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CHARACTERISATION OF ANTIOXIDANT PROPERTY OF ROOT EXTRACT OF *SPHAGNETICOLA TRILOBATA* IN RECOVERY OF OXIDATIVE STRESS

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ABSTRACT

Excessive production of free radicals during metabolic processes, disrupt the antioxidant defense mechanisms of the body. This leads to oxidative stress, which is associated with the molecular damage of DNA, and increased lipid peroxidation of bio-membranes. The so-caused oxidative damage give rise to the acute conditions of pathogenecity. Such harmful conditions are prevented by the neutralizing agents, the antioxidants, that constitute the antioxidant defense system. The naturally occurring antioxidants occur in many of the medicinal plants. They are capable of scavenging free radicals, thereby inhibiting lipid peroxidation and reducing oxidative stress. One of such antioxidant rich plants known is *Sphagneticola trilobata.* The present study deals with the characterization of antioxidant property of the root extract of *Sphagneticola trilobata,* prepared in Methanol and Chloroform separately, in recovery of H_2O_2 induced oxidative stress in lymphocytes of *Oryctolagus cuniculus* L. The oxidative stress increase lipid peroxidation in terms of MDA (Malondialdehyde), and alter adversely the activity of glutathione system and antioxidant enzymes, *viz.,* GSH (Reduced Glutathione), GPx (Glutathione peroxidase), SOD (Superoxide dismutase) and CAT (Catalase) as well. The present work indicated that the antioxidant effectivity is restored with the pretreatment of Methanol root extract (MDA – 1.97 ± 0.03; GSH – 4.83 ± 0.04; SOD – 2.64 ± 0.09 ; CAT – 4.07 ± 0.04 ; GPx – 8.19 ± 0.05), and Chloroform root extract (MDA – 0.98 ± 0.04 ; GSH – 4.99 ± 0.06 ; SOD – 2.99 ± 0.03 ; CAT – 4.79 ± 0.07 ; GPx – 8.45 ± 0.15). However, the Chloroformic root extract was found more effective with antioxidant property than the Methanolic root extract.

KEYWORDS: Free radicals, Oxidative stress, Redox stress, Lipid peroxidation, Antioxidant enzymes, Scavengers.

The inhaled oxygen, an extreme essential element for life, often exhibits toxic effect by becoming part of potentially damaging molecules called free radicals (Mohammed *et al.,* 2004). These are highly reactive compounds produced continuously during cellular oxidation and metabolic reactions in the body (Ebadi *et al.,* 2001). These free radicals cause harmful effects by increasing lipid peroxidation in bio-membranes, and also adversely alter lipid, protein and DNA constitution of cell (Mc Cord *et al.,* 2000 ; Ridnour *et al.,* 2005). The so caused oxidative damages of the cells are efficiently taken care of by the highly powerful antioxidant systems. Endogenous antioxidant defenses include a network of enzymic and non-enzymic antioxidants, that are capable of deactivating free radicals (Vertuani *et al.,* 2004). The excessive and continuous generation free radicals gradually decrease the activity of antioxidants. When the balance between free radical production and antioxidant defences is lost, 'oxidative stress' results. It deregulates the cellular functions, which is responsible for the development of many ailments and early ageing (Irshad *et al.,*2002 ; Rao *et al.,* 2006). The state of oxidative stress is overcome by the intake of antioxidants from the natural sources, such as plants. This fact correlates to the use of plants in the olden times as the major component of the traditional and 'Ayurvedic' medicinal system (Scartezzinic *et al.,* 2000; Rekha *et al.,*2001).

There are a variety of plants existing around us, commonly and wildly growing, which are being constantly explored for their antioxidant and medicinal values. One of the such plants spreading rapidly in certain areas or fields of Durg, Chhattisgarh, is *Sphagneticola trilobata.* The plant is known for being used in medicine. The crushed leaves are used as poultice in tea to reduce the symptoms of cold and flu. The various extracts are used for the effective treatment of hepatitis, cirrhosis, reproduction disorders and inflammation (Xuesong *et al.,* 2006).

The present work emphasizes on the antioxidant characterization of root extract of *Sphagneticola trilobata,* prepared in methanol and chloroform solvents separately, efficient enough in recovery of H_2O_2 induced oxidative stress in the lymphocytes of *Oryctolagus cuniculus*. L.

MATERIALS AND METHODS

Plant Extract

Some plants of *Sphagneticola trilobata* were collected from the open grounds or fields of the twin city, i.e., Durg and Bhilai cities of Chhattisgarh, India. The roots were separated and washed in70% alcohol and shade dried. The root extract was prepared in 59% alcohol by Soxhlet Extraction apparatus for the use of present investigation. The solvents used for the preparation of the root extract were Methanol and Chloroform separately.

In vitro study

Blood sample was collected from *Oryctolagus cuniculus,* and stored in heparinized sterilized tube. Lymphocytes were then isolated through centrifugation and washed in phosphate buffer saline. The lymphocyte culture was prepared by using DMEM medium alongwith 10% fetal serum, and maintained in a

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humidified CO_2 incubator at 37˚C temperature and 5% CO_2 for 18 hours. After incubation, the lymphocytes / cells were exposed to oxidative stress with 100µM H_2O_2 for 2 hours (Sohi *et al.,* 2003).

Experiment Design

The experimental design constituted five groups of cultured lymphocytes, for the analysis of each of the antioxidant enzymes considered for the investigation. Sampling was done in replicates of five.

Group I	Only lymphocytes (Control)
Group II	Lymphocytes with 100µM H_2O_2 for 2 hours
Group III	H_2O_2 treated lymphocytes +pretreated with 5µL/10,000 cells of *Sphagneticola trilobata* root extract
Group IV	H_2O_2 treated lymphocytes + pretreated with 10µL/10,000 cells of *Sphagneticola trilobata* root extract
Group V	H_2O_2 treated lymphocytes + pretreated with20µL/ 10,000 cells of *Sphagneticola trilobata* root extract

The cells were collected, washed in icecold phosphate buffer and used for biochemical assay.

Enzymatic Parameters

The enzymatic parameters considered to evaluate the effect of root extract (Methanolic / Chloroformic) on oxidative stressed cultured lymphocytes were:

MDA (Malondialdehyde) - Okhawa *et al.,* 1979

GSH (Reduced Glutathione) - Moron *et al.,* 1979

SOD (Superoxide Dismutase) - Misra *et al.,* 1972

CAT (Catalase) - Bergmeyer *et al.,* 1974

GPx (Glutathione peroxidase) - Rotruck *et al.,* 1973

Statistical Analysis

The collected data for all antioxidant enzymatic parameters were statistically validated by ANOVA.

RESULTS AND DISCUSSION

Oxidative stress was developed in H_2O_2 treated lymphocytes, as the hydroxyl radicals triggered lipid peroxidation. This resulted in increased levels of MDA (Group II – Table 1 & 2) as compared to the control values (Group I Table 1 & 2). The state of oxidative stress also resulted in much decreased radical-scavenging activity of Glutathione system, i.e. GSH and GPx (Group II – Table 1 & 2). The significant antioxidant enzymes, SOD and CAT, too showed reduced activity due to overwhelming free radicals (Group II – Table 1 & 2).

In the further process, the H_2O_2 induced lymphocytes were treated with Methanol and Chloroform root extracts of *Sphagneticola trilobata,* in separate sets of experiments for about 18 hours. With the pretreatment of root extract in gradual increasing doses, i.e., 5µl, 10 µl and 20 µl / 10,000 cells, the recovery from oxidative stress was observed. The increased MDA levels were reduced with the extract treatment, indicating the inhibition of lipid peroxidation ($P<0.5$). Also, the activity of GSH and GPx, as well as that of antioxidant enzymes was enhanced with the extract treatment (Group III, IV & V – Table 1 & 2). Almost similar results were obtained with both Methanolic and Chloroformic extract pretreatments. However, root extract prepared in Chloroform exhibited more effectively in recovery from oxidative stress as compared to Methanol root extract of *Sphagneticola trilobata*.

The increased MDA levels indicated the activation of lipid peroxidation in H_2O_2 treated lypmphocytes. During the process, the free radicals generated, attack the fatty acid components of membrane lipid leading to membrane rigidity and receptor realignment (Nowak *et al.,* 2003).

The medicinal plant, *Sphagneticola trilobata,* considered for the present study is well known for its antimicrobial, anti-inflammatory and anti-cancerous activities. Limited literature about the plants antioxidant activity is available. Bhargava *et al.* in (1974) reported the pharmacological and antioxidant properties of the plant, extracts of *Sphagneticola trilobata.* This attributes to the presence of natural products in the form of flavonoids, terpenoids and steroids. The studies of Subramonium *et al.,* (1999) inferred the use of the plant in the treatment of liver disorders, due to its efficient antioxidant property owing to the presence of iso-flavonoids. The abundant presence of terpenoids and tannins in all extracts of *Sphagneticola trilobata* was reported to be responsible for its antioxidant and other potent bioactivities (Singh *et al.,* 2003 ; Kaur *et al.,* 2009).

Plant extracts prepared in different solvents have been known to exhibit variety of biological activities. The Ethanol extract of *Sphagneticola* is known for analgesic activity, and the Methanol extract for anti-bacterial activity (Lans *et al.,* 2006 ; Chetan *et al.,* 2012).

The most significant work about *Sphagneticola trilobata* and its various activities was carried out by Govindappa *et al.,* 2011. They reported the antioxidant property of the Ethanol extract of leaf, stem and flower of the plant. The inhibition of protein denaturation was

brought about by the Ethanol leaf extract (87.14%), stem extract (86.76%) and flower extract (61.63%). Water extracts of fresh parts of stem and flower exhibited high scavenging activity than that of dry extracts of *Sphagneticola trilobata,* thereby serving as free radical inhibitors, possibly acting as primary antioxidants (Govindappa *et al.,* 2011). High antioxidant activity was reported from the Methanol extract of leaf (Kataki *et al.,* 2012).

In the present study, Methanol and Chloroform root extracts were evaluated for their antioxidant characterization, effective in recovery of H_2O_2 induced oxidative stress. The antioxidant properties of the plant extracts of *Sphagneticola trilobata* have been reported, but the assay methods mostly used for evaluation are DPPH and Superoxide assays. But the fact is that glutathione system and the antioxidant enzymes, *viz.,* MDA, GSH, SOD, CAT and GPx are well known for free radical scavenging and antioxidant activities. So, in the present work, the evaluation of antioxidant property of root of *Sphagneticola trilobata* was accomplished by enzymatic assay in, *in vitro* cultured and H_2O_2 induced lymphocytes, which is a unique feature. It was concluded that the Methanol and Chloroform extracts of root showed high antioxidant activity. However, the Chloroform root extract of *Sphagneticola trilobata* exhibited better efficacy in reducing oxidative stress, in comparison to Methanol root extract.

Table 1: Effect of Methanolic Root Extract of *Sphagneticola trilobata* on the antioxidant activity of different enzymes

Enzymatic Parameters	Grp. I (Control)	Grp II (H_2O_2 treated)	Grp III (5µL MSRE + H_2O_2)	Grp IV (10µL MSRE + H_2O_2)	Grp V (20µL MSRE+H_2O_2)
Malondialdehyde (MDA) [Mole MDA / mg protein]	0.87 ± 0.02	3.96 ± 0.04*	3.19 ± 0.04#	2.65 ± 0.05#	1.97 ± 0.03#
Red. Glutathione (GSH) [µ moles / mg protein]	6.16 ± 0.02	2.52 ± 0.04*	3.21 ± 0.06#	4.09 ± 0.04#	4.83 ± 0.04#
Sup. Dismutase (SOD) [Units / mg protein]	3.70 ± 0.03	1.60 ± 0.03*	1.96 ± 0.04#	2.31 ± 0.04#	2.64 ± 0.09#
Catalase (CAT) [µ moles H_2O_2 / mg protein]	5.90 ± 0.04	2.74 ± 0.04*	3.62 ± 0.08#	3.84 ± 0.04#	4.07 ± 0.04#
Glutathione Peroxidase (GPx) [µg utilized / mg protein]	10.96 ± 0.04	5.70 ± 0.03*	6.08 ± 0.04#	7.36 ± 0.06#	8.19 ± 0.05#

MSRE – Methanolic *Sphagneticola* Root Extracts
* - Compared with control
# - Compared with H_2O_2

Table 2: Effect of Chloroformic Root Extract of *Sphagneticola trilobata* on the antioxidant activity of different enzymes

Enzymatic Parameters	Grp. I (Control)	Grp II (H_2O_2 treated)	Grp III (5µL CSRE + H_2O_2)	Grp IV (10µL CSRE + H_2O_2)	Grp V (20µL CSRE + H_2O_2)
Malondialdehyde (MDA) [Mole MDA / mg protein]	0.87 ± 0.02	3.57 ± 0.07*	2.96 ± 0.04#	2.56 ± 0.07#	0.98 ± 0.04#
Red. Glutathione (GSH) [µ moles / mg protein]	5.80 ± 0.04	2.47 ± 0.04*	3.04 ± 0.02#	3.41 ± 0.05#	4.99 ± 0.06#
Sup. Dismutase (SOD) [Units / mg protein]	3.49 ± 0.04	1.54 ± 0.03*	1.78 ± 0.04#	2.73 ± 0.05#	2.99 ± 0.03#
Catalase (CAT) [µ moles H_2O_2 / mg protein]	5.54 ± 0.08	2.62 ± 0.02*	2.77 ± 0.08#	3.76 ± 0.08#	4.79 ± 0.07#
Glutathione Peroxidase (GPx) [µg utilized / mg protein]	10.08 ± 0.06	5.29 ± 0.14*	5.88 ± 0.04#	7.27 ± 0.13#	8.45 ± 0.15#

CSRE – Chloroformic *Sphagneticola* Root Extracts
* - Compared with control
# - Compared with H_2O_2

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