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Enzymic Levels and Its Biological Significance of *Chlorophytum borivillianum*

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Chlorophytum borivillianum (safed musli), a medicinal plant is being known for its use from ancient age. The present study was designed to assess the activities of various enzymes viz., catalase, peroxidase, superoxide dismutase, polyphenol oxidase and glutathione-s-transferase. Results indicate that the tuber contained good levels of catalase (5.8 U), peroxidase (0.4 U), superoxide dismutase (6.1 U) which may be useful in the management of oxidative stress mediated ailments.

Key words: safed musli,Chlorophytum borivillianum, catalase, peroxidase, superoxide dismutase, polyphenol oxidase, glutathione-S-transferase

Phytotherapy is being recognised for healing qualities and gaining worldwide acceptance. A large number of people who are sceptical or apprehensive about conventional drugs have now turned to alternative treatments which makes use of medicinal plants. The World Health Organization (WHO) has estimated that over 80% of the populations in the developing countries depend on traditional medicines. The curative parts of a medicinal plant are not simply its woody stem or its leaves, but the number of chemical compounds (phytochemicals) it produces and uses for its own growth and development. *Chlorophytum borivillianum* (safed musli), a medicinal plant is being known for its use from ancient age. The Indian Pharmacopoeia (1966) has recognized safed musli as one of the 85 drug plants, whose ingredients are used in various pharmaceutical preparations. The National Medicinal Plants Board, set up by the Government of India has ranked safed musli as the 6th among the 28 selected priority medicinal plants, for cultivation and export (Purohit and Prajapathi, 2003). The present study was designed to assess the level of various enzymic components *viz.,* catalase, peroxidase, super oxide dismutase, polyphenol oxidase and glutathione-s-transferase of safed musli that may contribute to its medicinal value.

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Materials and Methods

Collection and processing

Tubers of *Chlorophytum borivillianum* were collected from the cultivators in Erode district. The tubers were washed thoroughly to remove the residual soil particles. The fingers were peeled using scalpel and were dried in shade for 2-3 days. The dried fingers were powdered and used for analysis.

Enzymes of Chlorophytum borivillianum

Assay of various enzymes like catalase, peroxidase, superoxide dismutase, polyphenol oxidase, and glutathione-S-transferase was performed in the tuber of *C.borivillianum*.

Catalase

The sample (tuber) was homogenized in a pre-chilled mortar and pestle with 0.1M phosphate buffer pH- 7.0 (assay buffer diluted 10 times) at 1 - 4ºC and centrifuged. The sediment was stirred with cold phosphate buffer, kept in ice with occasional shaking and then the extraction was repeated once or twice. The combined super natants were used for the assay. The activity of this enzyme in the tuber was assayed by the method of Luck (1974) following the UV light absorption of hydrogen peroxide solution at 240 nm. On decomposition of hydrogen peroxide by

catalase, the absorption decreased with time and the enzyme activity was calculated based on the decrease in absorbance. One unit of catalase is defined as the amount of enzyme that brings about a decrease in absorbance of 0.05 at 240nm.

Peroxidase

One part of the tuber was macerated with 5 parts (w/v) of 0.1M phosphate buffer (pH 6.5) in a pre-chilled mortar and pestle. The homogenate was centrifuged at 300g for 15 minutes. The supernatant was used as the enzyme source. All procedures were carried out at 0 - 5º C. Peroxidase was assayed by the method of Reddy *et al*. (1995). The oxidation of pyrogallol or dianisidine to a coloured product called purpurogallin was measured colorimetrically at 400nm and the enzyme activity calculated based on the increase in absorbance. The enzyme activity was expressed as Units/ mg protein. One unit of peroxidase is defined as the amount of enzyme that brings about an increase in absorbance of 0.05 at 400 nm.

Superoxide dismutase

One part of the tuber was macerated with 5 parts (w/v) of 0.1M phosphate buffer (pH 6.5) in a pre-chilled mortar and pestle. The homogenate was centrifuged at 300g for 15 minutes. The supernatant was used as the enzyme source. All procedures were carried out at 0-5º C. Superoxide dismutase was assayed by the method of Misra and Fridovich (1972) by measuring the extent of inhibition of NBT (Nitro Blue Tetrazolium) reduction, spectrophoto metrically at 600nm and enzyme activity calculated. One unit is defined as the amount of SOD that cause 50% reduction in the extent of NBT oxidation.

Phenol oxidases

5g of the tuber was extracted in a medium containing 50mM TrisHCl (pH 7.2), 0.4M sorbitol and 10mM NaCl and made up to 20 ml. The homogenate was centrifuged at 2000g for 10 minutes and the supernatant was used for the assay. Extraction was performed under ice-cold conditions. The polyphenol oxidase comprised of catechol oxidase and laccase. The activities of these enzymes was assayed by the method of Esterbauer *et al*, (1977), following the oxidation of the substrate catechol to catechin, colori metrically at 495 nm for every 30 sec for 5 min. Enzyme activity was calculated based on the increase in absorbance. One Unit of either catechol oxidase or laccase is defined as the amount of enzyme that transforms 1µM of dihydrophenol to 1µM of quinone/ min under the assay conditions

Glutathione-S-transferase

5g of the tuber was extracted in a medium containing 50mM TrisHCl (pH 7.2), 0.4M sorbitol and 10 mM NaCl and made up to 20 ml. The homogenate was centrifuged at 2000g for 10 minutes and the supernatant was used for the assay. Extraction was performed under ice-cold conditions. Glutathione-S-transferase activity was determined by measuring the absorbance at 350 nm as per the method of Beutler (1984). The activity in the extract was expressed as µmoles of CDNB-GSH conjugate/min/mg protein.

Results and Discussion

Activities of various enzymes viz., catalase, peroxidase, superoxide dismutase, polyphenols oxidase and glutathione-S-transferase are presented in Table 1.

The activities of catalase, peroxidase and superoxide dismutase are higher than glutathione-S-transferase and polyphenol oxidase in the tuber of *Chlorophytum borivillianum*. Catalase is an enzyme, which is present in most cells and catalyses the decomposition of hydrogen peroxide to water and oxygen. Catalase is a heme - containing protein and is found to act 10^4 times faster than peroxidase.

Superoxide dismutases are all naturally occurring metalloenzymes that convert superoxide ion to H_2O_2. SOD is the most important enzyme because it is found virtually in all aerobic organisms. There are four types of SOD: Cu-SOD, Cu-Zn-SOD, Mn-SOD and Fe-SOD. The transition metal of the enzyme reacts with superoxide ion taking its electron. The function of SOD is primarily to eliminate rapidly the biologically generated free radical species, the superoxide anion (Mahesh *et al.,* 2003). SOD is

Table 1. Activities of various enzymes in *Chlorophytum borivillianum*

Enzyme	Enzyme activity μ/mg protein
Catalase•	5.8
Peroxidase•	0.4
Superoxide dismutase ❍	6.1
Glutathione -s-transferase ★	0.7
Polyphenol oxidase c	
(i) Catechol oxidase	0.3
(ii) Laccase	0.2

• Amount of enzyme that brings about a decrease in absorbance of 0.05 at 240nm.
c Amount of enzyme that brings about an increase in absorbance of 0.05 at 400nm
❍ Amount of SOD that cause 50% reduction in the extent of NBT oxidation.
★ μ mole of CDNB – GSH conjugate / min/ mg protein
c Amount of enzyme which transforms 1μ mole of dihydro phenol to 1 μ mole of quinone /min.

reported to inhibit hydroxyl radical production. SOD can act as an anti-carcinogen and inhibitor at initiation and promotion / transformation stages in carcinogenesis (Yamukara, 1998).

Catalase, peroxidase and superoxide dismutase are well known antioxidant enzymes that act as endogenous defence for scavenging free radicals. Free radicals have been implicated in the causation of ailments such as liver cirrhosis, atherosclerosis, cancer, diabetes and other diseases. Antioxidants that scavenge free radicals have great potential in ameliorating these disease processes. Reactive Oxygen Species (ROS) have been known to cause tissue injury through covalent binding and lipid peroxidation. Antioxidants have an important role in inhibiting the reactions induced during the damage of cells (Naik, 2003).

Degenerative diseases like atherosclerosis, carcinogenesis and cataractogenesis associated with diabetes mellitus have been found to correlate with decreased levels of endogenous antioxidant system (Kamble *et al.,* 1998). The tuber of *C. borivillianum* is found to be a rich source of enzymic antioxidants, and it would be useful in the treatment of various patho physiologies like diabetes mellitus associated with free radical damage. Antioxidants have an important role in inhibiting the reactions induced during the damage of cells (Naik, 2003).

Phenol oxidases catalyse the aerobic oxidation of certain phenolic substrates to quinones, which are autooxidized to dark brown pigments generally known as melanins. The polyphenol oxidase comprises of catechol oxidase and laccase. The activities of these enzymes are important with regard to appearance; palatability and use of plant products Low levels of phenol oxidases improve the appearance and palatability of the tubers of safed musli. Glutathione-S-transferase helps in the xenobiotic metabolism.

The present study reveals that good level of enzymic-antioxidants *viz.,* catalase, peroxidase and superoxide dismutase which may attribute to the antioxidant potential of *Chlorophytum borivillianum* (safed musli).

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