World Journal of Pharmaceutical ReseaRch
SJIF Impact Factor 5.045
Volume 3, Issue 6, 1815-1829. Research Article ISSN 2277 – 7105

EVALUATION OF ENZYMATIC AND NON-ENZYMATIC ANTIOXIDANT ACTIVITIES OF *IN VIVO* AND *IN VITRO* REGENERATED *ALOE VERA* L.

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Article Received on
22 June 2014,

Revised on 17 July 2014,
Accepted on 12 August 2014

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ABSTRACT

Since ancient time a great number of aromatic, medicinal, spice and other plants contain chemical compounds exhibiting antioxidant properties, such as their secondary metabolites, which play an important role in protecting the cell or tissues from oxidative damage. In the present investigation *in vitro* enzymatic and non-enzymatic antioxidant activities in the leaves of *in vivo* and *in vitro* regenerated *Aloe vera* L. were tested. The plant leaves were analysed for the selected enzymatic antioxidants such as superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), glutathione S-transferase (GST), polyphenol oxidase (PPO), and for the non-enzymatic antioxidants such as ascorbic acid, tocopherol, total carotenoids, total phenols, reduced glutathione, flavonoids were examined. Results were showing that the leaves from *in vitro* propagated *Aloe vera* L. were having higher level of antioxidants when compared to antioxidant levels in the leaves of *in vivo* grown *Aloe vera* L.

KEY WORDS: *Aloe vera* L., *in vitro* and *in vivo*, enzymatic antioxidants, non-enzymatic antioxidants.

INTRODUCTION

Recently there has been an upsurge of interest in the therapeutic potential medicinal plants as antioxidants in reducing oxidative stress-induced tissue injury [1] and damages rather than looking for synthetic ones [2]. As molecular oxygen is an essential component for all living organisms, where it helps in the process of oxidation, which is a basic component of aerobic

life and of our metabolism [3]. A part of the oxygen taken into living cells is converted to several harmful reactive oxygen species and free radicals. Once formed, free radicals can start a chain reaction, leading to the formation of more free radicals [4]. Oxidative process is one of the most important routes for producing free radicals in foods, drugs and even in living systems [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. Among the numerous naturally occurring antioxidants; ascorbic acid, carotenoids and phenolic compounds are more effective [6]. Natural antioxidants such as α-tocopherol and ascorbic acid are widely used because they are regarded as safer and causing fewer adverse reactions but their antioxidant activities are lower than the synthetic antioxidants such as butylated hydroxyanisol (BHA) and butylated hydroxytoluene (BHT) which have been restricted by legislative rules because they are suspected to have some toxic effects and as possible carcinogens [7-9]. Therefore, there is a considerable interest in finding new and safe antioxidants from natural sources to replace these synthetic antioxidants [10,11]. The medicinal properties of plants have been investigated in the recent scientific developments throughout the world, due to their potent antioxidant activities, no side effects and economic viability. The systematic screening of plant species with the purpose of discovering new bioactive compounds has been a routine activity in many laboratories [12,13]. *Aloe vera* L. has a long ethno botanical and medicinal history around the world. As plant-derived drugs always remains an important resource, especially in developing countries, to combat serious diseases. Approximately 60% to 80% of the world's population still relies on traditional medicines for the treatment of common illness [14]. Medicinal plants are also cheaper and more accessible to most of the population in the world [15]. Therefore, many pharmacognostical and pharmacological investigations are carried out for the development of novel therapeutic agents for the treatment of human ailments such as cancer and infectious diseases [16]. Medicinal plants have regained a wide recognition due to an escalating faith in herbal medicine in the last few decades contributed by its lesser side effects compared to allopathic medicine [17]. The previous studies done on medicinal plants and vegetables strongly supports the idea that plant constituents with antioxidant activity are capable of exerting protective effects against oxidative stress in biological systems [18]. In present investigation an *in vitro* study was conducted to examine and compare the enzymatic and non-enzymatic antioxidant activities in the leaves of both *in vivo* and *in vitro* regenerated *Aloe vera* L.

MATERIALS AND METHODS

Initial preparation of test samples of *Aloe vera* L. Leaves (*in vitro* and *in vivo* grown)

Leaves were collected from 9 months old (from initial culture) *in vitro* regenerated plants of *Aloe vera* L., maintained on MS medium in combination with different growth regulators such as BAP (6-benzylaminopurine), NAA (Naphthalene acetic acid) and IBA (Indole-3-butyric acid) followed by proper acclimatization [19]. At the same time leaves from 9 months old *in vivo* grown *Aloe vera* L. plants were also collected. Both *in vitro* and *in vivo* grown leaves were washed with distilled water, followed by disinfecting with 70% ethanol. Later they 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 and transferred separately into sterile containers and stored at 4ºC for further use.

Determination of enzymatic antioxidants

The enzymatic antioxidants analysed in *in vitro* and *in vivo* grown *Aloe vera* L. leaves were superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), glutathione S-transferase (GST) and polyphenol oxidase (PPO).

Assay of superoxide dismutase (SOD)

SOD in *Aloe vera* L. leaves was determined by the method of Kakkar *et al.* [20]. The colour formed at the end of the reaction can be extracted into butanol and measured at 560nm. An exact amount (0.5 g) of *Aloe vera* L. leaves samples were mixed with 3.0ml of potassium phosphate buffer separately and centrifuged at 2000g for 10 minutes and the supernatants were used for the assay as enzyme preparation. The assay mixture contained 1.2ml of sodium pyrophosphate buffer, 0.1ml of Phenazine methosulphate (PMS), 0.3ml of Nitroblue tetrazolium (NBT), 0.2ml of the enzyme preparation and water in a total volume of 2.8ml. The reaction was initiated by the addition of 0.2ml of NADH. The mixture was incubated at 30°C for 90 seconds and arrested by the addition of 1.0ml of glacial acetic acid. The reaction mixture was then shaken with 4.0ml of n-butanol, allowed to stand for 10 minutes and centrifuged. The intensity of the chromogen in the butanol layer was measured at 560nm. One unit of enzyme activity is defined as the amount of enzyme that gave 50% inhibition of NBT reduction in one minute.

Assay of catalase (CAT)

Catalase activity was assayed according to the method given by Luck [21]. The UV absorption of hydrogen peroxide can be measured at 240nm.The absorption of hydrogen peroxide

decreases on degradation by the enzyme catalase. An exact amount (0.5 g) of *Aloe vera* L. leaves samples were homogenized in 2.5 ml of phosphate buffer. The homogenate was centrifuged and the supernatant was used for the enzyme assay. Hydrogen peroxide in phosphate buffer (3.0ml) was taken followed by the rapid addition of 40µl of enzyme extract and mixed thoroughly. The time interval required for a decrease in absorbance by 0.05 units was recorded at 240nm. The enzyme solution containing H_2O_2-free phosphate buffer served as control. The concentration of hydrogen peroxide was calculated using the extinction coefficient 0.036 per µM cm^{-1}. One enzyme unit was calculated as the amount of enzyme required to decrease the absorbance at 240nm by 0.05 units.

Assay of peroxidase (POD)

Peroxidase activity was assayed by the method of Reddy *et al.* [22]. The oxidation of pyrogallol to a coloured product called purpurogalli can be measured at 430nm. An exact amount (0.5 g) of *Aloe vera* L. leaves samples were homogenized in 2.5 ml of phosphate buffer. The homogenate was centrifuged and the supernatant was used for the enzyme assay. 0.1ml of the enzyme extract was added to 3.0ml of pyrogallol solution. and the baseline was adjusted at 430 nm in a spectrophotometer. To the test cuvette, 0.5ml of hydrogen peroxide was added and mixed. The change in absorbance was recorded every 30 seconds up to 3 minutes. One unit of peroxidase is defined as the change in absorbance per minute at 430nm.

Assay of glutathione S-transferase (GST)

The method proposed by Habig *et al.* [23] was adopted for assaying the activity of GST. The enzyme can be assayed by its ability to conjugate GSH (Glutathione) and CDNB (1-chloro-2,4-dinitrobenzene), the extent of conjugation causing a proportionate change in the absorbance at 340nm. An exact amount (0.5 g) of *Aloe vera* L. leaves samples were mixed with 5.0ml of potassium phosphate buffer separately and centrifuged at 5000rpm for 10 minutes and the supernatants were used for the assay as enzyme preparation. The reaction mixture contained 0.1ml of GSH, 0.1ml of CDNB and phosphate buffer in a total volume of 2.9ml. The reaction was initiated by the addition of 0.1ml of the enzyme extract. Distilled water was used as the blank and the readings were recorded every 15 seconds at 340nm against the blank. The assay mixture without the extract served as the control to monitor nonspecific binding of the substrates. GST activity was calculated using the extinction coefficient of the product formed ($9.6mM^{-1}cm^{-1}$) and was expressed as nmoles of CDNB conjugated/minute.

Assay of polyphenol oxidase (PPO)

Polyphenol oxidase activity was determined by the method given by Esterbauer *et al.* [24]. In this method catechol oxidase and laccase ativities were simultaneously estimated spectrophotometrically. Phenol oxidases are copper containing proteins that catalyse the aerobic oxidation of phenolic substrates to quinones, which are autooxidized to dark brown pigments known as melanins that can be estimated spectrophotometrically at 495nm. *Aloe vera* L. leaves samples (0.5g) were homogenized in the medium, containing Tris-HCl, sorbitol and NaCl and was made upto 2.0 ml. The homogenate was centrifuged at 2000g for 10 minutes and the supernatant was used for the assay. An aliquot of 2.5 ml of phosphate buffer and 0.3ml of catechol solution were added in the cuvette and the spectrophotometer was set at 495nm. The enzyme extract (0.2ml) was added and the change in absorbance was recorded for every 30 seconds up to 5 minutes. One unit of either catechol oxidase or laccase is defined as the amount of enzyme that transforms one µmole of dihydrophenol to one µmole of quinone per minute.

The activity of PPO can be calculated using the formula

Enzyme unit in the sample = K × (ΔA/min)

where, K for catechol oxidase = 0.272

K for laccase = 0.242

Determination of non-enzymatic antioxidants

The non-enzymatic antioxidants analysed in *in vitro* and *in vivo* grown *Aloe vera* L. leaves were ascorbic acid, tocopherol, total carotenoids, reduced glutathione, total phenols and flavonoids.

Estimation of ascorbic acid

Ascorbic acid was estimated by the spectrophotometric method proposed by Roe and Keuther [25]. Ascorbate is converted into dehydroascorbate when treated with activated charcoal. Dehydroascorbic acid then form osazones by reacting with 2,4-dinitrophenyl hydrazine. When dissolved in sulphuric acid these osazones produce an orange coloured solution, whose absorbance can be measured spectrophotometrically at 540nm. An accurate amount (1g) of *Aloe vera* L. leaves samples were homogenized in 4%TCA and the volume was made upto 10 ml. After centrifugation at 2000rpm for 10 minutes the supernatant obtained was treated with a pinch of activated charcoal, shaken vigorously and kept for 10 minutes. Centrifuged again to remove the charcoal residue and the supernatant obtained

was used for the estimation. Aliquots of 0.5-1.0 ml of this supernatant and 0.2-1.0 ml of standared ascorbic acid were taken and the volume was made up to 2.0 ml with 4% TCA. 0.5 ml of DNPH reagent was added to all the tubes, followed by 2 drops of 10% thiourea solution. The content was mixed and incubated at 37^{o}C for 3 hours resulting in the formation of osazone crystals. The crystals were dissolved in 2.5 ml of 85% of sulphuric acid, in cold. To the blank alone, DNPH reagent and thiourea were added after the addition of sulphuric acid. The tubes were cooled in ice and the absorbance was read at 540nm spectrophotometrically. A standard graph was constructed using an electronic calculator set to the linear regression mode. The concentration of ascorbate in the samples were calculated and expressed in terms of mg ascorbate /g leaf sample.

Estimation of tocopherol

Tocopherol was analyzed in the leaves samples by Emmerie-Engel reaction as reported by Rosenberg [26]. The Emmerie-Engel reaction is based on the reduction of ferric to ferrous ions by tocopherols, which forms red colour with 2,2'-dipyridyl. An exact amount (2.5g) of *Aloe vera* L. leaves samples was homogenized in 50ml of 0.1N sulphuric acid and allowed to stand overnight. The contents of the flask were shaken vigorously and filtered through Whatman No.1 filter paper. Aliquots of the filtrate were used for the estimation. 1.5ml of plant extract, 1.5ml of the standard solution (D,L-α-tocopherol, 10mg/L in absolute alcohol) and 1.5ml of water were pipetted out separately into 3 stoppered centrifuge tubes. To all the tubes, 1.5ml of ethanol and 1.5ml of xylene were added, mixed well and centrifuged. Xylene (1.0ml) layer was transferred into another stoppered tube. To each tube, 1.0ml of dipyridyl reagent was added and mixed well. The mixture (1.5ml) was pipetted out into a cuvette and the extinction was read at 460nm. Ferric chloride solution (0.33ml) was added to all the tubes and mixed well. The red colour developed was read exactly after 15 minutes at 520nm in a spectrophotometer. The concentration of tocopherol in the leaves samples were calculated using the following formula,

$$\text{Tocopherols } (\mu g) = \frac{\text{Sample } A_{520} - A_{460}}{\text{Standard } A_{520}} \times 0.29 \times 0.15$$

Estimation of total carotenoids

Total carotenoid was assayed by the method described by Zakaria *et al.* [27]. Total carotenoids can be extracted in the samples using petroleum ether and estimated at 450nm. The experiment was carried out in the dark to avoid photolysis of carotenoids once the

saponification was complete. An accurate amount (0.5g) of *Aloe vera* L. leaves sample was homogenized and saponified with 2.5ml of 12% alcoholic potassium hydroxide in a water bath at 60°C for 30 minutes. The saponified extract was transferred to a separating funnel containing 10- 15ml of petroleum ether and mixed well. The lower aqueous layer was then transferred to another separating funnel and the upper petroleum ether layer containing thecarotenoids was collected. The extraction was repeated until the aqueous layer became colourless. A small amount of anhydrous sodium sulphate was added to the petroleum ether extract to remove excess moisture. The final volume of the petroleum ether extract was noted. The absorbance of the yellow colour was read in a spectrophotometer at 450nm using petroleum ether as blank. The amount of total carotenoids was calculated using the following formulae,

$$\text{Amount of total carotenoids (mg/g)} = \frac{A_{450} \times \text{Volume of the sample} \times 100 \times 4}{\text{Weight of the sample}}$$

Estimation of total phenols

Total phenols in the plant tissues were analyzed by the method given by Mallick and Singh [29]. Phenols react with phosphomolybdic acid in Folin-Ciocalteau reagent in alkaline medium to produce a blue-coloured complex, which can be estimated spectrophotometrically at 650nm. An accurate amount (0.5g) of leaves samples was homogenized in 100X volume of 80% ethanol. The homogenate was centrifuged at 10,000rpm for 20 minutes. The extraction was repeated with 80% ethanol. The supernatants were pooled and evaporated to dryness. The residue was then dissolved in a known volume of distilled water and 0.5ml of Folin-Ciocalteau reagent was added to it. After 3 minutes, 2.0ml of 20% sodium carbonate solution was added, mixed thoroughly and placed in a boiling water bath for exactly one minute, cooled and measured the absorbance at 650nm in a spectrophotometer against a reagent blank. Standard catechol solutions (0.2-1ml) corresponding to 2.0-10µg concentrations were also treated as above by adding Folin-Ciocalteau reagent and sodium carbonate solution. A standard curve was constructed using an electronic calculator on the linear regression mode, using which the concentrations of phenols in the samples were read. The values are expressed as mg phenols/g leave samples.

Estimation of reduced glutathione

The amount of reduced glutathione was determined by the method of Moron *et al.* [28]. On reaction with DTNB (5,5'-dithiobis nitro benzoic acid) reduced glutathione produces a

yellow coloured product which can be measured spectrophotomertrically at 412nm. *Aloe vera* L. leaves samples (0.5g) were homogenized with 2.5ml of 5% TCA. The precipitated protein was centrifuged at 1000rpm for 10 minutes. The supernatant was used for the estimation of GSH. 0.1 ml of supernatant was made up to 1.0ml with 0.2M sodium phosphate buffer. Standard GSH corresponding to concentrations ranging between 2 and 10 n moles were also prepared. Two ml of freshly prepared DTNB solution was added and the intensity of the yellow colour developed was measured in a spectrophotometer at 412nm after 10 minutes. The values are expressed as nmoles GSH/g leaves samples.

Estimation of flavonoids

Method proposed by Cameron *et al.* [30] was used to estimate the flavonoids in *Aloe vera* L. leave samples. After reacting with vanillin, flavonoids produced a coloured product, which can be measured spectrophotometrically at 340nm. The *Aloe vera* L. leaves samples (0.5g) were first extracted with methanol : water mixture (2:1) and secondly with the same mixture in the ratio 1:1. The extracts were shaken well and they were allowed to stand overnight. The supernatants were pooled and the volume was measured. This supernatant was concentrated and then used for the further estimation. A known volume of the extract was pipette out and evaporated to dryness. Vanillin reagent (4.0ml) was added and the tubes were heated in a boiling water bath for 15 minutes. Varying concentrations of the standard were also treated in the same manner. The optical density was read in a spectrophotometer at 340nm. A standard curve was constructed and the concentration of flavonoids in each sample was calculated. The values of flavonoids were expressed as mg flavonoids/g leaves samples.

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 test. P values less than 0.05 were considered statistically significant.

RESULTS AND DISCUSSION

There are many investigators who have worked on the extraction of valuable constituents of medicinal use from the plants or herbal resources, because these are traditionally trusted, natural, having lesser side effects, cheaper and more accessible. Therefore, plant extracts and their isolated constituents have always been an important part of various therapeutic systems [31].

In the present investigation leaves from *in vivio* and *in vitro* regenerated *Aloe vera* L. were analyzed for their enzymatic and non-enzymatic antioxidant activities. Results showed that the leaves of *in vitro* and *in vivo* grown *Aloe vera* L. are the good source of both enzymatic and non-enzymatic antioxidants.

The enzymatic antioxidant in leaves of *in vitro* and *in vivo* grown *Aloe vera* L. were SOD, catalase, peroxidise, glutathione S-transferase, polyphenols including catechol oxidase and laccase as shown in table-1. In which leaves from *in vitro* regenerated *Aloe vera* L. were possessing considerably higher activities of enzymatic antioxidants when compared to the leaves of *in vivo* grown *Aloe vera* L.

The non-enzymatic antioxidants such as ascorbic acid, tocopherol, total carotenoids, total phenols, reduced glutathione and flavonoids were also analyzed in the leaves of both *in vivo* and *in vitro* regenerated *Aloe vera* L. as shown in the table-2 and found that non-enzymatic antioxidants were also considerably higher in the leaves of *in vitro* regenerated *Aloe vera* L. as compared to the leaves from *in vivo* grown *Aloe vera* L. Ascorbic acid (a scavenger of oxyradicals), provides first line of defence against oxidative stress [32]. Ascorbic acid is a key antioxidant, partially protecting lipids from peroxidative damage and it has many biological activities in the human body [33]. Tocopherol is used in combating free radicals and the most active form of vitamin E is present in the cellular membrane and acts as a protective lipid soluble agent, generating the poorly reactive tocopherol radicals [34]. Carotenoids have the capacity of quenching singlet oxygen and acting as free radical scavengers and antioxidants [35].

Carotenoids and other antioxidant pigments are involved in several physiological processes and signalling in animals that cannot synthesize them and therefore, must acquire them from food [36]. Plant phenolic constitutes are one of the major group of compounds acting as primary antioxidants or free terminators [37]. Phenolic compounds are commonly found in both edible and inedible plants, and have been reported to have multiple biological effects, including antioxidant activity. Phenolics are able to scavenge reactive oxygen species due to their electron donating properties. Their antioxidant effectiveness depends on the stability in different systems, as well as number and location of hydroxyl groups [38].

Table 1: Enzymatic antioxidant activities of *in vivo* and *in vitro* regenerated *Aloe vera* L.

Enzymatic antioxidants		Leaves samples from *in vivo* grown *Aloe vera* L.	Leaves samples from *in vitro* grown *Aloe vera* L.
SOD (U^{*}/g)		21.07 ± 0.47	24.16 ± 0.33[a]
Catalase ($U^{\#}$/g)		57.29 ± 0.08	63.05 ± 0.12[a]
Peroxidase ($U^{@}$/g)		12.32 ± 0.54	15.28 ± 0.42[a]
Glutathione- S-transferase ($U^{\$}$/g)		2.29 ± 0.16	3.43 ± 0.25[a]
Polyphenols (PPO) ($U^{\&}$/g)	Catechol oxidase	18.74 ± 0.52	24.13 ± 0.18[a]
	Laccase	18.21 ± 0.06	23.57 ± 0.02[a]

Values are mean ± SD of triplicates.

a - Statistically significant (P≤0.05) compared to *in vivo* grown *Aloe vera* L. leaves.

* 1unit - Activity of enzyme that gives 50% inhibition of the extent of NBT reduction in 1minute.

\# 1unit - Amount of enzyme required to decrease the absorbance at 240nm by 0.05 units/ minute.

@ 1unit - Change in absorbance / minute at 430nm.

$ 1unit - nmoles of CDNB conjugated / minute.

& 1unit - Amount of catechol oxidase/laccase which transforms 1µmol of dihydro-phenol to quinone / minute.

Table 2: Non-enzymatic antioxidant activities of *in vivo* and *in vitro* regenerated *Aloe vera* L.

Non-enzymatic antioxidants	Leaves samples from *in vivo* grown *Aloe vera* L.	Leaves samples from *in vitro* grown *Aloe vera* L.
Ascorbic acid (mg/g)	1.63 ± 0.02	2.82 ± 0.03[a]
Tocopherol (µg/g)	2.8 ± 0.25	4.91 ± 0.37[a]
Total carotenoids (mg/g)	9.01 ± 0.33	11.58 ± 0.55[a]
Total phenols (mg/g)	14.2 ± 0.43	19.1 ± 0.08[a]
Reduced glutathione (nmoles/g)	57.7 ± 0.28	63.3 ± 0.47[a]
Flavonoids (mg/g)	7.01 ± 0.50	7.92 ± 0.31[a]

Values are mean ± SD of triplicates.

a - Statistically significant (P≤0.05) compared to *in vivo* grown *Aloe vera* L. leaves.

The phenolic compounds such as phenolic acid and flavonoids are most important antioxidant food source. Flavonoids are also described as scavengers of reactive oxygen species, via inhibition of oxido-reductases [36]. Flavonoids and other phenolic compounds of plant origin have been reported as scavengers and inhibitors of lipid peroxidation as well [39]. Similar work has done by Miladi and Damak [40] who reported the efficient total antioxidant capacity

in *Aloe vera* L. leaf skin extracts. There are many other medicinal plants on which different workers investigated the same. According to Karthikeyen and Rani [41], a comparative study of antioxidant levels in different Piper species showed a differential showed a differential antioxidant status with reference to both enzymatic and non-enzymatic antioxidants. The composition of antioxidants varies widely with several factors like the variety, climatic conditions, part of plant analyzed, post harvest handling, processing and storage [42]. Viji and Parvatham [43] studied on *in vitro* and *in vivo* regenerated leaf, stem and root tissues of *Withania somnifera*-Poshita variety for their enzymatic and non-enzymatic activities and found that the leaves were showing the considerable enzymatic and non-enzymatic activities. Montavon [44] reported green coffee samples to have high catalase activity. In another study *Teucrium polium* [45], whilst rush crimp [46], raspberries [47] and *Cassia fistula* seeds extracts [48] have been reported to possess higher level of phenolic and flavonoid contents. Padmaja *et al*., [49] evaluated enzymatic amd non-enzymatic antioxidant activities of *Adhatoda vasica* and *Sasbania grandiflora* (L.) and found both as a good source of enzymatic and non-enzymatic antioxidant activities. Matkowaski *et al*., [50] evaluated aerial parts of herb extracts from five medicinal plants from *Lamiaceae*, subfamily of *Lamioideae* including *Ballota nigra*, *Lamium maculatum*, *Leonurus cardiaca*, *Marrubium vulgare*, and *Galeopsis tetrahit* for their antioxidant activities and polyphenols content and found to have remarkable activities.

CONCLUSION

In the present study *in vivo* and *in vitro* regenerated *Aloe vera* L. plant leaves were analyzed for their enzymatic and non-enzymatic antioxidant activities. The results showed that the both *in vivo* and *in vitro* regenerated *Aloe vera* L. plant leaves having adequate antioxidant activities but *in vitro* regenerated plant leaves were more significant in having antioxidant properties when compared to *in vivo* grown plant leaves. Therefore, the assessment of such properties remains an interesting and useful task, particularly for finding new sources for natural antioxidants.

ACKNOWLEDGEMENT

Authors are highly thankful to Dr. B. R. Singh, Principal Scientist, IVRI, Izzatnagar Bareilly (U.P.) India, for his kind co-operation and skilful guidance during the whole work.

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