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Antioxidant Properties of *Chlorophytum borivillianum*

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Safed musli (*Chlorophytum borivillianum*) is an important ayurvedic medicinal plant. The tubers are said to possess several medicinal values. Hence in an attempt to assess the antioxidant properties of safed musli, the present study was designed to study the radical scavenging antioxidants viz., ascorbic acid, alpha-tocopherol, phenols, tannins, flavonoids and reduced glutathione. Results indicate that the tuber contained good level of radical scavenging antioxidants. The tuber apart from being a rich source of carbohydrate and protein is also a good source of radical scavenging antioxidants, which may be useful in the management of free radical mediated ailments.

Key words: *Chlorophytum borivillianum* (safed musli), antioxidants, phenols, tannins, flavonoids, ascorbic acid, reduced glutathione

World is endowed with a rich wealth of medicinal plants. Herbs have always been the principal form of medicine in India. The herbal medicines are being used by about 80% of the world population for primary health care, particularly in the developing countries. These drugs are popular for its safety and efficacy and are used in the treatment of diseases that have long defied synthetic drugs (Lakshmanan, 2003). Scientific studies on the medicinal plants are likely to provide invaluable drugs (Babu *et al*., 2002).

Chlorophytum borivillianum (safed musli), a medicinal plant is being known for its use from ancient age Safed musli has been associated with “CAITHA” in the ATHARVAVEDA as one of the divine herbs, offering cure for many ailments and health related problems. It is a rich source of over 25 alkaloids, vitamins, minerals, proteins, carbohydrates, steroid saponins, and polysaccharides (Mittal and Ajay, 2001). No scientific examination has been so far done to study the antioxidant effect of the tuber of *C.borivillianum.* Hence the study was designed to assess the level of various radical scavenging antioxidants like ascorbic acid, a-tocopherol, polyphenols, flavonoids, tannins and reduced glutathione.

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Materials and Methods

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. The radical scavenging antioxidants like ascorbicacid, α-tocopherol, polyphenols, flavonoids, tannins and reduced glutathione were analyzed in the tuber.

Ascorbic acid

One gram of the sample (tuber) was homogenized in 4% TCA, made up to 10 ml and centrifuged at 2000 rpm for 10 minutes. The supernatants obtained were treated with a pinch of activated charcoal, shaken well and kept for 10 minutes. The charcoal residue was removed by centrifugation. The supernatant served as the sample for analysis. Ascorbic acid in the sample was determined by the method of Roe and Kuether (1953) and the content was calculated from the standard curve for ascorbic acid.

α-tocopherol (vitamin E)

Accurately 2.5g of the homogenized tissue was weighed into a conical flask. 50ml of 0.1 N H_2SO_4 was added slowly without shaking, stoppered and allowed to stand over night .The next day the contents of the flask were shaken vigorously and filtered through Whatman No:1 filter paper discarding the initial 10-15 ml of the filtrate. Aliquots of the filtrate were used for the estimation of vitamin E by the method of Rosenberg (1992) and the content of vitamin E was arrived from the standard graph for tocopherol.

Polyphenols

One gram of the sample (tuber) was homogenized using 20 ml of 80% ethanol. The homogenate was centrifuged at 10,000 rpm, for 20 minutes. The supernatant was saved and the residue was re-extracted with 10ml of 80% ethanol, centrifuged and the supernatant was collected. The combined supernatants were evaporated to dryness and the residue was dissolved in a known volume of distilled water (5.0ml) Polyphenols in the sample was estimated by the method of Malick and Singh (1980). From the standard graph for catechol, the amount of polyphenols present in the tuber was calculated and expressed as mg of polyphenols per g of the sample.

Flavonoids

The extraction was carried out in two steps, firstly with 9:1 of methanol: water mixture and secondly with 1:1 of methanol: water. Sufficient solvent was added and the mixture was left for 6-12 hrs. The sample was filtered, the extracts combined and evaporated until most of the methanol was removed. The resultant aqueous extract was extracted (in a separating funnel) with hexane or chloroform. It was repeated several times and the extracts were combined. The solvent-extracted aqueous layer was concentrated and the amount of flavonoids was estimated by the method of Cameron *et al*. (1943).

Tannins

To one g of the sample 50ml of water was added, boiled for 30 min and centrifuged. The supernatant was collected and made up to 100.0 ml with distilled water. The tannin content in the sample was estimated by the method of Schanderl (1970) and the amount of tannin was calculated from the standard curve for tannic acid.

Reduced glutathione

One gram of the tuber was homogenized in 5% TCA and centrifuged at 1000 rpm for 10 minutes; the homogenate was cooled and estimated for reduced glutathione by the method of Moron *et al*. (1979).

Results and Discussion

Radical scavenging antioxidants

The level of various radical scavenging antioxidants in the tuber of *C.borivillianum* is presented in Table 1.

Table 1. Radical scavenging antioxidants in *Chlorophytum borivillianum* tuber

Radical scavenging antioxidants	Tuber (mg/100g)*
Ascorbic acid	43.5
Vitamin E	47.9
Polyphenols	357.6
Flavonoids	20.3
Tannins	162.4
Reduced glutathione	192.6

*mean of triplicates

It is evident from Table 1 that the tuber of *C.borivillianum* is a good source of radical scavenging antioxidants like vitamin E, polyphenols, tannins and reduced glutathione.

Ascorbic acid

Ascorbic acid content in the tuber was found to be 43.5mg / 100g. Ascorbic acid (vitamin C) is an important water-soluble antioxidant in biological fluids and an essential micronutrient required for normal metabolic functioning of the body. It is known to be a potential antioxidant essential for the functioning of the central nervous system (Naik, 2003). Plasma devoid of vitamin C, but no other endogenous antioxidant, is extremely vulnerable to oxidant stress and

susceptible to peroxidative damage to lipids. Vitamin C readily scavenges reactive oxygen metabolites $ONOO^-$, NO_2, NO and hypochlorous acid. Vitamin C reduces oxidative DNA damage and genetic mutations. It can also protect lipid and lipoprotein against oxidative damage. Vitamin C can act as a co-antioxidant by regenerating α-tocopherol from the α-tocopheroxyl radical produced during scavenging of reactive oxygen metabolites. It has also been shown to regenerate urate, glutathione and β-carotene from their respective one-electron oxidation product (Ray and Hussain, 2002).

Vitamin E

The vitamin E content of the tuber was found to be 47.9 mg / 100g. Vitamin E is a lipid soluble vitamin, occurring in plasma as a variety of tocopherols, of which the α and γ isomers are usually the major ones. Vitamin E has been considered as an antioxidant and free radical scavenger in the suppression of cell membrane unsaturated lipid peroxidation (Mallika *et al*., 2000). The antioxidant property of vitamin E neutralizes reactive oxygen metabolites, reduces oxidative DNA damage and genetic mutations. Vitamin E is thought to be an important chain-breaking antioxidant, which plays an important role in carcinogenesis through its contribution to immuno competence, membrane and DNA repair and decreasing oxidative DNA damage. Vitamin E can directly act with a variety of oxyradicals, including the peroxy radical ($ROO^\bullet$), $CCl_3^\bullet$, OH, $O^\bullet$ and singlet oxygen. The lipophilic antioxidant, α-tocopherol has been shown to protect LDL from oxidation. It regenerates ascorbic acid from its oxidized form dehydroascorbic acid and acts synergistically with ascorbic acid to prevent lipid peroxidation (Serafini *et al.,* 2002). Antioxidant plant nutrients like, vitamin C and vitamin E have been suggested to exert a protective role against degenerative diseases and gastric cancer (Mallika *et al*., 2000).

Glutathione (GSH)

GSH is an important antioxidant that limits oxidative damage caused by Reactive Oxygen Species (ROS), many of which are generated as a consequence of normal metabolic activity. It acts as both a nucleophilic scavenger of toxic compounds and as a substrate in the glutathione-peroxidase mediated destruction of hydroperoxides (Parihar and Taruna, 2003). It is a major non-protein thiol in living organisms, which plays a central role in coordinating the body's antioxidant defense processes. Perturbation of GSH status of a biological system may thereby allow H_2O_2 to accumulate to toxic levels and this can lead to serious consequences (Venukumar and Latha, 2002). The thiols are important compounds, which are required to keep up the cellular levels of active forms of other antioxidants, vitamin C and vitamin E (Anuradha and Ravikumar, 2001). The tubers of musli were found to be a good source of reduced glutathione (192.6 mg / 100 g).

Polyphenols, flavonoids and tannin

The tubers of musli is a good source for polyphenol, flavonoids and tannin (357.6 mg / 100g, 20.3 mg / 100g and 162.4 mg / 100g) compounds. Polyphenols possess a wide range of biological properties including free radical scavenging property. Dugas *et al*. (2000) have shown that dietary intake of flavonoids, containing foods potentially lower the risk of certain free radical related pathophysiology. These are a major group of antioxidative compounds, more powerful than vitamin E, and act to regenerate vitamin E after it becomes oxidized. They offer protection against LDL oxidation and inhibition of platelet aggregation. Apart from their antiatherogenetic properties, these non-essential dietary components appear to elicit promising anti-carcinogenic effects (Della Regione *et al.,* 2000). The anticarcinogenic activity of phenols may be due not only to their antioxidant properties but also to their ability to reduce the bioavailability of food carcinogens and to inhibit their metabolic activation. A wide array of phenolic substances present in dietary and medicinal plants have been reported to possess powerful antimutagenic activity apart from the antioxidant property (Patil *et al.*, 2003). Recently, increasing evidences support the hypothesis that the phenolic compounds could play an essential health promoting role. Hayashi *et al.* (2001) have proved

that certain tannins (ellagitannins from *Lagerstroemia speciosa*) stimulate glucose uptake. They exhibit insulin like activity acting as glucose transport activators of fat cells. Flavonoids are a group of phenolic antioxidants found in vegetables, fruits and beverages. Flavonoids have been shown to inhibit oxidative modification and the cytotoxicity of LDL and also due to their amphipathic nature, flavonoids may act within the LDL particle, in a manner similar to vitamin E, or may act comparable with ascorbic acid in the extra particle environment of LDL. Flavonoids, due to their lower redox potential have been shown to suppress superoxide anion, hydroxyl radicals and lipid peroxyl radical (Safari *et al*., 2003). Increased intake of flavonoids might reduce the risk of cardiovascular diseases, cancer and many chronic degenerative diseases (Haxley and Neul, 2003; Shetgiri and Mello, 2003).

Natural antioxidants strengthen the endogenous antioxidant defences from ROS ravage and restore the optimal balance by neutralizing the reactive species. They are gaining immense importance by virtue of their critical role in disease prevention (Venukumar and Latha, 2002). The antioxidant properties of green tea extract, blueberry, spinach, strawberry, *Allium sativa* and *Glycyrriza glabra* have already proven beneficial in reducing oxidative tissue injury (Parihar and Taruna, 2003). The secondary metabolites like phenolics (phenylproponaoids, coumarines, flavonoids, tannins, proanthocyanidins), alkaloids, phytosterols, carotenoids and chlorophyll derivatives contribute to the antioxidant properties of many spices, medicinal and aromatic plants.

Numerous epidemiological studies has demonstrated that increased intake of natural antioxidants like vitamin A, C, E, flavonoids is very promising in reducing the level of free radicals and the overall severity of free radical mediated pathophysiologies like diabetic complications, cancer and other inflammatory diseases (Bechen, 1995).

The present study revealed that the tubers of safed musli are a rich source of radical scavenging antioxidants like phenols, tannins, flavonoids, vitamin E and reduced glutathione. Medicinal plants with good antioxidant potential are very promising for the treatment of diseases due to oxidative stress. The tubers of musli could be used to lower the risk of several patho physiologies associated with free radicals like diabetes mellitus, atherosclerosis, several degenerative diseases and cancer. The antioxidant property of *Chlorophutum borivillianum* (safed musli) may add to its enormous medicinal values.

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