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Review Paper
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A Peer Reviewed of General Literature on Chlorophytum borivilianum Commercial Medicinal Plant

R HAQUE[1]*, S SAHA[2], T BERA[2]
*Department of Biotechnology Technology, Institute of Technology & Marine Engineering , DH Road, West Bengal, India
Division of Plant Biotechnology, Department of Pharmaceutical Technology, Jadavpur University, Kolkata-700032, India

Abstract

Chlorophytum borivilianum Santapau & Fernandes (Liliaceae) is a very popular herb in traditional Indian medicine and constitute a group of herbs used as 'Rasayan' or adaptogen Thirteen species of Chlorophytum have been reported from India [2,14,17,18,] All these species differ in appearance, native species are sold as 'Safed musli' in the Indian drug market. Major biochemical constituents of safed musli are carbohydrates 42%, protein 80-90%, fibres 3 - 4%, saponins 2 -17% and alkaloids 15 - 25% [3]. Primarily saponins and alkaloids impart medicinal value. Chlorophytum borivilianum has therapeutic application in ayurvedic system of medicine [2,3,17] Fasciculated roots of Chlorophytum borrivillianum is used as tonic and constitute and important ingredient of 20 ayuervedic and unani preparation. Roots are used for the preparation of nutritional tonic used in general sexual weakness. Administration of Chlorophytum borivilianum root power also increased the activities of antioxidant enzymes and vitamin C levels which may have enhanced the anti-oxidant capacity of the live]. Chlorophytum tuberosum Baker commonly referred as Safed Musli has been widely used as a potent "Rasayna" drug in Ayurveda as a rejuvenator and tonic . The aqueous extract of Chlorophytum borivilianum(250mg/kg for 7 days) significantly reverted levels of plasma glucose, triglycerides, cholesterol and serum corticosterone and also reduced the ulcer index, adrenal gland weight more as effectively as standard drugs(diazepam) in rats.

*Corresponding author, Mailing address:
E-mail: ruuubil_haque@yahoo.com

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Chlorophytum borrivillianum, Medicinal herb, Shoot culture, Callus culture, Michropropagation, Plant Regeneration

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Introduction

Scientific literature is continuously reporting plant drugs having immunomodulatory activity. Most of the leads for this activity are from traditional

medicines from different parts of the world [1,2]. The Indian system of medicine 'Ayurveda', conceptualizes a category of drug activity known as 'Rasayana'. The word Rasayana is composed of two words 'Rasa' meaning elixir and 'Ayana' meaning house. The word therefore signifies property of the plant that helps to rejuvenate the system, i.e. adaptogenic activity [3]. 'Rasayan' therapy prevents diseases and counteracts the aging process by means of optimization or homeostasis. Many plants have been extensively used as 'Rasayana' drugs in Ayurveda for the management of neurodegenerative diseases, as rejuvenators, immunomodulators, aphrodisiac and nutritional supplements [4–7]. Safed Musli has been described in ancient Indian literature

such as Bhavaprakash nighantu, Rasendra Sarsangrah, Raja Ballabh Nighantu as 'Vajikaran' or aphrodisiac which isa special type of immunomodulator [8–10]. Safed Musli is a controversial drug and various herbs are employed as Safed Musli by practitioners of Indian system of medicine. One of the popular and marketed herb under this nomenclature is Chlorophytum borivilianum Santapau & Fernandes (Liliaceae) [11,12]. Since the roots of C. borivilianum are employed as 'Rasayana' [13,14]and also because Safed Musli is a constituent of 'Chyawanprash', an outstanding rejuvenator [8]. It was considered worthwhile to investigate this drug forimmunomodulatory activity.

Materials and Methods

Plant Plant Material

C. borivilianum roots were obtained from Jeevan Agro farms (Sagar, Madhya Pradesh, India). A herbarium of the source was made and identified. A voucher specimen no. CB-MT16 has been submitted at the departmental herbarium of Department of Pharmaceutical Sciences Dr H.S. Gour Vishwavidyalaya, Sagar (MP, India). The roots were dried in sunlight and coarsely powdered for extraction.

Extraction and Fractionation: Powdered roots were defatted with petroleum ether (60–80_C). Ethanolic extract of the drug was prepared by extracting defatted roots with 95% ethanol in Soxhlet extractor (yield 16% w/w). For isolation of sapogenin, ethanolic extract as obtained above was suspended in water (100 ml). It was then extracted with n-butanol (300 ml), the volume of n-butanol soluble portion was then reduced to half under reduced pressure and finally saponins were precipitated by addition of diethyl ether. The collected precipitate was hydrolyzed using hydrochloric acid (2 N HCl) and the precipitated sapogenins were collected (15). Alternatively, 500 g defatted drug powder was hydrolyzed by using 200 ml of 4 N H_2SO_4 in a round bottom flask which was kept overnight at room temperature and filtered to remove acidic solution, marc was washed three to four times with cold distilled water. After washing and drying the marc was refluxed for 30 min using fresh 200 ml of 4 N H_2SO_4 to ensure complete hydrolysis of drug. The mixture was filtered and filtrate was discarded. Hydrolyzed drug powder was further washed with distilled water three times to ensure complete removal of acid. The hydrolyzed drug powder was dried in an air oven to ensure completely dried drug powder. The hydrolyzed drug powder was fed in a Soxhlet extractor and subjected to extraction with ethanol (95%). Ethanol was removed from the extract under reduced pressure and sapogenins were collected (16). The sapogenins isolated by this method were used for pharmacological studies. Sapogenins isolated by either method exhibited identical TLC profile suggesting suitability of both the process for isolation of sapogenins from the drug. HPTLC analysis of the ethanolic extract as well as isolated sapogenin fraction was performed. In brief, the chromatographic analysis of ethanolic extract was performed on silica gel-GF254 precoated plates (E Merck, Germany) using chloroform: glacial acetic acid: methanol: water (16:8:3:2, v/v) as mobile

phase, 10 spots were visualized upon derivatization with anisaldehyde sulfuric acid reagent. Sapogenin fraction was characterized on chloroform: diethylether (1:1, v/v) mobile phase against b-sitosterol as standard marker. The pattern and standardization of the sapogenins against standard marker (b-sitosterol) has been reported by us previously [17]. Materials Sheep red blood cells (SRBC's) were obtained from Haffkine Biopharmaceuticals Ltd, Mumbai, India, and were washed thrice with large volumes of pyrogen-free sterile saline andm adjusted to a concentration of 5 · 109 cells per ml for immunization and challenge. Azathioprine was obtained as gift sample from Troika Pharmaceuticals, Ahmedabad, India. Candida albicans was purchased from IMTECH (Chandigarh, ndia). Rats Wistar strain albino rats weighing between 140–150 gm of either sex were used. They were housed in departmental animal room under standard condition of temperature (24 ± 1_C), 12/12 light/dark cycle and fed on standard pellet diet. Ethanolic extract and sapogenin were administered orally as suspension in 2% polyvinyl pyr pyrolidine solution using metal canula. Toxicity studies were performed and dose of 1 gm kg_1 body weight (bw) of ethanolic extract or sapogenins did not cause any toxic effect. Statistical Analysis Data are expressed as mean ± SEM and analyzed for significance by Dunnet's test (comparing all versus control) using Instat v.2.02 software (Graphpad software Inc.) residing in Pentium IV processor run on Windows Xp.

Experimental Treatment

Albino rats were divided into groups comprising of six animals each. Group I served as control and was administered vehicle only. Group II was administered 200 mg kg_1 bw ethanolic extract. Group III received 100 mg kg_1 bw sapogenin extract. Each experiment was performed on fresh group of animals unless specified. Non-Specific Immunity Determined by Survival Rate Against Fungal Infection Treatments of all the three groups began 14 days before challenge. On the day of challenge all groups were injected with 5 · 107 viable C. albicans cells and observed daily for mortality for a period of 10 days. In Vivo Phagocytosis Using Carbon Clearance Method. The method of Biozzi et al. (1953) [18,19] was used. Treatments of all groups started 14 days before experimentation. On 15th day of treatment, mice were injected with 0.1 ml of carbon suspension (Pellikan Tuschea Ink, Germany) intravenously through tail vein. Blood samples (25 ml) were collected from retro-orbital plexus just before and at 4, 8, 12 and 16 min after injection. Blood samples were lyzed with 2 ml of 0.1% acetic acid and absorbance of samples recorded at 675 nm [20]. The graph for absorbance versus time was plotted for each animal in respective test group and phagocytic index was calculated using the formula: Phagocytic IndexðPIÞ¼Ksample Kstandard where Ksample represents the slope of absorbance versus time curve for extract-treated samples and Kstandard represents the slope of absorbance versus time curve for blood sample collected before treatment. SRBC-Induced Delayed-Type Hypersensitivity Reaction (DTH Response) The method of Lagrange et al. (1974) [21] was used.

Treatments with extracts began 14 days before challenge. All the groups were immunized by injecting 20 ml of 5 · 109 SRBC per ml subcutaneously into the right foot pad. After 420 Immunostimulant activity of Safed Musli 14 days of treatment the thickness of left foot pad was measured using calipers (Schnelltester, Germany) reading to 0.01 mm. The mice were then challenged by injecting 20 ml of 5 · 109 SRBC per ml intradermally on the left hind foot pad (time 0). Foot thickness was measured after þ24 and þ48 h of challenge. The difference between the thickness of left foot just before and after challenge in mm was taken as a measure of DTH [22]. Neutrophil Adhesion Test. The

method described by Wilkonson (1978) [23] was used for evaluating the effect of extracts on neutrophil adhesion. After 14 days of treatment of all the three groups, blood samples were collected by retroorbital puncture in heparinized vials and subjected to total as well as differential leukocyte count. After initial counts the blood samples were incubated with 80 mg ml_1 of nylon fibers at 37_C for 15 min. The incubated samples were again analyzed for total and differential leukocyte count. The product of total leukocyte count and % neutrophil known as neutrophil index was determined for each of the respective groups [24]. The % neutrophil adhesion for each of the test groups was determined as follows, %Neutrophil adhesion¼ Difference of neutrophil count in untreated and fiber-treated blood Neutrophil count of untreated blood· 100 Activity Against Drug-Induced Immuno suppression. The methods of Doherty (1981) [25] modified by Ziauddin et al. [26] was used. Six groups of six albino mice each were taken. Group I served as control. Group II was administered ethanolic extract 200 mg kg_1 bw. Group III was given sapogenin extract 100 mg kg_1 bw. Group IV received 100 mg kg_1 bw azathioprine. In Group V animals, 100 mg kg_1 bw azathioprine and 200 mg kg_1 bw of ethanolic extract was administered, whereas Group VI was treated with 100 mg kg bw azathioprine and 100 mg kg_1 bw of sapogenin extract. All the animals were sensitized by injecting 5 · 109 SRBC's intraperitoneally before experimentation. On 15th of treatment, all the mice were sacrificed and blood was collected in heparinized vials. Blood samples for animals of each group were subjected for hematological and serological studies such as hemoglobin content, total RBC, total and differential WBC count and platelet count .

Results

Survival Rate Studies

The survival rate of the treated animals was considerably enhanced after treatment with C. borivilianum extract.

Administration of ethanolic extract and sapogenin exhibited 94 ± 5.56 and 88.86 ± 5.6 percent survival after infection with C. albicans. Sapogenin extract was 50% ($P < 0.05$) and ethanolic extract 60 % ($P < 0.05$) more effective in reducing mortality as compared to control group animals (Table 1). The results suggest potentiation of non-specific immune response on treatment with ethanolic extract and sapogenins. DTH Using SRBC as an Antigen

In the control group animals, after þ48 and þ72 h of challenge the DTH response was either equal or slightly more than the 0 h response; therefore, the peak edema after þ24 h of challenge was the evaluating parameter. Ethanolic extract (200 mg kg_1 per orally) was most effective ($P < 0.05$) compared to sapogenin (100 mg kg_1 per orally) treatment in increasing the delayed-type hypersensitivity response.

Rate of Carbon Clearance

Rate of carbon clearance is the measure of competency of the reticuloendothelial system and its granulopoetic activity [14], the faster removal of carbon particles has been correlated with the enhanced phagocytic activity. In the present study an increased phagocytic activity was observed in treated groups as compared to control. The rate of carbon clearance which was determined as phagocytic index was _20 % (1.2 ± 0.03) and _25% (1.36 ± 0.51) greater in sapogenins and ethanolic extract-treated groups, respectively, clearly indicating an enhanced in vivo phagocytic activity .

Effectiveness against Drug-Induced Immunosuppression Administration of ethanolic extracts and sapogenins in general had a beneficial effect on hematological profile wherein all the parameters such as hemoglobin, platelets, RBC and WBC counts were increased. Simultaneous

administration of ethanolic extract and sapogenins along with azathioprine resulted in restoration of suppressed values observed after azathioprine treatment alone. Platelet, hemoglobin, RBC and WBC values observed were better than untreated control groups. Among the two test drugs ethanolic extract was pronouncedly more effective ($P < 0.005$) followed by sapogenin treatment ($P < 0.05$) .

Neutrophil Adhesion Test

This test is an indicative of the marginalization of phagocytic cells in the blood vessels, i.e. an indication of immunostimulation. The % neutrophil adhesion in control group animals was 23.34 ± 2.1, in ethanolic extract-treated group it was 27.21 ± 1.6 whilst for sapogenin extract-treated group it was 25.62 ± 1.4. As is evident from the results of neutrophil adhesion test, nearly 10% ($P < 0.5$) increase in neutrophil adhesion is observed after administration of sapogenin extract, whilst a significant 17% ($P < 0.05$) increase in neutrophil adhesion is observed in ethanolic extract.

Discussion

The scientific evidences collected from the study supports the traditional claims behind usage of the herb C. borivilianum, which is being cultivated and marketed extensively in India and abroad for medicinal purposes [12]. The study affirms that C. borivilianum root extract is an effective immunostimulatory principle. The inference that can be drawn from the present study is that the total ethanolic extract is superior over sapogenin fraction of the plant as far as immunostimulatory balancing and adaptogenic effectiveness of extracts. activity is concerned. The extract does not only potentiate nonspecific immune response, but is also effective in improving humoral as well as cell-mediated immunity. The effectiveness of extract-treated animals in overcoming the side effects of drug-induced immunosuppression provides evidence for balancing and adaptogenic effectiveness of extracts.

Use of herbs for improving the overall resistance of body against common infections and pathogens has been a guiding principle of Ayurveda [27,28]. Chlorophytum spp. have been used and reported in many such formulations. The increase in survival rate is a general marker exhibiting potency of the ethanolic extract to overcome infectious condition. Increased carbon clearance is an indicator of enhanced in vivo phagocytic activity and competency of granulopoetic system in removal of foreign particle, thereby an indicator of enhanced immunological response against foreign particles or antigens. Increase in percent neutrophil adhesion is attributed due to marginalization of phagocytic cells, i.e. improved defensive response under normal circumstances. This study, apart from confirming the immunostimulant activity of C. borivilianum also, presents evidence for the presence of the substance other than sapogenins which induce stimulation of immune response in treated animals. Therefore, the plant holds promise for being used as an immunostimulating agent and an in-depth study on various fractions of the extract effective as immunomodulating entities from the plant is warranted to determine the most potent immunostimulating fraction from C. borivilianum. Thus, the study validates the traditional use of herb as a 'Rasayana' in Ayurvedic system of medicine [23].

Antibacterial Activities of Crude Extracts of *Chlorophytum borivilianum* to Bacterial Pathogens[29]

Antibacterial properties of different extracts of *Chlorophytum borivilianum* were studied. Ethanol, ethyl acetate, acetic acid and water were used to prepare the extract. The antibacterial activity of different extracts were carried out against four bacteria, *Staphylococcus aureus, Escherichia coli*, *Pseudomonas aeruginosa* and *Bacillus subtilis*, by

agar cup diffusion method. Zone of inhibition produced by different extracts were measured. Acetic acid extract of *C. borivilianum* showed antibacterial activity against all the tested bacteria in the order of sensitivity as *Staphylococcus aureus>Pseudomonas aeruginosa>Escherichia coli>Bacillus subtilis*. The antibacterial activity of *Staphylococcus aureus* was sensitive with 6, 24, 12 and 8 mm zone of inhibition at 10 mg mL^{-1} of water, acetic acid, ethanol and acetone extract respectively. For, *Pseudomonas aeruginosa* zone of inhibition is 8, 20, 12 and 10 mm for water, acetic acid, ethanol and acetone. *Escherichia coli* revealed no zone of inhibition for water extract whereas it possess 18, 10, 2 mm zones of inhibition at 10 mg mL^{-1} for acetic acid, ethanol and acetone respectively. *Bacillus subtilis* showed 3, 20, 9 and 4 mm zone of inhibition at 10 mg mL^{-1} for different extracts. These results showed that the extract has a wide range of antibacterial property than the other extracts.

Identification of *Chlorophytum* species (*C. borivilianum*, *C. arundinaceum*, *C. laxum*, *C. capense* and *C. comosum*) using molecular markers [30]

Dried tubers of the genus *Chlorophytum* (Liliaceae) are used in herbal formulations. Out of 200 species of the genus, 13 species are available in India. Adulteration of medicinally important *Chlorophytum* species (*C. borivilianum, C. arundinaceum, C. laxum*) with its garden species (variegated form of *C. comosum*, *C. capense*) is an important question to be addressed. Identification of these species in herbal formulations is difficult on the basis of phenotypic characters. In the present study, five *Chlorophytum* species (*C. borivilianum, C. arundinaceum, C. laxum, C. capense* and *C. comosum*) were identified and the phylogenetic relationship has been established between them using molecular markers. Plants of five species of *Chlorophytum* were subjected to molecular profiling using RAPD marker and the sequences of plastid *ribulose bisphosphate carboxylase (rbcL)* region and the gene *rpl16* and the *rpl16–rpl14* spacer region. Species-specific RAPD markers were identified from the amplicons obtained with nine primers. Coefficient of similarity ranged between 0.6 and 0.85. Similarly comparisons of the *rbcL* region sequences allowed identification of each of the five species unequivocally, whereas the gene *rpl16* and the *rpl16–rpl14* spacer region could identify only two species *C. borivilianum* and *C. comosum*.

Aphrodisiac activity of safed musli [32,33,34]

Effects of *Chlorophytum borivilianum* (Safed musli) on sexual behavior and sperm count is observed. Thakur for their folkloric claims as aphrodisiac and sexual stimulant. Ethanolic extract of roots as well as sapogenins isolated from the roots were studied for effect on sexual behavior and spermatogenesis in albino rats. Administration of 100 mg/Kg and 200 mg/Kg b.w. of the sapogenin and ethanolic extract respectively had pronounced anabolic and spermatogenic effect in treated animals as evidenced by weight gains in the body and reproductive organs and histological studies. The treatment also markedly affected sexual behavior of animals as reflected in reduction of mount latency, ejaculation latency, post ejaculatory latency, intromission latency and an increase of mount frequency and attraction towards female. The study therefore, validated the overall claim for utilization of this herb as potent sexual stimulant.

This study was designed to evaluate the aphrodisiac and spermatogenic potential of the aqueous extract of dried roots of safed mulsi in rats. Male Wistar albino rats were divided into four groups. Rats were orally treated with (1) Control group: distilled water; (2) CB 125 mg/kg/day; (3) CB 250 mg/kg/day; and (4) Viagra group: 4 mg/kg/day sildenafil citrate and their sexual behavior was monitored 3 h later using a receptive female. Their sexual behavior was

evaluated on days 1, 7, 14, 21 and 28 of treatment. Safed musli had a marked aphrodisiac action, increased libido, sexual vigor and sexual arousal. Similarly, at the higher dose (250 mg/kg) all the parameters of sexual behavior were enhanced, but showed a saturation effect after day 14. On day 60 the sperm count increased significantly in both the Safed Musli groups, 125 mg/kg and 250 mg/kg, in a dose dependent manner. Thus, roots of mafed musli (Chlorophytum borivilianum) can be useful in the treatment of certain forms of sexual inadequacies.

A comparative study on aphrodisiac activity of some ayurvedic herbs in male albinoRats[34]

The roots of Asparagus racemosus, *Chlorophytum borivilianum* (safed musli), and rhizomes of Curculig orchioiodes are popular for their aphrodisiac properties. The herbs have been traditionally used as Vajikaran Rasayana herbs because of their putative positive influence on sexual performance in humans[313]. Lyophilized aqueous extracts obtained from the roots of A. racemosus, safed musli, and rhizomes of Curculigo orchioides were studied for sexual behavior effects in male albino rats and compared with untreated control group animals. Administration of 200 mg/kg body weight of the aqueous extracts had pronounced anabolic effect in treated animals as evidenced by weight gains in the body and reproductive organs. There was a significant variation in the sexual behavior of animals as reflected by reduction of mount latency, ejaculation latency, post ejaculatory latency, intromission latency, and an increase of mount frequency. Penile erection (indicated by Penile Erection Index) was also considerably enhanced. The present results support the folklore claim for the usefulness of safed musli and these herbs as natural aphrodisiacs and provide a scientific basis for their traditional usage.

Antioxidant and anti-stress effects of roots of *Chlorophytum borivilianum*[32]

Safed musli animal studies, antioxidant and anti-stress effects is observed 11]

The aqueous extract of safed musli significantly reverted the elevated levels of plasma glucose, triglycerides, cholesterol and serum corticosterone and also reduced the ulcer index, adrenal gland weight more as effectively as the standard drug (diazepam) in rats. Their results suggest that safed musli could be used for the treatment of oxidative stress-induced disorders.

Free radical scavenging potential of Chlorophytum tuberosum baker is observed [35]

Chlorophytum tuberosum Baker commonly referred as 'Musli' has been widely used as a potent 'Rasayana' drug in 'Ayurveda' as a rejuvenator and tonic. Antioxidant potential of Chlorophytum tuberosum has been investigated for their ability to scavenge 1,1,diphenyl picryl hydrazyl (DPPH), nitric oxide radical along with their capacity to reduce lipid peroxidation in rat liver homogenate, chelation of ferrous ion, radical scavenging potential using chemiluminescence and their total antioxidant capacity. Sugar, starch, protein, and vitamin C content were estimated spectrophotometrically along with the percentages of the individual amino acids by HPLC and individual sugars by using HPTLC as standardization tool. The extract has been found to possess antioxidant activity in all the models tested as evident by IC50 values being 225.31, 888.44, 809.22 and 422.97 µg/ml for scavenging of DPPH, nitric oxide, lipid peroxidation and ferry bi-pyridyl complex, respectively.

Rapid plant regeneration and analysis of genetic fidelity of in vitro derived plants of Chlorophytum arundinaceum Baker—an endangered medicinal herb is observed [36]

An efficient in vitro multiplication system via multiple shoot bud induction and regeneration has been developed in Chlorophytum arundinaceum using shoot crown explants. Optimum regeneration frequency (87%) and desirable organogenetic response in the form of de novo organized multiple shoot buds without an intervening callus phase was obtained on Murashige and Skoog's (MS) minimal organics medium containing 3% sucrose (w/v) supplemented with 4×10^{-6} M Kn and 2×10^{-6} MIBA. Axenic secondary explants with multiple shoot buds on subculturing elicited best response with 1×10^{-5} M Kinetin (Kn) and 5×10^{-6} M indole-3-butyric acid (IBA) giving rise to an average of 18.74 shoots per culture with mean shoot length of 7.6 cm ± 1.73. Varying molar ratios of either Kn/IBA or Kn/NAA revealed statistically significant differences in the regeneration frequencies among the phytohormone treatments. It was observed that the shoot bud differentiation and regeneration was influenced by the molar ratios of cytokinins/auxin rather than their relative concentrations. Healthy regenerated shoots were rooted in half strength MS basal medium containing 3% sucrose (w/v) supplemented with 5×10^{-6} M IBA. Following simple hardening procedures, rooted plantlets, were transferred to soil-sand (1:1; v/v) with more than 90% success. Genetic fidelity was assessed using random amplified polymorphic DNA (RAPD), karyotype analysis and meiotic behaviour of in vitro and in vivo plants. Five arbitrary decamers displayed same banding profile within all the micropropagated plants and in vivo explant donor. The cytological and molecular analysis complemented and compared well and showed no genomic alterations in the plants regenerated through shoot bud differentiation. High multiplication frequency, molecular, cytological and phenotypic stability ensures the efficacy of the protocol developed for the production and conservation of this important endangered medicinal herb.

Micropropagation; a tool for the production of high quality plant-based medicines is observed[7

Medicinal plants are the most important source of life saving drugs for the majority of the world's population[37] . The biotechnological tools are important to select, multiply and conserve the critical genotypes of medicinal plants. Plant tissue culture techniques offer an integrated approach for the production of standardized quality phytopharmaceutical through mass-production of consistent plant material for physiological characterization and analysis of active ingredients. Micropropagation protocols for cloning of some medicinal plants such as Catharanthus roseus (Apocynaceae), Chlorophytum borivilianum (Liliaceae), Datura metel (Solanaceae), and Bacopa monnieri (Scrophulariaceae) have been developed. Regeneration occurred via organogenesis and embryogenesis in response to auxins and cytokinins. The integrated approaches of our culture systems will provide the basis for the future development of novel, safe, effective, and high-quality products for consumers.

Genetic Divergence and Correlations Study in Chlorophytum borivilianum is observed

Continuous shrinking of natural resources (forest) led to the cultivation of Chlorophytum borivilianum, an ayurvedic crude drug, has necessitated the genetic improvement program of the aforesaid crop[38]. The plant is a member of family liliaceae and naturally occurring populations are open-pollinated, with varying levels of performance. The objective of this study was to assess, characterize, quantify and suitably utilize available genetic variability. Genetic divergence among 31 genotypes was determined using nine characters of C. borivilianum of indigenous origin via Mahalanobis D^2 statistic. The genotypes were grouped into eight clusters. Intra-cluster distance was largest for cluster VIII (nine

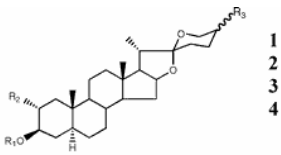
genotypes), followed by cluster I (six genotypes). Inter-cluster D^2 values recorded between cluster II and III and those between cluster III and VI indicated the possibility of raising transgressed hybrids from cross hybridization programs using divergent parents of these four clusters. The clustering pattern indicated that geographical diversity was not necessarily related with genetic diversity. Leaf number contributed most toward divergence (15.8%), followed by finger number, length and width (each with at least 13% contribution) and leaf length (12.1%). Correlation analysis for root yield (dependent variable) and the remaining seven plant traits (independent variables) revealed that leaf number, leaf length and finger number, which had contributed highly to divergence, had also significant associations with root yield. The D^2 and correlation results suggested that the variability for the three traits (leaf number, leaf length and finger number) could be reliable selection criterion for root yield in C. borivilianum.

Antidiabetic And Antihyperlipidaemic Effect of Alcoholic extract of Chlorophytum borivilianum Roots in Alloxan Induced Diabetic Albino Rats [39]

Chlorophytum has also been acclaimed for its antidiabetic activity traditionally. In a recent study the herb was studied for its antidiabetic activity against streptozotocin induced diabetes [38]. The study thus provides evidence for the effectiveness of drug in managing diabetic stress[37]. Also, fructans have been reported for their ability to alleviate diabetes by normalizing the blood glucose level. Fructans themselves serve as source of energy . Therefore, thepresence of fructans in the herb may have a major role to play in reducing glucose level in diabetic individuals. In a study conducted by the authors the aqueous extract of C. rich in polysaccharides could ameliorate the sexual dysfunction induced by streptozotocin and alloxan induced oxidative stress. Thus, the study did not only validatethe concept of Vajikaran but also substantiated the role of the plant as a Rasayana herb [316] Chlorophytum borivilianum, commonly known as 'safed musli', is widely used in different parts of India for the treatment of diabetes mellitus. The present study was designed to evaluate the antidiabetic and antihyperlipidaemic effect of alcoholic extract of Chlorophytum borivilianum root (CREt) in alloxan induced diabetic rats. Diabetes was induced by single intraperitoneal injection of alloxan (150 mg/kg of body weight). Oral administration of alcoholic

CREt to diabetic rats, at a dose of 100 mg/kg body weight, resulted in a significant reduction in blood glucose, urine sugar and serum lipids in alloxan diabetic rats. The extract also increases the total haemoglobin lever. The extract effect was similar to that of insulin. Thus, the investigation clearly shows that alcoholic CREt has both antidiabeticand antihyperlipidaemic effects.

Cytotoxic spirostane-type saponins from the roots of *Chlorophytum borivilianum* [40,41]



	R_1	R_2	R_3
1	S_1	H	eq Me
2	S_1	H	exo CH_2
3	S_2	OH	exo CH_2
4	S_3	OH	exo CH_2

S_1 = Rha-(1→4)-[Glc-(1→3)]-Glc-(1→3)-Ara-(1→3)-[Xyl-(1→2)]-Glc-(1→2)-[Rha-(1→6)]-Gal-
S_2 = Glc-(1→3)-[Glc-(1→2)]-Glc-(1→4)-Gal-
S_3 = Xyl-(1→3)-[Glc-(1→2)]-Glc-(1→4)-Gal-

Four new spirostane-type saponins named borivilianosides E–H (1–4) were isolated from an ethanol extract of the roots of *Chlorophytum borivilianum* together with two known steroid saponins [40,41 The structures of 1–4 were elucidated using mainly 2D NMR spectroscopic techniques and mass spectrometry. The cytotoxicity of borivilianosides F (2), G (3), and H (4) and three known compounds was evaluated using two human colon cancer cell lines (HT-29 and HCT 116).

Chemical constituents

A lot of chemical analysis has been carried out on the roots of *C. arundinaceum* the major reported constituents include 4 hydroxy- 8,11 oxidoheniconesol and pentacosanol, docasonoic acid,pentacosonyl docosanoate, n-nonacosane,tetracosanoic acid, stigmasterol and stigmasterol Z-Dglucopyranoside were the major constituents reported[42]. Arundinoside A and B have also been reported as major glycosidic portions from *C. arundinaceum*. Presence of such constituents straight chain alcohols with tetrahydrofuran moiety in saponin containing drugs are a rarity :[.Chemical Constituents reported from *C. borivilianum*. Although, there is a paucity of scientific work on specific characterization and standardization of Safed Musli, still there are few reports on its chemical constituents: Inulin type 2_ 1 linked fructans have been reported by Thakur and Dixit, 2004 [43] by a comparative RP-HPAE Chromatography in a Dionex system. The structure of the fructans have also been characterized by Maldi-MS and NMR studies as identified as O- Z-D- fructofuranosyl – (2_1)- (Z-Dfructofuranosyl) n- (2_1)-D-glucopyranoside (n = 5-30) [10] and is shown in Fig. 1. The total fructan content of the herb was found to be nearly 14% [323] . Presence of mannans of pure type have also been determined by us in a separate set of experiments (unpublished data). Saponins and sapogenins of *Chlorophytum borivilianum* (Sant.& F.) were standardized using HPTLC [324] and HPLC [325]. In HPTLC analysis the sapogenins isolated from powder hydrolyzed safed musli by ethanolic extraction were standardized against Z-sitosterol in Chloroform : Diethylether (1:1 v/v) mobile phase Fig.2. A HPLC method in order to detect adulteration/substitution and for identification of different species, was developed using UV spectrum of four synonyms of Safed Musli viz. Asparagus adscendens, *Chlorophytum borivilianum* , *Chlorophytum laxum* and *Chlorophytum tuberosum* were used as diagnostic markers.This method can be used to differentiate different species based on the absorbance and the spectral pattern, which was found to be different in all the species . Apart from biologically effective steroidal and triterpenoidal saponins, sapogenins and fructans having prebiotic importance [43], the other phytoconstituents reported from the plant are, high quantities of simple sugars mainly sucrose , glucose, fructose, galactose, mannose and xylose [44] . Proteins, phenolics, Triterpenoids, gallo-tannins and mucilage are also reported from *Chlorophytum borivilianum* [43] . Medicinal importance of *C. borivilianum*

Safed Musli has been traditionally acclaimed and advocated for its aphrodisiac activity [45]. In a recent study, ethanolic extract of roots as well as sapogenins isolated from the roots were studied for effect on sexual behavior and spermatogenesis in albino rats. Treatment had pronounced anabolic and spermatogenic effect in treated animals, evidenced by weight gains of body and reproductive organs.Administration of extracts markedly affected sexual behavior of animals reflected in reduction of mount ejaculation, post ejaculatory and intromission latency. An increase in mount frequency and attractability towards female was observed [46] .

Antiulcer activity[47]

Treatment with ethanolic extract of *C. arundinaceum* rendered significant protection in gastric ulceration which was evident from reduction in ulcer-index in all the models. It showed increased mucin activity in pylorus ligation model. In stress induced ulceration model, proved antioxidant activity was also observed, where it reversed the increase in lipid peroxidation and decreased the catalase levels, however, the extract did not produce any change in SOD levels, which was significantly increased in stressed condition. Further there was a significant reduction in vascular permeability and gastric emptying rate [47] . Adaptogenic activity, antiobesity and Inhibition

Antioxidant activity[48]

Antioxidant activity of the ethanolic extract was evaluated by DPPH radical and hydroxyl radical scavenging activity . The capacity to reduce lipid peroxidation in rat liver tissue along with chelating potency towards ferrous ion was also evaluated. Chemiluminiscence activity was also performed. Ethanolic extract exhibited potent antioxidant activity as evidenced by scavenging of 85.51% of DPPH radical, 48.95% of hydroxyl radical, ferryl bipyridyl complex (84.53%). The % inhibition of lipid peroxidation was found to be 67.17% at 100µg/ml concentration. Significant inhibition of superoxide radical was also exhibited in photochemiluminiscense activity [8]. The studies affirm for the potent antioxidant potential of this traditional Rasayana drug.. Srividya et al., 2006 have also reported[99] a potent free radical scavenging activity in *C. tuberosum* which is also designated as Safed musli and is some times referred to as Safed musli as well. hepatic lipid profiles. An increases in faecal cholesterol, neutral sterol and bile acid excretion with elevated hepatic 3- hydroxy-3-methylglutaryl coenzyme was also reported by the authors. Furthermore, the hypercholesteraemic rats treated with both doses of *C. borivilianum* also exhibited increases in dismutase and ascorbic acid levels. There was no evident variation in lipid or anti-oxidant profiles in control normocholestremic animals. Therefore the herb was significantly effective in ameliorating the lipid metabolism in hypercholestremic animals which remained normal and unaltered in untreated animals. The present activity corroborate with the previous findings reporting antioxidant activity of *C. borivilianum* extracts as well. Also the presence of fructans as reported by Thakur and Dixit 2006 could also be considered as the major contributing factor in better management of hypercholestramia .

Analgesic activity[49,50,51,52,53]

The effectiveness of methanolic extract of *C. borivilianum* in treating pain is observed . Their study was based on the traditional claim of utilization of this herb against rheumatoid arthritis. This activity could in part be attributed to the steroidal components in the plant. Biotechnological and agricultural perspective according to a recent report the total demand of Safed musli world over is 35000 tons compared to a meager 100 tons supply . Since India is a leader in production and supply of the herb so there is a recent upsurge in biotechnological and agricultural exploration for improvement in variety and quality of the drug. Germ plasm of Safed musli have been procured and biochemical traits have been determined the technique is being explored for conservation and spawning of the herbal drug. There is an increased awareness of herbal community for husbandry of the nutraceutically and medicinally important herb. Nearly 25-30% germination percentage has been found in *Chlorophytum borivilianum* with nearly 8 month dormancy period. Clonal propogation techniques have also been used for determining optimum growth requirements in the plant . In the studies conducted this far, there has been a desperate effort in reducing the cultivation and plantation cost and increasing the benefits for farmers. The plant grows well in loamy soil with good drainage and aeration. Although the plant has good regeneration capacity still, biotechnological agronomy and use of modern techniques need to be pushed for considerable reduction in the cost factor involved in cultivation and processing of Safed musli . The task for the agronomists in the near future is to further the good work in the field of reducing the cultivation cost and blend it with greater yield and production using proper manure and fertilizing techniques. Maintaining the desired level of macro and micronutrients required for an optimal growth of the plant.

Composition for anti-obesity, health-restorative and health-promotional benefits

An obesity control agent with health-restorative and health-promotional benefits to humans comprising the extract of *Chlorophytum* species, more particularly, *Chlorophytum arundinacceum*, is disclosed. The bioactive principles responsible for anti-obesity property have been determined to be mainly due to spirosta-steroidal saponins, spirosta-steroidal alkaloids and galacto-glucan oligosaccharides. Most effective as an obesity control agent is the spirosta-steroidal saponins. Pharmaceutical, nutritional and veterinary use of this inventive composition is also diclosed. [http://www.freepatentsonline.com/WO2006036638.html]

Effectiveness against lipid metabolism

Visavadiya and Narsimhcharya, 2007[54] reported the efficacy of *Chlorophytum borivilianum* root (powder) in modulating the hyperlipaemic/hypercholesteraemic conditions in male albino rats. The whole root powder of *C. borivilianum* was administered in two dose i.e. 0.75 and 1.5 g root powder/rat per day for 4 weeks to hypercholesteraemic rats. The administration significantly increased high-densit lipoprotein-cholesterol levels and decreased plasma and containing herbs are antitussive activity [2],prevention of post gastrectomy anemia and osteopenia [55] antidiabetic activity , immunomodulatory activity [56]. They have also been found useful for targeting drugs to colon [57] and prevention of colon cancer [58]. Since safed musli contains appreciable quantity of fructans so there is an ample possibility for exploration of mentioned medical attributes in the herb[346]. These polymers may have a role in the purported Rasayana action of the herb. This virgin aspect needs to be thoroughly investigated to enhance the commercial value of the herb. Therapeutic and medicinal values of a plant are major concerns for imparting a prominence and propelling the sal of any medicinal herb in the global market. Although, Indian share has not gained the desired global prominence and has been overrun by superpowers in the field like Germany, China and Japan still it is not a dooms day situation . In modern context, a thorough identification of biologically active marker compound, a complete and systematic chemical identification and determination of medicinally useful components from the herb is very important for developing a standardization profile of the herb. Proper standardization of any medicinal herb is very important as per the WHO guidelines before any herb can truly find its potential market in the global arena [47 59]. An important aspect that has to be dealt with utmost care is of creating awareness amongst the state farmers growing safed musli. The farmers must be well versed with pros and cons of growing safed musli, they must be cognizant of a possible fiasco that may occur if an equal heed is not paid to the processing and formulation development from the herb [60]. From, the current trends available it can rightly be said that if assenting and quick steps are not taken for the preparation of commercially viable products from safed musli then no sooner the roots of gold may just loose their shine and glitter. It is not just by promoting the agricultural aspect that a true value of herbal drug may be recognized. It is a blend of cultivation, adequate processing, formulation, marketing and subsequent globalization that makes any herbal drug judiciously successful in the market. To keep the white tubers glowing and golden a firm step in increasing research input onthe plant is the need of the hour. Other *Chlorophytum* species of importance *Chlorophytum malyanese*[62,63] is another important plant group which has been evaluated extensively for various botanical perspectives as well as medicinal properties. Chromaloside A isolated from this plant is reported as a major cytotoxic agent and is being

explored for a potential anticancer agent. *C. tuberosum* another important plant has been reported to possess potent antioxidant activity by Srividya et al., 20065 . *C.arundinaceum* is also an important plant commonly designated as safed musli which has been reported to possess adaptogenic activity . Other plants or species designated as safed musli *Asparagus adescendens* Linn. Family Liliaceae an *chlorophytum arundinaceum* Baker Family Liliaceae are two other important plants which have been designated as safed musli by some of the workers. The plants are reported to possess immunomodulatory and adaptogenic properties as well. Saponins of *Chlorophytum* spp. have been reviewed extensively by Kaushik, 2005. The important property that has been ascribed to saponins of this particular species is there bidesmosidic nature which in part may also be responsible for better bioavailability of steroidal saponins in-vivo. Although, since most of the plants of this specie remain potentially unexplored, it would be apt to state that further research on some traditionally acclaimed plants of this specie may provide some important insights.

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