro* regenerated) whole leaf and only gel extracts were examined for their free radical scavenging properties against hydrogen peroxide and super oxide free radicals. Results were showing that among the different extracts (prepared with the solvents of different polarities such as aqueous, methanol, ethyl acetate, chloroform and n-hexane) of *in vivo* and *in vitro* regenerated *Aloe vera* L. whole leaf and only gel, methanol extracts with *in vitro* regenerated *Aloe vera* L. leaves (only gel extract followed by whole leaf extract) were showing higher degree of radical scavenging activities.

The maximum hydrogen peroxide radical scavenging activity in the *in vitro* regenerated *Aloe vera* L. whole leaf samples was 41.33 ± 3.54 of methanol extract with 1000 µg/ml. sample and minimum was 19.34 ± 1.16 of *n*-hexane extract with 100 µg/ml. sample (shown in the Table 1). Simultaneously, the highest hydrogen peroxide radical scavenging activity in the *in vitro* regenerated *Aloe vera* L. only gel samples was 46.83 ± 1.54 of methanol extract with 1000 µg/ml. sample and lowest was 23.14 ± 2.83 of *n*-hexane extract with 100 µg/ml. sample (shown in the Table 2).

Similarly, the highest hydrogen peroxide radical scavenging activity in the *in vivo* grown *Aloe vera* L. whole leaf samples was 30.76 ± 3.36 of methanol extract with 1000 µg/ml. sample and lowest was 11.62 ± 1.24 of *n*-hexane extract with 100 µg/ml. sample (shown in the Table 3). At the same time, highest hydrogen peroxide radical scavenging activity in the *in vivo* grown *Aloe vera* L. only gel samples was 33.33 ± 2.14 of methanol extract with 1000 µg/ml. sample and lowest was 15.35 ± 3.58 of *n*-hexane extract with 100 µg/ml. sample (shown in the Table 4).

The highest superoxide radical scavenging capacity of *in vitro* regenerated *Aloe vera* L. whole leaf samples was 82.28 ± 4.04 of methanol extract with 1000 µg/ml. sample and lowest was 40.32 ± 4.26 of *n*-hexane extract with 100 µg/ml. sample (shown in the Table 5). Subsequently, the maximum superoxide radical scavenging ability exhibited by the *in vitro* regenerated *Aloe vera* L. only gel samples was 93.37 ± 2.83 of methanol extract with 1000 µg/ml. sample whereas minimum superoxide radical scavenging ability was 48.02 ± 1.28 of *n*-hexane extract with 100 µg/ml. sample (shown in the Table 6).

At the same time, highest superoxide radical scavenging potential of *in vivo* grown *Aloe vera* L. whole leaf samples was 62.34 ± 4.57 of methanol extract with 1000 µg/ml. sample and lowest was 29.56 ± 2.33 of *n*-hexane extract with 100 µg/ml. sample (shown in Table 7). Likewise, highest superoxide radical scavenging effects in the *in vivo* grown *Aloe vera* L. only gel samples was 70.29 ± 4.73 of methanol extract with 1000 µg/ml. sample and lowest was 34.83 ± 1.92 of *n*-hexane extract with 100 µg/ml. sample (shown in the Table 8).

Ak and Gulcin[13] found that curcumin (diferuoyl methane), a phenolic compound and a major component of *Curcuma longa* L. had an effective hydrogen peroxide

scavenging activity. Raja and Pugalendi[14] have reported that the aqueous extract of *Melothria maderaspatana* was capable of scavenging H_2O_2 in a dose-dependent manner. The grape seed extracts possessed strong antioxidant activity by scavenging hydrogen peroxide when compared to bagasse extract.[15] Gulcin *et al*.[16] showed that the water and ethanolic extracts of *Ocimum basilicum* had strong antioxidant activity and were effective in scavenging H_2O_2.

Hydrogen peroxide scavenging effects of aqueous extract of *Strychnos henningsii* Gilg. indicated a concentration-dependent activity against H_2O_2.[17] The petroleum ether fraction of *Coccinia grandis* showed strong H_2O_2 scavenging activity followed by chloroform and ethyl acetate fractions.[18] The enzymatic extracts from seven species of brown seaweeds exhibited more prominent effects in hydrogen peroxide scavenging activity.[19]

Saito *et al*.[20] reported that the extracts from *Punica granatum* (peel), *Syzygium aromaticum* (bud), *Mangifera indica* (kernel) and *Phyllanthus emblica* (fruit) scavenged superoxide anions, which was comparable to that of L-ascorbic acid. Kim *et al*.[21] reported that α-chymotrypsin extract from the center of the root of Korean Elk velvet antler showed high superoxide scavenging activity. The crude extract and fractions of *Vaccinium uliginosum* L. containing polyphenol and pigment exhibited superoxide scavenging activity.[22] The superoxide anion radical scavenging activity was found to be significantly higher in the raw and dry heated seed extracts than the hydrothermally processed seed samples of cowpea (*Vigna unguiculata*).[23]

The aqueous and ethanolic extracts of *Iris germanica* was found to exhibit strong super oxide scavenging activity.[24] The methanol extracts of bark and leaves of *Cassia siamea* and *Cassia javanica* plants showed effective superoxide anion radical scavenging activity.[25]

Table 1: Hydrogen peroxide radical scavenging activity of different extracts of *in vitro* regenerated *Aloe vera* L. whole leaf extracts.

S. No.	Concentration of extract (µg/ml.)	% Inhibition				
		n-Hexane	Chloroform	Ethyl Acetate	Methanol	Aqueous
1	100	19.34 ± 1.16^{a*}	24.03 ± 1.83^{b}	22.65 ± 3.37^{b}	27.46 ± 4.83^{b}	20.18 ± 2.83^{b}
2	200	21.81 ± 1.39^{b}	26.57 ± 2.42^{b}	25.23 ± 2.86^{b}	30.16 ± 3.33^{c}	22.24 ± 3.02^{b}
3	400	23.16 ± 3.33^{b}	29.34 ± 2.36^{b}	28.67 ± 1.39^{b}	33.83 ± 3.37^{c}	24.91 ± 2.18^{b}
4	600	26.48 ± 2.43^{b}	32.19 ± 4.83^{c}	31.25 ± 3.42^{c}	35.98 ± 2.86^{c}	27.62 ± 1.42^{b}
5	800	29.17 ± 1.86^{b}	35.04 ± 3.24^{c}	33.72 ± 1.09^{c}	38.46 ± 1.19^{c}	30.43 ± 4.63^{c}
6	1000	31.16 ± 1.19^{c}	37.28 ± 1.10^{c}	36.12 ± 4.36^{c}	41.33 ± 3.54^{d}	32.08 ± 2.14^{c}

The values are given as Mean ± SD of triplicates

*****Values having different letters in superscript in each column are significantly different from each other (P≤0.05) according to Duncan's Multiple Range Test (DMRT).

Table 2: Hydrogen peroxide radical scavenging activity of different extracts of *in vitro* regenerated *Aloe vera* L. only gel extracts.

S. No.	Concentration of extract (µg/ml.)	% Inhibition				
		n-Hexane	Chloroform	Ethyl Acetate	Methanol	Aqueous
1	100	23.14 ± 2.83^{a*}	30.75 ± 1.42^{b}	27.89 ± 3.42^{a}	33.16 ± 4.17^{b}	25.91 ± 2.43^{a}
2	200	27.35 ± 2.54^{a}	31.80 ± 3.36^{b}	30.12 ± 1.84^{b}	36.68 ± 2.64^{b}	28.36 ± 4.82^{a}
3	400	29.61 ± 1.37^{a}	33.18 ± 2.82^{b}	32.67 ± 4.19^{b}	38.31 ± 1.06^{b}	31.28 ± 2.93^{b}
4	600	31.42 ± 4.28^{b}	36.33 ± 3.24^{b}	35.26 ± 4.33^{b}	41.15 ± 2.57^{c}	34.42 ± 4.86^{b}
5	800	33.80 ± 3.86^{b}	39.24 ± 1.19^{b}	38.21 ± 3.86^{b}	43.20 ± 4.02^{c}	37.08 ± 2.17^{b}
6	1000	35.28 ± 2.45^{b}	42.62 ± 4.83^{c}	40.36 ± 1.74^{c}	46.83 ± 1.54^{c}	39.33 ± 3.12^{b}

The values are given as Mean ± SD of triplicates

*****Values having different letters in superscript in each column are significantly different from each other (P≤0.05) according to Duncan's Multiple Range Test (DMRT).

Table 3: Hydrogen peroxide radical scavenging activity of different extracts of *in vivo* grown *Aloe vera* L. whole leaf extracts.

S. No.	Concentration of extract (µg/ml.)	% Inhibition				
		n-Hexane	Chloroform	Ethyl Acetate	Methanol	Aqueous
1	100	11.62 ± 1.24^{a*}	17.46 ± 4.18^{a}	15.48 ± 3.33^{a}	20.23 ± 3.36^{b}	12.58 ± 2.51^{a}
2	200	13.49 ± 1.60^{a}	19.45 ± 3.83^{a}	18.56 ± 2.24^{a}	22.91 ± 1.54^{b}	14.23 ± 4.83^{a}
3	400	14.18 ± 3.33^{a}	21.33 ± 2.19^{b}	20.24 ± 4.57^{b}	23.57 ± 2.36^{b}	15.61 ± 1.24^{a}
4	600	16.54 ± 4.82^{a}	23.26 ± 2.36^{b}	21.95 ± 1.27^{b}	25.82 ± 1.86^{b}	17.53 ± 3.16^{a}
5	800	18.26 ± 2.46^{a}	25.72 ± 1.18^{b}	24.32 ± 3.82^{b}	27.46 ± 4.19^{b}	19.24 ± 2.90^{a}
6	1000	20.33 ± 2.27^{b}	28.84 ± 4.02^{b}	27.47 ± 1.39^{b}	30.76 ± 3.36^{c}	22.18 ± 1.82^{b}

The values are given as Mean ± SD of triplicates

*****Values having different letters in superscript in each column are significantly different from each other (P≤0.05) according to Duncan's Multiple Range Test (DMRT).

Table 4: Hydrogen peroxide radical scavenging activity of different extracts of *in vivo* grown *Aloe vera* L. only gel extracts.

S. No.	Concentration of extract (µg/ml.)	% Inhibition				
		***n*-Hexane**	**Chloroform**	**Ethyl Acetate**	**Methanol**	**Aqueous**
1	100	15.35 ± 3.58^{a*}	20.54 ± 2.64^{b}	18.37 ± 2.04^{a}	23.05 ± 1.82^{b}	16.25 ± 4.32^{a}
2	200	16.12 ± 1.42^{a}	22.38 ± 1.82^{b}	19.33 ± 4.39^{a}	25.83 ± 4.71^{b}	18.87 ± 3.64^{a}
3	400	18.46 ± 2.14^{a}	23.55 ± 3.37^{b}	21.24 ± 1.57^{b}	26.15 ± 2.36^{b}	21.93 ± 1.18^{b}
4	600	20.83 ± 2.18^{b}	25.28 ± 2.46^{b}	23.86 ± 1.17^{b}	28.36 ± 4.13^{b}	23.03 ± 3.26^{b}
5	800	22.19 ± 1.18^{b}	26.11 ± 4.82^{b}	25.71 ± 3.33^{b}	30.28 ± 2.32^{c}	24.95 ± 1.83^{b}
6	1000	23.75 ± 4.21^{b}	29.62 ± 1.02^{b}	27.42 ± 2.46^{b}	33.33 ± 2.14^{c}	26.14 ± 3.29^{b}

The values are given as Mean ± SD of triplicates

*****Values having different letters in superscript in each column are significantly different from each other (P≤0.05) according to Duncan's Multiple Range Test (DMRT).

Table 5: Superoxide radical scavenging activity of different extracts of *in vitro* regenerated *Aloe vera* L. whole leaf extracts.

S. No.	Concentration of extract (µg/ml.)	% Inhibition				
		***n*-Hexane**	**Chloroform**	**Ethyl Acetate**	**Methanol**	**Aqueous**
1	100	40.32 ± 4.26^{a*}	53.28 ± 2.39^{b}	47.36 ± 1.63^{a}	60.72 ± 3.33^{c}	44.57 ± 4.36^{a}
2	200	42.04 ± 3.33^{a}	57.39 ± 1.24^{b}	50.12 ± 4.22^{b}	64.19 ± 2.27^{c}	47.13 ± 1.72^{a}
3	400	45.37 ± 1.82^{a}	60.46 ± 4.33^{c}	56.04 ± 3.78^{b}	68.33 ± 2.88^{c}	51.36 ± 2.77^{b}
4	600	48.19 ± 4.53^{a}	66.83 ± 3.91^{c}	62.35 ± 2.39^{c}	73.48 ± 1.62^{d}	54.21 ± 2.41^{b}
5	800	50.25 ± 2.98^{b}	69.13 ± 1.83^{c}	65.42 ± 4.68^{c}	77.36 ± 3.57^{d}	56.78 ± 1.33^{b}
6	1000	53.47 ± 1.63^{b}	74.65 ± 2.27^{d}	68.39 ± 1.13^{c}	82.28 ± 4.04^{e}	60.83 ± 3.57^{c}

The values are given as Mean ± SD of triplicates

*****Values having different letters in superscript in each column are significantly different from each other (P≤0.05) according to Duncan's Multiple Range Test (DMRT).

Table 6: Superoxide radical scavenging activity of different extracts of *in vitro* regenerated *Aloe vera* L. only gel extracts.

S. No.	Concentration of extract (µg/ml.)	% Inhibition				
		***n*-Hexane**	**Chloroform**	**Ethyl Acetate**	**Methanol**	**Aqueous**
1	100	48.02 ± 1.28^{a*}	68.24 ± 2.17^{c}	63.32 ± 4.92^{c}	73.52 ± 3.33^{d}	56.77 ± 2.52^{b}
2	200	54.32 ± 2.14^{b}	72.18 ± 3.39^{d}	65.80 ± 4.78^{c}	78.24 ± 2.42^{d}	59.32 ± 1.68^{b}
3	400	58.64 ± 4.63^{b}	75.02 ± 1.28^{d}	68.19 ± 2.16^{c}	81.39 ± 3.33^{e}	64.68 ± 3.27^{c}
4	600	65.29 ± 3.82^{c}	78.63 ± 3.86^{d}	72.36 ± 1.62^{d}	84.12 ± 4.36^{e}	69.38 ± 2.06^{c}
5	800	71.88 ± 1.57^{d}	82.57 ± 2.24^{e}	76.83 ± 3.86^{d}	88.06 ± 1.19^{e}	73.24 ± 4.32^{d}
6	1000	75.13 ± 4.36^{d}	86.48 ± 4.18^{e}	80.27 ± 1.57^{e}	93.37 ± 2.83^{f}	78.83 ± 3.18^{d}

The values are given as Mean ± SD of triplicates

*****Values having different letters in superscript in each column are significantly different from each other (P≤0.05) according to Duncan's Multiple Range Test (DMRT).

Table 7: Superoxide radical scavenging activity of different extracts of *in vivo* grown *Aloe vera* L. whole leaf extracts.

S. No.	Concentration of extract (µg/ml.)	% Inhibition				
		***n*-Hexane**	**Chloroform**	**Ethyl Acetate**	**Methanol**	**Aqueous**
1	100	29.56 ± 2.33^{a*}	38.93 ± 2.62^{b}	35.16 ± 3.49^{b}	43.24 ± 1.37^{c}	32.63 ± 2.80^{b}
2	200	33.13 ± 4.67^{b}	42.19 ± 1.43^{c}	38.40 ± 2.57^{b}	47.63 ± 4.82^{c}	34.02 ± 3.19^{b}
3	400	38.64 ± 2.23^{b}	45.83 ± 4.36^{c}	41.78 ± 3.42^{c}	52.16 ± 1.15^{d}	39.47 ± 4.29^{b}
4	600	41.39 ± 1.56^{c}	48.32 ± 3.33^{c}	44.24 ± 4.68^{c}	56.48 ± 2.04^{d}	43.33 ± 2.72^{c}
5	800	45.27 ± 3.82^{c}	52.83 ± 3.15^{d}	47.82 ± 1.39^{c}	60.55 ± 2.72^{e}	46.82 ± 4.53^{c}
6	1000	49.82 ± 2.39^{c}	55.60 ± 2.36^{d}	52.39 ± 1.98^{d}	62.34 ± 4.57^{e}	50.16 ± 3.28^{d}

The values are given as Mean ± SD of triplicates

*****Values having different letters in superscript in each column are significantly different from each other (P≤0.05) according to Duncan's Multiple Range Test (DMRT).

Table 8: Superoxide radical scavenging activity of different extracts of *in vivo* grown *Aloe vera* L. only gel extracts.

S. No.	Concentration of extract (µg/ml.)	% Inhibition				
		***n*-Hexane**	**Chloroform**	**Ethyl Acetate**	**Methanol**	**Aqueous**
1	100	34.83 ± 1.92^{a*}	49.46 ± 4.28^{b}	44.29 ± 3.33^{b}	53.15 ± 3.67^{c}	40.82 ± 2.47^{b}
2	200	38.57 ± 3.34^{a}	52.33 ± 2.63^{c}	47.24 ± 1.36^{b}	57.83 ±1.45^{c}	43.14 ± 4.21^{b}
3	400	40.16 ± 4.55^{b}	55.62 ± 2.39^{c}	50.68 ± 2.45^{c}	60.64 ± 2.88^{d}	46.29 ± 3.15^{b}
4	600	43.78 ± 2.23^{b}	58.53 ± 1.59^{c}	54.32 ± 3.78^{c}	64.57 ± 2.37^{d}	49.72 ± 4.48^{b}
5	800	47.34 ± 2.78^{b}	63.72 ± 3.47^{d}	58.50 ± 1.82^{c}	67.36 ± 3.92^{d}	53.57 ± 1.59^{c}
6	1000	52.40 ± 1.37^{c}	67.37 ± 2.60^{d}	61.83 ± 3.19^{d}	70.29 ± 4.73^{e}	55.49 ± 2.32^{c}

The values are given as Mean ± SD of triplicates

*****Values having different letters in superscript in each column are significantly different from each other (P≤0.05) according to Duncan's Multiple Range Test (DMRT).

CONCLUSION

In the present investigation *in vivo* and *in vitro* regenerated *Aloe vera* L. whole leaf and only gel extracts were analyzed for their hydrogen peroxide and super oxide free radical scavenging activities. The results showed that the both *in vivo* and *in vitro* regenerated *Aloe vera* L. plant leaves having adequate antioxidant activities. Whereas, methanol extracts of both *in vitro* and *in vivo* grown *Aloe vera* L. whole leaf and only gel extracts were more significant. According to results found, if we compare between methanol extract of *in vitro* and *in vivo* grown plants leaf, then, *in vitro* regenerated *Aloe vera* L. leaf extract (only gel extract followed by whole leaf extract) were more significant in having hydrogen peroxide and super oxide free radical scavenging properties than *in vivo* grown plant leaves extracts (only gel extract followed by whole leaf extract). Therefore, the assessment of such properties remains an interesting and useful task, particularly finding for new natural sources of free radical scavengers.

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