Natural Product Sciences
11(3) : 165-169 (2005)

In vitro Antioxidant Activity of Ethanolic Extract of *Chlorophytum borivilianum*

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Abstract – *Chlorophytum borivilianum* Baker (Antharicaceae) commonly referred as 'Safed Musli' has been widely used in the Indian traditional systems of medicine to treat various diseases like rheumatism apart from having immunomodulating property and is used as general tonic. It is also known as 'Ayurvedic viagra' for its aphordisiac properties. *C. borivilianum* was screened for the first time to determine its antioxidant activity, isolation of the sapogenins and standardization of the isolated sapogenin fraction using HPTLC. Potent antioxidant activity of ethanolic extract was found by their ability to scavenge DPPH (84.51%), hydroxyl radical (48.95 %), ferryl bi-pyridyl complex (84.53%) along with the inhibition of lipid peroxidation (67.17%) at 100 μg/ml concentration. The ethanolic extract also exhibited significant inhibition of superoxide anion radical generated by photochemiluminescence. Thus, the potent antioxidant activity validates the innumerable therapeutic claims of the plant in the traditional system especially its use as a Rasayana drug.
Keywords – *Chlorophytum borivilianum*, antioxidant, fructan, sapogenin, Rasayana

Introduction

Ayurveda has a clinical specialty called 'Rasayana' therapy, which prevents diseases and counteracts the aging process by means of optimization of homeostasis. It has been reported that number of plants have been extensively used as 'Rasayana' drugs in Indian traditional systems of medicine (Ayurveda) for the management of neurodegenerative diseases, as rejuvenators, immunomodulators and nutritional supplements (Govindarajan *et al.*, 2005, Puri, 2003).

The tuber of 'Safed musli' which is botanically equated to *Chlorophytum* sp. (Liliaceae) are also considered as one of promising 'Rasayana' drug of Ayurveda and is described as 'Ayurvedic viagra' for its aphordisiac properties including the treatment of erectile dysfunction (Puri, 2003). It is also used as general tonic, in treating rheumatism apart from having immunomodulating property (Puri, 2003). As most of the immunomodulators possesses antioxidant potential, therefore, in the present investigation efforts have been made to determine the antioxidant activity of *C. borivilianum* Sant & Fern. which includes the 1,1-diphenyl picryl hydrazyl (DPPH), hydroxy radical scavenging activity, their capacity to reduce lipid peroxidation in rat liver tissue, chelation of ferrous ion, integral radical scavenging potential using chemiluminescence and its total antioxidant capacity simultaneously to validate the ethobotanical claims too. The isolation of the sapogenins and standardization of the isolated sapogenin fraction using HPTLC has also been part of the study.

Experimental

Plant material and extraction – Tubers of *C. borivilianum* were collected in and around Pune, Maharashtra, India during the month of August 2002, identified and authenticated by taxonomist Dr. R. L. S. Sikarwar. A voucher specimen was deposited in the departmental herbarium (Voucher specimen No. LWG-221271). One kg of the plant material was air dried at room temperature and powdered coarsely. The powdered material (250 g) was macerated with petroleum ether to remove the fatty substances; further it was extracted by cold percolation with 80% aqueous ethanol for 3 days and filtered. The extract was concentrated under reduced pressure and lyophilised (Labconco, USA) to get dry

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extract 80 g (CbEt).

Isolation and estimation of fructans – Qualitative phytochemical screening of various extracts of the plant material was carried out for qualitative determination of the groups of secondary metabolites present in them according to the methods in Harborne (1973), Peach and Trachey (1955). The polymeric sugar was extracted using method of Paech and Tracey (1955) with minor modification. 20 g of powdered drug previously extracted with ethanol (95%) was extracted with 50 ml of distilled water at 100 °C. Ethanol (3×40 ml) was added to the filtrate with intermittent stirring resulting in precipitation of polymeric sugars. The mixture was refrigerated overnight. The precipitate formed was collected, washed thrice with ethanol (95%) to ensure complete removal of free sugars. The precipitate was dried using acetone.

Quantitative analysis of fructan was performed by spectrophotometrically (Sadasivam and Manickam, 1999). Aliquots of standard fructose (2-20 μg/ml) were taken in 10 ml volumetric flasks. To each flask resorcinol reagent (1 ml) was added along with 7.0 ml of dilute HCl. Flasks were heated on a water bath at 80°C for a period of 10 minutes and subsequently cooled under running water. The standard curve was plotted by reading the color at 520 nm (Cintra GBC, DB-UV spectrophotmeter). Quantification of fructan from *C. borivilianum* was carried out by the following method, 500 mg of ethanol extracted drug powder was taken in a 100 ml volumetric flask along with 20 ml of water and heated on water bath for 10 min. The extract was collected and 70 ml of water was

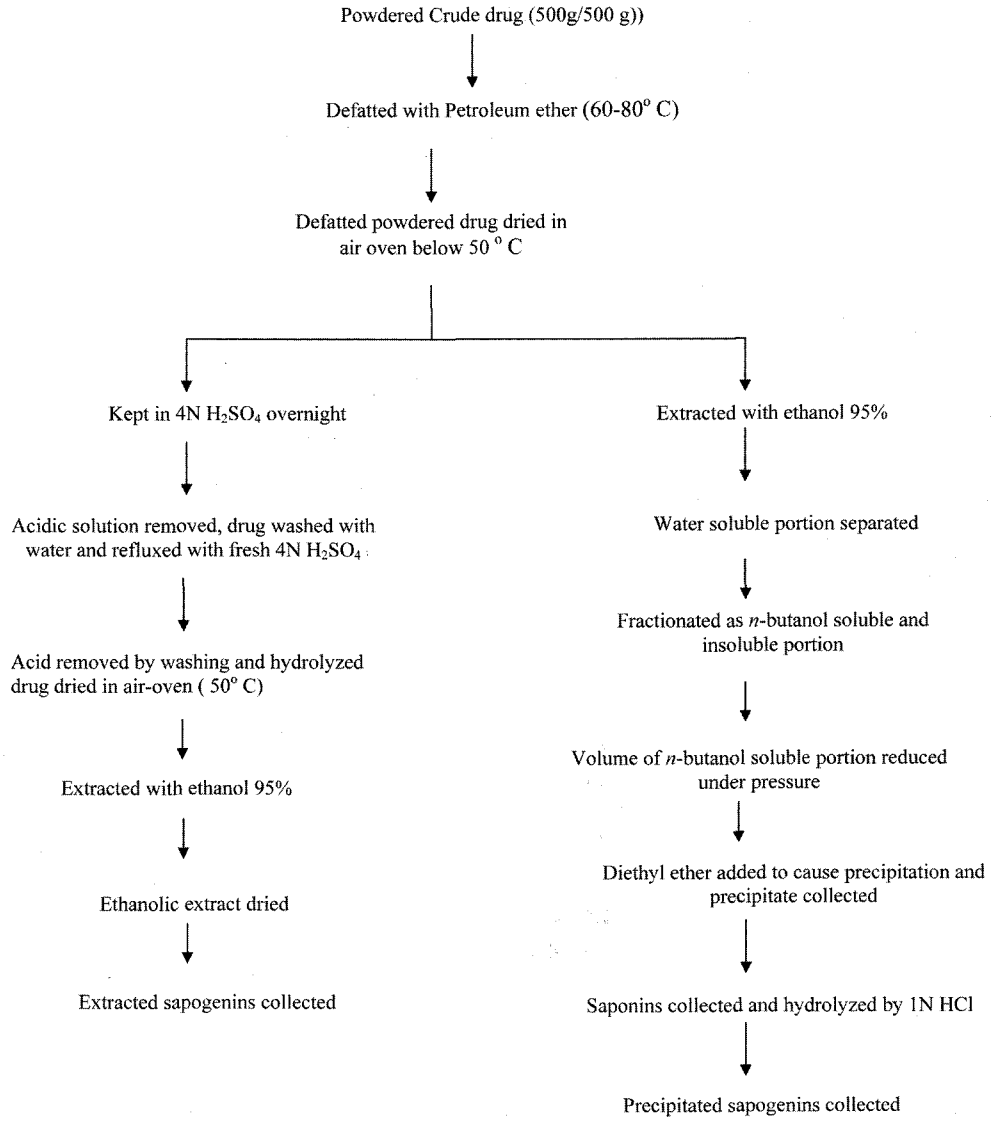

Fig. 1. Isolation scheme of sapogenin fraction

added to the mixture. The mixture was further heated for 30 min with occasional shaking. Subsequently the flask was cooled, and the extract was filtered. The volume of the flask was made up to 100 ml. One ml of extract was taken along with 1 ml distilled water, 1ml resorcinol reagent and 7 ml of dilute HCl. The color of the solution was read at 520 nm λ_{max} akin to the method for standard fructose solution against a standard blank.

Isolation and Standardization of Sapogenins – Five hundred grams of powdered drug was defatted with petroleum ether (60-80°C) in a Soxhlet apparatus. After removal of the solvent, marc was extracted with ethanol (95%) till complete extraction. The solvent free ethanolic extract was fractionated as water-soluble portion, which was further fractionated as *n*-butanol soluble and insoluble portions. The volume of *n*-butanol soluble portion was then reduced to half under reduced pressure. From the *n*-butanol soluble portion (14.4 gm) saponins were precipitated by addition of diethyl ether (100 ml). The collected precipitate was hydrolyzed using 10% HCl and then the precipitated sapogenins were collected (Fig. 1).

Saponin and sapogenins were characterized for their chemical nature by qualitative phytochemical screening (Peach and Tracy, 1955), co-TLC and HPTLC using scanning densitometric analysis (CAMAG III, Switzerland). Sapogenins obtained from fractionated ethanol extract as well as those obtained by ethanolic extraction of hydrolyzed drug powder along with standard β-sitosterol (Sigma-Aldrich) dissolved in petroleum ether were applied on TLC plate (Silica gel F_{254} Merck) with Camag linomat applicator IV and TLC plate was developed in previously saturated TLC chamber using Chloroform: Diethyl Ether (50 : 50 v/v) as mobile phase and was scanned densitometrically at 200 nm.

Antioxidant activity – Hydrogen donating activity was quantified in presence of stable DPPH radical on the basis of Blois method (1958). The degree of discoloration indicates the scavenging efficacy of the extract at 517 nm. The ability of extract to scavenge the OH radical was determined using ascorbic acid iron-EDTA model OH generating system. The decrease in formaldehyde formation due to scavenging or decreased formation of OH was assayed spectrophotometrically by the method of Nash (1953). Concentration of free iron ions (Fe^{2+}) was estimated using chelating agent 2,2-bipyridyl (Govindarajan *et al.*, 2003). Absorbance of ferrous-bipyridyl complex was measured at 525 nm against the blank devoid of ferrous sulphate. Ascorbic acid was used as positive control. Total antioxidant capacity was measured as per spectrophotometric method (Preito *et al.*, 1999). The total antioxidant capacity of the extract was calculated based on the formation of the phosphomolybdenum complex, which was measured. Ascorbic acid was used as the positive control and the total antioxidant capacity expressed as equivalents of ascorbic acid.

Male Sprague – Drawley rats (160-180 g) were purchased from the animal house of the Central Drug Research Institute, Lucknow, India. These were kept in the departmental animal house at 26 ± 2°C and relative humidity 44-55% light and dark cycles of 10 and 14 h. respectively for one week before the experiment. Animals were provided with rodent diet (Amruth, India) and water *ad libitum*. Randomly selected male rats were fasted overnight and were sacrificed by cervical dislocation, dissected and abdominal cavity was perfused with 0.9% saline. Whole liver was taken out and visible clots were removed and weighed amount of liver was processed to get 10% homogenate in cold phosphate buffered saline, pH 7.4 using glass teflon homogeniser and filtered to get a clear homogenate. The degree of lipid peroxidation of ethanol soluble extractive of *C. borivilianum* was assayed by estimating the thiobarbituric acid-reactive substances (TBARS) by using the standard method (Okhawa *et al.*, 1979) with minor modifications (Tripathi and Sharma, 1998). The values of TBARS were calculated from a standard curve (absorption against concentration of Tetraethoxy propane) and expressed as nmoles/mg of protein. The percentage inhibition of lipid peroxidation was calculated by comparing the results of the test with those of controls not treated with the extracts. Tocopherol was used as positive control.

For the determination of the integral antioxidative capacity (AC) of the water soluble substances in CbEt, method of photochemiluminscence (Govindarajan *et al.*, 2004) was used. A standard plot of ascorbic acid was plotted and the results were calculated in ascorbic acid equivalents (μmol ascorbic acid/g equivalent).

Statistical analysis – Data were subjected to statistical analysis using SPSS 11.0 for Windows. The values are represented as mean ± SEM for six experiments. Paired t-test was used for reporting the p value and significance with respect to the control group.

Results and Discussion

The qualitative phytochemical analysis indicates that saponin, steroids, alkaloids, phenolics, triterpenoids and more polar compounds like sugars, proteins, gallo-tannins along with mucilage were present in *C. borivilianum*. The presence of steroids, triterpenoids was observed in hexane

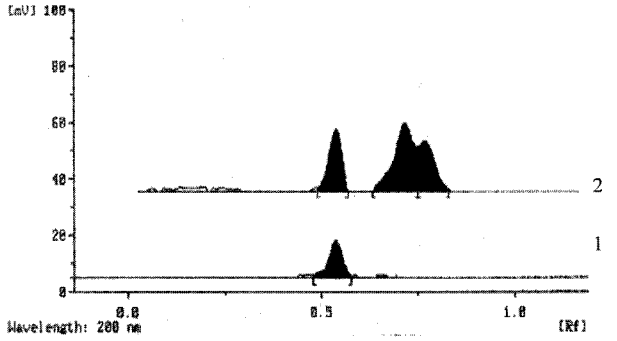

Fig. 2. Standardization of the sapogenins of *C. borivillianum* with β-sitosterol as standard. Track 1: β-sitosterol, Track 2: Isolated sapogenins. Mobile phase: Chloroform: diethyl ether (50 : 50)

soluble extractive while chloroform extractive contained alkaloids and saponins, sugars, proteins, gallotannins and mucilage were present in ethanol and water soluble extractives.

Chemical test and chromatographic analysis of the sapogenin extract obtained by hydrolysis of precipitated saponins as well as from the hydrolyzed powdered drug showed the presence of β-sitosterol. The co-TLC profile of sapogenin samples (80 μg/ml) and β-sitosterol (20 μg/ml) confirmed the presence of β-sitosterol which was identified as blue spot at R_f 0.5. The densitometric scanning data suggests that the β-sitosterol content in *C. borivilianum* is high (3.66 %) (Fig. 2). The presence of β-sitosterol was utilized as a standard marker for the steroidal saponins of *C. borivilianum*. Since there has been no previous report on the presence of fructan in the plant as a source of storage carbohydrate, confirmation of fructan and its quantification performed in the present study envisages towards the possibility of using inulin type fructan as a new parameter for the quality evaluation of *C. borivilianum*. The total fructan concentration in the plant was 14.25%.

The ethanolic extract scavenged the DPPH radical in a dose-dependent manner with maximum activity of 84.51% at 100 μg/ml (Table 1). The extract exhibited potent hydroxyl radical scavenging activity (Table 1). The hydroxyl radical is highly reactive and can damage biological molecules, when it reacts with polyunsaturated fatty acid moieties of cell membrane phospholipids producing lipid hydroperoxides (Miller *et al.*, 1992). Potent inhibition of DPPH, hydroxyl and ferryl-bipyridyl complex shows that CbEt is a potent radical scavenger (Table 1).

Some simple sugars have also been reported to possess free radical scavenging activity via the Fenton-Dependent damage (Morelli *et al.*, 2003). Since the plant has been found to contain high quantities of the simple sugars like, sucrose, glucose, fructose, galactose, mannose and xylose, they may also be responsible for the inhibition of the ferryl-bipyridyl complex.

CbEt inhibited the lipid peroxidation in rat liver homogenate in a dose-dependent manner as exhibited by the decrease in the TBARS level as compared to the control (Table 2). Initiation of lipid peroxidation by $FeSO_4$ takes place either through ferryl perferryl complex or through OH radical by Fenton's reaction (Tripathi and Sharma, 1998). Since Rasayana drugs are reported to be good antioxidants (Govindarajan *et al.*, 2005), the inhibition

Table 2. Effect of *C. borivilianum* on ferrous sulphate induced lipid peroxidation in rat liver tissue

Concentration (μg/ml)	TBARS (nmoles/mg protein)	% inhibition of lipid peroxidation
20	1.81 ± 0.27	30.92
40	1.72 ± 0.41[a]	34.35
60	1.48 ± 0.61[b]	43.51
80	1.15 ± 0.10[b]	56.10
100	0.86 ± 0.09[c]	67.17
Control	2.62 ± 0.41	–
Tocopherol (10)	0.006 ± 0.001[c]	99.77

The values represent the means ± S. E. M for six rats per group [a]P < 0.05, [b]P < 0.01 and [c]P < 0.001 compared to respective control group Tocopherol was used as positive control

Table 1. Antioxidant potential of *C. borivilianum* at various concentrations

Concentration (μg/ml)	Percentage inhibition		
	DPPH	Hydroxyl radical	Ferryl bipyridyl complex
20	40.12 ± 0.96	–	40.03 ± 2.17
40	51.39 ± 0.99[a]	14.71 ± 0.51	51.14 ± 1.15[a]
60	66.17 ± 0.72[a]	29.19 ± 0.79	69.63 ± 1.25[b]
80	77.57 ± 1.11[b]	42.80 ± 0.13[a]	76.37 ± 1.29[b]
100	84.51 ± 0.23[c]	48.95 ± 1.02[b]	84.53 ± 1.01[c]
AA (100 μM)	87.82 ± 0.45	–	91.73 ± 1.17
Mannitol (50 μM)	–	79.86 ± 0.42	–

The values represent the means ± S. E. M for six determinations per group [a]P < 0.05, [b]P < 0.01 and [c]P < 0.001 compared to blank AA: Ascorbic acid was used as positive control for DPPH and Ferryl bipyridyl complex Mannitol was used as positive control for hydroxyl radical scavenging

of lipid peroxidation by CbEt validates the use of the plant as Rasayana. Thus the use of the plant as an adaptogen in the traditional systems of medicine may be suggested because of the potent antioxidant activity.

The integral antioxidative capacity of the extract quantified by the ability to quench photo-induced chemiluminescence was found to be 3.19 nmoles ascorbic acid/g equivalents at 1 μg/ml. The total antioxidant capacity of CbEt was found to be 195.17 nmol ascorbic acid/g thus establishing the extract as an antioxidant.

On the basis of the obtained results and the discussion, it may be concluded that tubers of *C. borivilianum* possess potent antioxidant activity and validating the innumerable therapeutic claims of the plant in the traditional system especially its use as a Rasayana drug as many reports indicates the role of Rasayana drugs as antioxidants (Govindarajan *et al.*, 2005). The traditional claim of the plant as an immunomodulator and aphrodisiac may be in part due to the potent antioxidant activity. The qualitative phytochemical screening revealed the presence of saponin, which are of greater interest owing to their structural relationship with sex hormones and biological activity (Gulcin *et al.*, 2004). Pharmacological evaluation of the plant in future for adaptogenic and aphrodisiac property may prove its use traditionally as Ayurvedic Viagra.

Acknowledgement

M. Thakur is thankful to University Grants Commission for providing financial support. N. Sreevidya and M. Vijayakumar are thankful to CSIR, New Delhi for providing Senior Research Fellowships.

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(Accepted August 21, 2005)