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DPPH & ABTS FREE RADICAL SCAVENGING PROPERTIES OF *Aloe vera* L.(IN *Vivo* & IN *Vitro* REGENERATED) WHOLE LEAF AND INNER GEL EXTRACTS

NEELOFAR KHANAM[a1] AND G.K. SHARMA[b]

[a]School of Biotechnology, IFTM University, Moradabad, Uttar Pradesh, India
[b]Division of Biotechnology, Department of Botany, Hindu College, Moradabad, Uttar Pradesh, India

ABSTRACT

The production of oxygen free radicals is a natural consequence of aerobic metabolism. 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). In present investigation 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2'-azino-bis-3-ethyl benzthiazoline-6-sulphonic acid (ABTS) free radical scavenging properties of in vivo grown and in *vitro* propagated *Aloe vera* L. whole leaf and only gel extracts were examined. Results were showing that among the different extracts (prepared with the solvents of different polarities such as aqueous, methanol, ethyle 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.

KEYWORDS : *Aloe vera* L., free radicals, ROS, DPPH, ABTS

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 (Schipper, 1998).

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 (Ames et al., 1993).

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 (Halliwell, 1994).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 (Halliwell, 1994; Rackova et al., 2007) such as cancer (Kinnula and Crapo, 2004), cardiovascular disease (Singh and Jialal, 2006), neural disorders (Sas et al., 2007), Alzheimer's disease (Smith et al., 2000) mild congnitive impairment (Guidi et al., 2006), Parkinsons disease (Bolton et al., 2000), alcohol induced liver disease (Arteel, 2003), ulcerative colitis (Ramakrishna et al., 1997), ageing (Hyun et al., 2006), atherosclerosis (Upston et al., 2003).

The most effective path to eliminate and diminish the action of free radicals which cause the oxidative stress is antioxidative defense 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 (Halliwell, 2008). Recently there has been an upsurge of interest in the therapeutic potential medicinal plants as antioxidants in reducing oxidative stress-induced tissue injury (Pourmorad et al., 2006) and damages rather than looking for synthetic ones (McClements and Decker, 2000). Several naturally occurring antioxidants are there to scavenge free radicals and active oxygen species by propagating a reaction cycle

[1]**Corresponding author**

and to chelate heavy metal ions (Sudarajan et al., 2006).

Plants have been known to contain components of phytomedicine since times immemorial (Chiari, 2012). Man is able to obtain from them a wondrous assortment of industrial chemicals (Tiwari, 2011). 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 (Chiari, 2012). 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 (Tiwari, 2011).

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 cation 2,2'-azino-bis-3-ethyl benzthiazoline-6-sulphonic acid ($ABTS^+$ assay) or of the stable radical 2,2-diphenyl-1-picrylhydrazyl (DPPH assay). The presence of antioxidant species leads to the disappearance of these radical chromogens which can be followed by spectrophotometric methods (Magalhaes et al., 2006).

In the present study we have investigated DPPH & ABTS free radical scavenging ability, of several 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. plant 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 65ºC 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 (2005). 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, ethyle 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 DPPH Radical Scavenging Properties

The scavenging ability of the natural antioxidants

of the leaves towards the stable free radical DPPH was measured by the method of Mensor et al. (2001). The leaf extracts (20µl) were added to 0.5ml of methanolic solution of DPPH and 0.48ml of methanol. The mixture was allowed to react at room temperature for 30 minutes. Methanol served as the blank and DPPH in methanol, without the leaf extracts, served as the positive control. After 30 minutes of incubation, the discolouration of the purple colour was measured at 518nm in a spectrophotometer. The radical scavenging activity was calculated as follows:

$$\text{Scavenging activity \%} = 100 - \frac{A_{518}\,(\text{sample}) - A_{518}\,(\text{blank})}{A_{518}\,(\text{blank})} \times 100$$

Determination of ABTS Scavenging Properties

The antioxidant effect of the leaf extracts was studied using ABTS (2,2'-azino-bis-3-ethyl benzthiazoline-6-sulphonic acid) radical cation decolourisation assay according to the method of Shirwaikar et al. (2006). ABTS radical cations (ABTS+) were produced by reacting ABTS solution (7mM) with 2.45mM ammonium persulphate. The mixture was allowed to stand in the dark at room temperature for 12-16 hours before use. Aliquots (0.5ml) of the three different extracts were added to 0.3ml of ABTS solution and the final volume was made up to 1ml with ethanol. The absorbance was read at 745nm in a spectrophotometer and the per cent inhibition was calculated using the formula:

$$\text{Inhibition \%} = \frac{\text{Control-test}}{\text{Control}} \times 100$$

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 (Halliwell and Gutteridge, 1998; Good et al., 1996; Gassen and Youdim, 1997). 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 (Osawa et al., 1994; Noda et al., 1997).

As in present study *Aloe vera* L. (in *vivo* and in *vitro* regenerated) whole leaf and only gel extracts were examine for their free radical scavenging properties against DPPH and ABTS free radicals. Results were showing that among the different extracts (prepared with the solvents of different polarities such as aqueous, methanol, ethyle 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 highest DPPH scavenging activity in the in vitro regenerated *Aloe vera* L. whole leaf samples was 83.34 ± 2.34 of methanolic extract with 1000 µg/ml. sample and lowest was 32.89 ± 3.37 of n-hexane extract with 100 µg/ml. sample (shown in the Table 1). Subsequently, the highest DPPH scavenging activity in the in vitro regenerated *Aloe vera* L. only gel samples was 95.34 ± 1.72 of methanolic extract with 1000 µg/ml. sample and lowest was 35.39 ± 1.82 of n-hexane extract with 100 µg/ml. sample (shown in the Table 2).

Similarly, the highest DPPH scavenging activity in the in vivo grown *Aloe vera* L. whole leaf samples was 62.43 ± 2.86 of methanolic extract with 1000 µg/ml. sample and lowest was 21.83 ± 3.19 of n-hexane extract with 100 µg/ml. sample (shown in the Table 3). Subsequently, the highest DPPH scavenging activity in the in vivo grown *Aloe vera* L. only gel samples was 72.33 ± 3.54 of methanolic extract with 1000 µg/ml. sample and lowest was 26.54 ± 1.47 of n-hexane extract with 100 µg/ml. sample (Table 4).

Whereas, the highest ABTS scavenging activity in the in vitro regenerated *Aloe vera* L. whole leaf samples was 84.32 ± 2.32 of methanolic extract with 1000 µg/ml. sample

Table 1 : DPPH Radical Scavenging Properties 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	32.89 ± 3.37[a*]	45.32 ± 5.07[b]	41.03 ± 3.58[b]	48.53 ± 4.57[b]	36.40 ± 3.33[a]
2	200	35.07 ± 3.14[a]	49.63 ± 4.39[b]	47.52 ± 2.43[b]	55.42 ± 1.33[c]	38.63 ± 2.78[a]
3	400	41.28 ± 4.39[b]	56.33 ± 2.83[c]	52.37 ± 1.34[c]	68.57 ± 5.46[d]	46.17 ± 3.37[b]
4	600	48.33 ± 3.58[b]	67.42 ± 4.54[d]	59.83 ± 3.07[c]	74.36 ± 3.27[e]	50.38 ± 4.72[c]
5	800	52.14 ± 2.17[c]	72.39 ± 3.58[e]	66.42 ± 4.58[d]	79.82 ± 4.58[e]	58.07 ± 2.39[c]
6	1000	56.47 ± 3.33[c]	76.59 ± 3.72[e]	70.39 ± 3.17[e]	83.34 ± 2.34[f]	63.24 ± 1.67[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 (DNMRT)

Table 2 : DPPH Radical Scavenging Properties 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	35.39 ± 1.82[a*]	48.63 ± 3.17[b]	45.88 ± 4.07[b]	57.34 ± 4.17[c]	33.37 ± 2.83[a]
2	200	39.72 ± 4.07[a]	56.04 ± 1.34[c]	54.23 ± 1.19[c]	70.54 ± 3.08[e]	45.61 ± 3.57[b]
3	400	46.50 ± 2.56[b]	64.85 ± 2.87[d]	59.46 ± 3.42[c]	75.37 ± 1.87[e]	51.83 ± 2.19[c]
4	600	53.62 ± 3.33[c]	73.51 ± 2.58[e]	65.37 ± 2.04[d]	81.06 ± 3.36[f]	58.39 ± 2.36[c]
5	800	58.43 ± 3.87[c]	75.36 ± 2.68[e]	71.32 ± 2.57[e]	87.33 ± 2.54[f]	64.08 ± 1.42[d]
6	1000	62.82 ± 2.19[d]	79.83 ± 4.32[e]	74.96 ± 1.68[e]	95.34 ± 1.72[g]	68.52 ± 3.39[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 (DNMRT)

Table 3 : DPPH Radical Scavenging Properties 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	21.83 ± 3.19[a*]	38.14 ± 2.57[b]	32.53 ± 4.12[b]	40.57 ± 3.37[c]	26.39 ± 2.57[a]
2	200	24.18 ± 1.59[a]	41.13 ± 2.36[c]	38.16 ± 3.59[b]	43.32 ± 1.63[c]	31.57 ± 3.26[b]
3	400	30.52 ± 2.24[b]	46.62 ± 1.47[c]	43.33 ± 3.02[c]	49.18 ± 4.71[c]	37.42 ± 3.55[b]
4	600	35.63 ± 2.36[b]	51.39 ± 3.12[d]	47.42 ± 2.29[c]	55.37 ± 2.36[d]	40.36 ± 2.27[c]
5	800	42.19 ± 3.42[c]	55.13 ± 4.43[d]	50.36 ± 1.42[d]	58.83 ± 3.33[d]	45.91 ± 3.12[c]
6	1000	46.83 ± 2.16[c]	58.38 ± 1.19[d]	54.75 ± 4.36[d]	62.43 ± 2.86[e]	49.13 ± 1.82[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 (DNMRT)

Table 4: DPPH Radical Scavenging Properties 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	26.54 ± 1.47[a*]	40.52 ± 3.33[c]	37.19 ± 4.57[b]	44.75 ± 3.19[c]	32.02 ± 2.71[b]
2	200	31.02 ± 2.36[b]	44.16 ± 3.16[c]	42.33 ± 2.18[c]	50.13 ± 1.57[d]	35.32 ± 1.19[b]
3	400	34.57 ± 1.19[b]	49.35 ± 1.87[c]	45.62 ± 3.46[c]	54.27 ± 4.02[d]	40.18 ± 2.37[c]
4	600	39.45 ± 3.24[b]	53.62 ± 2.39[d]	51.43 ± 3.33[d]	59.43 ± 2.36[d]	44.39 ± 2.56[c]
5	800	45.10 ± 2.37[c]	57.83 ± 1.32[d]	56.36 ± 1.85[d]	65.05 ± 2.71[e]	49.27 ± 3.28[c]
6	1000	50.36 ± 2.91[d]	64.13 ± 4.17[e]	59.04 ±2.43[d]	72.33 ± 3.54[f]	54.30 ± 1.46[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 (DNMRT)

Table 5 : ABTS Radical Scavenging Properties 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	39.19 ± 2.19[a*]	57.93 ± 4.12[c]	52.36 ± 1.32[c]	64.81 ± 2.53[d]	46.02 ± 3.37[b]
2	200	44.58 ± 1.32[b]	62.43 ± 3.19[d]	57.14 ± 2.90[c]	69.24 ± 4.12[d]	50.37 ± 1.42[c]
3	400	48.33 ± 2.41[b]	66.52 ± 1.82[d]	61.28 ± 1.63[d]	73.13 ± 1.90[e]	53.82 ± 4.56[c]
4	600	51.03 ± 3.72[c]	71.02 ± 2.63[e]	65.91 ± 4.32[d]	77.56 ± 3.36[e]	58.19 ± 1.75[c]
5	800	56.49 ± 1.57[d]	74.99 ± 3.25[e]	69.41 ± 3.52[e]	80.47 ± 2.57[e]	63.72 ± 2.09[d]
6	1000	62.37 ± 1.33[d]	79.63 ± 4.57[e]	73.19 ± 3.23[e]	84.32 ± 2.32[f]	66.28 ± 2.16[d]

The values are given as Mean ± SD of triplicates

*Values having different letters in each column significantly differed from each other (P<0.05) according to Duncan's Multiple Range Test (DNMRT)

and lowest was 39.19 ± 2.19 of n-hexane extract with 100 µg/ml. sample (Table 5). Subsequently, the highest ABTS scavenging activity in the in vitro regenerated Aloe vera L. only gel samples was 96.67 ± 4.12 of methanolic extract with 1000 µg/ml. sample and lowest was 46.57 ± 3.33 of n-hexane extract with 100 µg/ml. sample (Table 6).

Likewise, the highest ABTS scavenging activity in the in vivo grown *Aloe vera* L. whole leaf samples was 68.14 ± 3.16 of methanolic extract with 1000 µg/ml. sample and lowest was 26.14 ± 2.13 of n-hexane extract with 100 µg/ml. sample (Table 7). Subsequently, the highest ABTS scavenging activity in the in vivo grown *Aloe vera* L. only gel samples was 75.32 ± 4.83 of methanolic extract with 1000 µg/ml. sample and lowest was 33.25 ± 1.82 of n-hexane extract with 100 µg/ml. sample (Table 8).

The screening of herbal extracts and their components by the DPPH scavenging assay has become a routine parameter for testing their antioxidant efficacy (Mothana et al., 2008). The aqueous, methanolic and ethanolic extracts of *Melissa officinalis*, *Matricaria recuttia* and *Cymbopogan citrates* were found to possess DPPH scavenging activity (Pereira et al., 2009). The methanolic extracts of leaves and flowers of *Lippia alba* exhibited very significant DPPH radical scavenging activity compared to the standard antioxidant ascorbic acid (Ara and Nur, 2009). The methanolic extract of *Manikara zapota* showed strong activity on scavenging DPPH radical, which implicates an essential defence against the free radicals (Kaneria et al., 2009).

Seed and shell essential oils extracted from *Abrus precatorius* (L) were found in having good antioxidant and free radical scavenging potential including DPPH and ABTS free radical scavenging capacity (Okoh et al., 2014).

ABTS is an excellent substrate for peroxidases and is frequently used to study the antioxidant properties of natural compounds (Reszka and Britigan, 2007). The ethyl acetate fraction of *Evax pygmaea* showed strong ABTS radical scavenging and it nearly fully scavenged ABTS+ (Boussaada et al., 2008). Gulcin et al. (2008) have shown that Ligustroside and Oleuropein, isolated from the methanolic extracts of the root bark of *Chinonanthus virginicus* exhibited good ABTS scavenging activity.

Table 6 : ABTS Radical Scavenging Properties 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	$46.57 \pm 3.33^{a*}$	64.38 ± 4.19^{c}	55.19 ± 1.83^{b}	68.29 ± 3.17^{c}	50.86 ± 2.14^{b}
2	200	51.03 ± 2.87^{b}	69.33 ± 1.06^{c}	63.83 ± 2.26^{c}	72.57 ± 4.46^{d}	56.13 ± 4.39^{b}
3	400	55.82 ± 2.34^{b}	73.21 ± 3.57^{d}	68.42 ± 3.62^{c}	77.32 ± 2.29^{d}	61.39 ± 1.57^{c}
4	600	59.36 ± 4.12^{b}	78.14 ± 2.83^{d}	72.01 ± 1.17^{d}	82.64 ± 3.33^{e}	65.82 ± 2.42^{c}
5	800	64.61 ± 1.72^{c}	81.62 ± 2.24^{e}	75.19 ± 4.43^{d}	89.20 ± 1.85^{e}	68.03 ± 3.33^{c}
6	1000	67.43 ± 2.04^{c}	85.20 ± 1.62^{e}	77.63 ± 3.89^{d}	96.67 ± 4.12^{f}	72.13 ± 1.45^{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 (DNMRT)

Table 7: ABTS Radical Scavenging Properties 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	$26.14 \pm 2.13^{a*}$	41.36 ± 4.53^{c}	38.12 ± 4.39^{b}	50.83 ± 3.02^{d}	32.46 ± 2.36^{b}
2	200	30.75 ± 2.46^{b}	44.15 ± 2.24^{c}	43.05 ± 3.57^{c}	54.41 ± 1.81^{d}	36.58 ± 1.43^{b}
3	400	35.12 ± 1.19^{b}	49.34 ± 3.09^{c}	47.83 ± 2.82^{c}	59.23 ± 2.62^{d}	40.19 ± 4.57^{c}
4	600	39.93 ± 4.32^{b}	54.68 ± 3.33^{d}	52.61 ± 1.19^{d}	62.77 ± 4.12^{e}	45.37 ± 3.33^{c}
5	800	42.37 ± 3.04^{c}	58.18 ± 1.84^{d}	56.39 ± 2.36^{d}	65.46 ± 3.47^{e}	49.83 ± 3.82^{c}
6	1000	47.01 ± 1.55^{c}	63.52 ± 2.63^{e}	60.74 ± 4.43^{e}	68.14 ± 3.16^{e}	54.71 ± 2.68^{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 (DNMRT)

Table 8 : ABTS Radical Scavenging Properties 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	33.25 ± 1.82^{a*}	47.92 ± 2.34^{b}	42.13 ± 4.23^{b}	54.39 ± 3.37^{c}	38.32 ± 1.18^{a}
2	200	37.16 ± 3.08^{a}	53.14 ± 1.83^{c}	48.57 ± 3.37^{b}	57.42 ± 1.19^{c}	41.86 ± 2.37^{b}
3	400	42.52 ± 1.33^{b}	58.36 ± 4.06^{c}	55.82 ± 3.06^{c}	63.19 ± 2.53^{d}	46.52 ± 1.42^{b}
4	600	45.32 ± 2.24^{b}	65.47 ± 3.82^{d}	59.16 ± 1.82^{c}	68.83 ± 3.33^{d}	53.16 ± 4.13^{c}
5	800	50.43 ± 4.03^{c}	68.24 ± 1.19^{d}	64.32 ± 2.73^{d}	73.04 ± 3.12^{e}	58.73 ± 3.67^{c}
6	1000	56.19 ± 2.63^{c}	72.16 ± 2.37^{e}	69.46 ± 1.39^{d}	75.32 ± 4.83^{e}	63.47 ± 1.53^{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 (DNMRT).

CONCLUSION

In the present investigation in vivo and in vitro regenerated *Aloe vera* L. whole leaf and only gel extracts were analyzed for their DPPH and ABTS free radical scavenging properties. 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 DPPH and ABTS 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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