



Review Article

***Chlorophytum borivilianum* (Safed musli): A Vital Herbal Drug**

Payal Sharma*, Kaushal K Chandrul

School of Pharmaceutical Science, Shri Venkateshwara University, Gajraula, Distt:- Amroha, Uttar Pradesh, India

ARTICLE INFO:**Article history:**

Received: 10 January, 2017

Received in revised form:

15 February, 2017

Accepted: 25 February, 2017

Available online: 28 February, 2017

Keywords:*Chlorophytum borivilianum*

Safed musli

Herbal

Musli

Aphrodisiac

ABSTRACT

The trend of using natural products is increasing steadily. The use of traditional medicines and medicinal plants in most developing countries as a normative basis for maintenance of good health has been widely observed. Further an increasing reliance on the use of medicinal plants in the industrialized societies has been related to the development of several drugs and chemotherapeutics from plant species as well as from traditionally used rural herbal preparations. *Chlorophytum borivilianum* Santapau & Fernandez also known as Safed Musli is a traditional herbal plant with assorted Ayurvedic relevance. This review is an attempt to summarise the potential applications of *Chlorophytum borivilianum* as a vital herbal drug.

1. Introduction

Herbal remedies have attained much more popularity in the treatment of minor ailments, due to increasing awareness of personal health maintenance through natural products. Indeed, the market and public demand has been so great that there is a huge extinction risk to many medicinal plants and obviously the loss of genetic diversity. The use of plants for treatment of various ailments dates back to over 5000 years. A great source of ancient information is contained in the 'Vedas' and more specifically 'Yajur Veda' is the main source of such information. In these 'Vedas' the medicinal importance of many plants has been mentioned.

The earliest monumental contribution on 'Ayurveda' is the 'Samhitas' of 'Charak' and 'Sushrut' (1000-700 B.C.), which included 500 plants with their therapeutic properties. Although with the invasion of Greeks, Sagthians, Huns, Mughals and Europeans, the progress of Ayurveda declined, but the plant based drugs caught the attention of the west from the early colonial days. 'Ayurvedic Rasayanas' have been designated as a special class of herbs having multifaceted action inside the human body. Their activities range from improvement in mental acuity, keenness to enhancement of anabolism, maintenance of homeostasis, prevention of degenerative diseases, antioxidant activity and improvement in failing sexual functions. 'Chyawanprash' is an excellent example of Rasayana which is a convergence of numerous therapeutic benefits and was formulated by 'Rishi Chyawan' after years of research put together, to keep the ailments at bay and look young and rejuvenated. Safed Musli is a part of this age old, time tested formulation. Now-a-days world-wide shift towards herbal preparations over synthetic pharmaceuticals has realized the importance of focused research in medicinal plants. Indian continent is the repositories of large number of medicinal plants, and most of these are available as wild plants in the forests of hills and planes. Disturbances of the natural

habitats of these plants, as a result of anthropogenic activities and invasion of the exotic species has resulted in the drastic decline in the population of these important plant species and many of these species are now listed among the rare, critically rare and endangered category.

Chlorophytum borivilianum has therapeutic application in Ayurvedic system of medicine. Generally, it is considered very good to increase general body immunity. Its aphrodisiac properties have proved very much useful for the people suffering from Erectile Dysfunction and to increase male potency. It has spermatogenic property and helpful in curing impotency as they are rich in glycosides. Its roots are widely used for various therapeutic applications in the Ayurvedic and Unani systems of medicine. It is known to cure many physical illness and weaknesses.

It is also reported to cure diabetes, arthritis and increasing general body immunity. However, in recent years its effectiveness in increasing male potency has become very popular and is now considered as an alternative to 'Viagra'. The roots are reported to contain 42% of carbohydrates, 8–9% of proteins, 3–4% fibres, and 2–17% of saponins. Among all the species of *Chlorophytum* present in India, *C. borivilianum* produces the highest yield of roots along with the highest saponin content as one of the important phytochemical constituents. Saponins consist of a sugar moiety, usually containing glucose, galactose, glucuronic acid, xylose, rhamnose or methylpentose, glycosidically linked to a hydrophobic aglycone (sapogenin) which may be triterpenoid or steroid in nature. The oligosaccharide chain is normally attached at C3 position (monodesmosidic) but many saponins have an additional sugar moiety at the C26 or C28 positions (bidesmosidic).

Chlorophytum borivilianum is considered as a curative of Natal and Post Natal Problems and a cure for Diabetes and Arthritis. Its root powder is fried in the ghee and chewed in case of apthae of mouth and throat. It is effective in curing rheumatism and joint pains. It is an essential part of a traditional diet of mothers (after delivery) in the form of "Laddoos". Efforts in countries like U.S.A and England are also on to convert it into chips/flakes to use it as a nutritious breakfast. *C. borivilianum* is traditionally used for lack of libido male impotency, oligospermia. It is also widely used as a general health promotive tonic and for delaying the ageing process. Varying its common use for health promotion, it is also used for increasing lactation, treating various gynaecological disorders, arthritic conditions and to control diabetes mellitus. As such Safed musli has no adverse effect if taken in a proper dose while hyper dosing may lead to gastrointestinal disorders. Safed Musli has been described in ancient Indian literature such as Bhavaprakash nighantu, Rasendra Sarsangrah, Raja Ballabh Nighantu as 'Vajikaran' or aphrodisiac which is a special type of immunomodulator.

An **antimicrobial** is an agent that kills microorganisms or inhibits their growth. Antimicrobial medicines can be grouped according to the microorganisms they act primarily against.

For example, antibiotics are used against bacteria and antifungals are used against fungi. They can also be classified according to their function. Agents that kill microbes are called microbicidal, while those that merely inhibit their growth are called biostatic. The use of antimicrobial medicines to treat infection is known as antimicrobial chemotherapy, while the use of antimicrobial medicines to prevent infection is known as antimicrobial prophylaxis.

***Chlorophytum borivilianum* (Safed musli)**

Botanical Classification

- Kingdom: Plantae
- Clade: Angiosperms
- Clade: Monocots
- Order: Asparagales
- Family: Asparagaceae
- Subfamily: Agavoideae
- Genus: Chlorophytum
- Species: *C. borivilianum*

Chlorophytum borivilianum is an herb with lanceolate leaves, from tropical wet forests in peninsular India. The Hindi name is **safed musli** (also commonly known as **musli**). It is cultivated and eaten as a leaf vegetable in some parts of India, and its roots are used medicinally as a sex tonic under the name *safed musli*. The medicinal value is thought to derive from its saponin content, up to 17 percent by dry weight. It has also recently been suggested that it may produce an aphrodisiac agent. As medicinal demand has increased, the plant has been brought under cultivation. The saponins and alkaloids present in the plant are the source of its alleged aphrodisiac properties. In traditional Indian medicine it is used as 'Rasayan' or adaptogen.

Chlorophytum borivilianum is a traditional rare Indian medicinal herb which has many therapeutic applications in Ayurvedic, Unani, Homeopathic and Allopathic system of medicines. Its roots (tubers) are very famous for various therapeutic applications viz. adaptogenic, aphrodisiac,

immunomodulatory and antidiabetic. It is used to cure physical illness and weakness, as an aphrodisiac agent and revitalizer, as general sex tonic, remedy for diabetes, arthritis and increasing body immunity, curative for natal and postnatal problems, for rheumatism and joint pains, increase lactation in feeding mothers, also used in diarrhea, dysentery, gonorrhoea, leucorrhoea etc. It has spermatogenic property and is found useful in curing impotency, now it is considered as an alternative 'Viagra'. Recent pharmacological studies on tubers of *C. borivilianum* has indicated antiviral, anticancer, antioxidant, antidiabetic, antistress, aphrodisiac, antimicrobial, hypolipidemic, hypocholesteremic, anti-inflammatory, immunomodulatory activities. Steroidal saponins, polysaccharides, fructans and fructooligosaccharides, Beta sitosterol, stigmasterol, hecogenin and mucilage are major constituents of *C. Borivilianum* which act as therapeutic agents in many therapeutic applications.

In the Ayurvedic literature, Safed Musli is celebrated as a Divya Aushad with unparalleled medicinal properties. It is a chief ingredient in the preparation of over a hundred Ayurvedic formulations. Safed Musli is also gaining increasing acceptance as a Vitalizer and health-giving tonic, a curative for pre-natal and post-natal problems, a restorative for immunity-improvement and as a remedy for diabetes and arthritis.

The recent discovery of Safed Musli as a natural and safe aphrodisiac agent has also opened up new channels of application and new demand for it. By virtue of being an herb, Safed Musli has been found to be an ideal aphrodisiac with no negative side-effects associated with chemical-based aphrodisiac



Figure No. 1: Whole plant of safed musli



Figure No. 2: Different Stages of safed musli

2. Climatic Requirements

As Safed Musli grows naturally in most parts of the Central Region of India, the normal climate of the Central region suits the crop most and it can grow successfully in the wide range of temperature and rainfall. Sandy loam soil with proper drainage system facilitates its growth. Safed Musli is found growing in thick forests in its natural form. It is partly an herb with sub-erect lanceolata leaves. There are about 256 varieties of *Chlorophytum* and 17 among them are found in India. Among these, *Chlorophytum borivilianum* has good market both indigenously and globally. The Medicinal Plants Board has recognized Safed Musli as the sixth important herb to be protected and promoted.

3. Classification, Origin and Distribution

Chlorophytum borivilianum Santapau & Fernandez (described as *C. arundinaceum* in old literature) is a threatened species belonging to the family 'Liliaceae' and genus 'Chlorophytum', is probably christened so because of the white milky texture of its tubers after peeling. Tribals in India have used Safed Musli since ages for enhancing their virility. The species was first described from India in 1954 and reached rare status in nature due to over exploitation. There are 215 species in the genus *Chlorophytum*. Most species are originated from Africa and distributed throughout warmer regions of the world. Safed Musli is available in deep forests or reserve sanctuaries in the Aravali Hills.

The genus *Chlorophytum* is distributed in the tropical and subtropical regions of the world. Tropical and subtropical Africa is probably the centre of origin of the genus, where about 85% of the species are found in India. *C. borivilianum* is mainly distributed in Southern Rajasthan, North Gujarat and Western Madhya Pradesh. In Genera Plantarum, Bentham and Hooker reported 40 species of *Chlorophytum* distributed in Asia, Tropical Africa, America and Australia. Cooke also mentioned about 40 species distributed in tropical and subtropical parts of the world. The species distributed in India are *C. heyneanum* Wall., *C. breviscapum* Dalz., *C. arundinaceum* Baker, *C. Glaucum* Dalz., *C. tuberosum* Baker, *C. khasianum* Hooker, *C. Attenuatum* Baker, *C. malabaricum* Baker, *C. undulatum* Wall. syn. *C. nepalense* (Lindl) Baker, *C. orchidastrum* Lindl., *C. laxum* Br. and *C. borivilianum* Sant. & Fernand .

Aundhe and Deokule reported 10 species from Maharashtra and classified them on the basis of their root morphology. These species are *C. borivilianum*, *C. bharuchae*, *C. orchidastrum*, *C. arundinaceum*, *C. glaucum*, *C. attenuatum*, *C. glaucoides*, *C. breviscapum*, *C. laxum* and *C. tuberosum*. *C. borivilianum* is distributed mainly in Southern Rajasthan, Northern Gujarat and Western Madhya Pradesh in India. The plants grow in a wide variety of places in nature, starting from open rocky places to shady and highly humusrich soil in the forest. Thirteen species of *Chlorophytum* have been reported from India. All these species differ in appearance, native species are sold as 'Safed musli' in the Indian drug market. Amongst these, *C. borivilianum* produces the highest yield and highest saponin content. Other important indigenous

species are *C. arundinaceum*, *C. tuberosum*, *C. laxum*, and *C. breviscapum*.

4. Botanical Description

Chlorophytum borivilianum is an herbaceous plant with a condensed stem disc from which a whorl of leaves originates. Leaves are sessile, 10-40 cm in length and 0.6-4.0 breadth. The inflorescence is racemes, flowers are pedicellate with joints, small, white, bracteate, zygomorphic. The perianth consists of six tepals arranged in two heteromorphous series having three tepals in each. The androecium consists of six stamens arranged opposite to tepals. Anthers are longer than filaments. The pistil is longer than the stamen and the stigma which is arranged away from the stamens. The flower clusters are dense on the upper part of the scape, bracts are linear, papery and purplish, 1.0-10.5 cms long, pedicle whitish and 6-10 mm long. It bears green to yellow coloured fruit which is almost equal in length and breadth. Seeds are endospermic, onion-like, black coloured and angular in shape.

The fruit is a capsule, which is trilobed and bears 3-12 seeds inside. The seeds are black and flat. The fibrous roots of the plant are modified into fascicular roots (fleshy root), comprising the economically useful part. Its root tubers are fleshy, fascicled and directly originate from the stem disc devoid of any fibrous structure. They are cylindrical and 5- 20 in number. It has 6-13 radical leaves spirally imbricate at the base, sessile in nature, linear or ovate with acute apex and slightly narrowed at the base. The leaves spread horizontally with smooth surfaces, wavy margins and parallel venation.

5. Morphology

Safed musli is a tiny annual herb that grows well in tropical and sub-tropical climates with altitudes upto 1500m. It grows to a maximum height of 1.5 ft. Tubers can grow upto a depth of 10 inches. Figure no.1 is the whole plant and figure no. 2 demonstrates different stages of *C. Borivilianum*.

5.1. Root

Roots pale brown to white colour with characteristic odour and are tasteless in nature. Root tubers are fleshy, fascicled and directly originate from the stem disc devoid of any fibrous structure and distinguished it from other species of *Chlorophytum* genus. The shape of tubers were cylindrical, the thickness being on the average 0.9 cm and the length 8cm. The number of tubers varies from plant to plant and on an average 5-30 tubers/ plant are observed and slightly tapering towards lower side look like pestle.

5.2 Leaf

It has 6 -13 radical leaves spirally imbricate at the base, sessile in nature, lanceolate and linear or ovate with acute apex and slightly narrowed at the base and less than 30cm long. The leaves spread horizontally, with smooth surfaces, wavy margins and parallel venation.

5.3. Flowers and fruit

Flowers of *C. borivilianum* (Figure No.3) are small, white, bracteate, pedicellate, zygomorphic usually arranged in

alternate clusters, each cluster comprising of 3 flowers. The flower clusters are dense on the upper part of the scape; bracts are linear, papery and purplish, 1.0 - 10.5cms long; pedicle whitish and 6 -10 mm long. It bears green to yellow coloured fruit which is almost equal in length and breadth. The seed is very small, black and enclosed in the holes. In one hole, there are about 10- 12 seeds. Seeds are endospermic, onion like, black coloured and angular in shape.



Figure No. 3: Flowers of *C. borivilianum*

6. Phytochemistry

Major biochemical constituents of *C. borivilianum* are carbohydrates 42%, protein 10%, fibres 20 - 30%, saponins 2 - 17% and alkaloids 15 - 25%. Primarily saponins and alkaloids impart medicinal value. It is a rich source of over 25 alkaloids, vitamins, proteins, calcium, magnesium, phenol, resins, mucilage, and polysaccharides and also contains high quantity of simple sugars, mainly sucrose, glucose, fructose, galactose, mannose and xylose. Recently Stigmasterol and saponin named as furostanol and Chlorophytoside-I (3b, 5a, 22R, 25R)-26-(β -D-glucopyranosyloxy)- 22-hydroxy-furostan-12-one-3yl O- β -D-galactopyranosyl (1-4) glucopyranoside has been isolated.

7. Biotechnological and Agricultural Perspectives

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.

Germplasm of Safed Musli has been procured and biochemical traits determined (Bhagat et al., 2003) 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 *C. borivilianum* with a nearly 8 month dormancy period[1]. In the studies conducted so 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 *C. borivilianum*[2]. 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.

Due to its therapeutic activity and diversified uses, demand for *C. borivilianum* is increasing in Indian and the international market. Its seeds have poor germination percentage (11–24%), low viability and long dormancy period. *C. borivilianum* is propagated vegetatively by fleshy tuberous roots bearing shoot buds. Due to large-scale and indiscriminate collection of its roots for gainful trade and insufficient attempts either to allow its replenishment or its cultivation, *C. borivilianum* has been enlisted in the list of National Medicinal Plant Board as one of the prioritized plant species. There is need for commercial cultivation of this species.

7.1 Micropropagation

In the past decade, plant tissue culture procedures have become useful tools for the horticultural industry. *In vitro* plant propagation methods developed allow for large-scale micropropagation, which increases breeding efficacy and decreases the breeding cycle for many crops. This practice allows for the development of high quality crops, which have been selected for horticulturally desirable traits for example disease or pest resistance and stress adaptability[3].

7.2 Micropropagation technique

Plants have the unique capability to develop into complete plants from a single cell. This phenomenon is termed as totipotency and each plantlet developed from these cells is likely to be similar to the parent plant[4]. Micropropagation of phytomedicinal species exploits this ability and, by careful manipulation of plant growth hormones and nutrients, provides us with the ability to produce many identical or clonal offspring expressing the same or greater concentrations of their valuable metabolites.

In the micropropagation process, small pieces of tissue called explants are excised from seedlings or plantlets and exposed to an induction medium that satisfies all of the requirements for plant growth and development. Disrupting the connection of

plant cells with the material tissue allows for the manipulation of the developmental pathways[5]. Addition of phytohormones to the culture media redirects the growth and differentiation of somatic cells[6]. New cell proliferation and differentiation in cultured plant cells can occur in two different developmental pathways: a) organogenesis or b) somatic embryogenesis. The organogenic mode of development results in renewed generation of shoots and/or roots from cultured tissue to asexually produce organs and eventually whole plants. Somatic embryogenesis is also an asexual method of propagation and the resulting clones are genetically identical unlike sexually produced embryos, which are recombination products of individual male and female gametes. In both organogenesis and somatic embryogenesis, the differentiation is either direct or indirect. Indirect morphogenesis is defined

as the formation of callus on explants and the subsequent development of shoots, roots or somatic embryos[7]. Development of callus or cell culture is the result of a dedifferentiation or reversion of the plant cells to the meristematic phase of cell development. In contrast, direct differentiation is the development of organogenesis or somatic embryogenesis directly from the explant tissue without a callus (dedifferentiation) stage. The developmental route and frequency of regeneration in any tissue culture system is dependent on several factors: a) selection of an appropriate explant, b) preparation of the explant, c) supplementation of plant tissue culture media with the optimal combination of growth regulating compounds and amendments, and d) optimization of environmental conditions for the development of regenerants[4,6].

7.3 Micropropagation and its cost effectiveness

Micropropagation technology is advantageous due to production of high-quality disease-free, true-to-type plants independent of seasonal and other environmental conditions in a comparatively smaller space, but higher cost of plant production has always limited the use and exploitation of this technique at industrial level[8]. So to fill the gap of demand and supply, and to provide genetically uniform planting material from a known source, micropropagation is one of the most desirable option. The technique of tissue and organ culture is being used for rapid multiplication of elite plants. In comparison to conventional propagation, micropropagation has the advantage of mass scale propagation in limited time and space, maintenance of disease free germplasm and round the year propagation of quality planting material[9,10]. Attempts have been made to develop *in vitro* propagation protocol for safed musli[11,12,13] wherein some of the parameters have been worked out. However, in order to propagate the quality planting material from the selected elite plants, further studies are required to investigate the various factors which influence large scale multiplication and subsequent acclimatization.

For scale-up cultures using bioreactors, the use of liquid culture medium has been recommended[14,15,16]. The physical state of the culture medium and its composition affect the *in vitro* growth of plants to a great extent. The most commonly used gelling agent—agar (adding up to 65% of the cost of the culture medium) results in local accumulation of heat and hinders the access of dissolved oxygen to the cultured cells[17]. By employing liquid culture medium, reduction in plant production cost can be achieved[18]. The advantages of liquid culture medium for enhancing shoot proliferation and growth have been reported in several plant species[19,20,21]. Liquid cultures are generally more desirable than solid support medium because of higher growth rates resulting from high medium to tissue contact, and can be employed in bioreactors for large-scale multiplication of plants. The response of cultured tissue to media manipulation and selection pressure is also more rapid.

7.4 Role of Clonal Propagation and Molecular Tools

Clonal propagation techniques have also been used or determining optimum growth requirements in the plant[22]. Micropropagation being a clonal method has the potential for

large-scale propagation of elite trees, but there is a risk of getting somaclonal variation. Therefore, a method has to be evolved to characterize deviant phenotypes and to determine the level of clonal fidelity (true-to-type plants) among *in vitro* propagated plants. Various technologies which can be applied to test the clonal fidelity are phenotypic studies, protein based markers (protein patterns and isoenzymes) and DNA based markers.

DNA fingerprinting is useful to assess genetic diversity among germplasms and could potentially overcome profiling limitations associated with morphological and biochemical data. DNA-based techniques have been widely used for authentication of plant species of medicinal importance. This is especially useful in case of those that are frequently substituted or adulterated with other species or varieties that are morphologically and/or phytochemically indistinguishable. Various types of DNA based molecular techniques are utilized to evaluate DNA polymorphism. These are hybridization-based methods, polymerase chain reaction (PCR)-based methods and sequencing-based methods. PCR-based techniques where random primers are used, include random amplified polymorphic DNA (RAPD).

Correlation between morphological/biochemical and molecular characters and the chemical quality among different varieties of this plant is need to be investigated. A wide variety of biological criteria such as morphological similarities are traditionally used to try and deduce relationships among varieties. Other criteria are similarities with respect to plant secondary metabolites, isozymes, and other protein systems. Methods that permit a direct assay of mutational differences at the level of DNA have great promise. Many different molecular techniques are now available, i.e. protein-techniques (amino acid sequencing, electrophoresis) and DNA-techniques {RFLPs (Restriction Fragment Length Polymorphisms), RAPDs (Random Amplified Polymorphic DNA), AFLPs (Amplified Fragment Length Polymorphisms), and sequencing of the DNA}.

Amongst the DNA based technology such as RAPD, ISSR, AFLP etc. has the potential to find out the variants if any amongst the regenerated plants at the genetic level. RAPD is the amplification of genomic DNA using at least one short oligonucleotide (random) primer, in low stringency conditions, results in multiple amplification products from loci distributed throughout the genome[23,24]. The attraction of RAPD is that there is no requirement of hybridization or of sequence information. It is considered the easiest and simple method for detecting variations in regenerated plants. This technique is based on the use of a single arbitrary primer (mostly 10 mers) in a PCR reaction to synthesize multiple copies of random genomic DNA regions.

In ISSR (Inter Simple Sequence Repeat), ISSR primers anchored at 5' or 3' are used. Micro satellite or SSR'S consist of tandemly repeated 2-7 base pair units which are distributed widely throughout the genome and the region flanking the ISSR are generally conserved among genotypes of same species. PCR primers similar to the flanking regions are used to amplify the ISSR-containing DNA fragments. Variability is due to the the difference in the number of repeat units.

7.5 Pathway Engineering

Increasing the production of active phytochemical constituents is a well-established target for genetic manipulation but presents some severe challenges. In particular, the metabolic pathways by which active compounds are biosynthesized are mostly poorly understood, and relatively few genes for key enzymatic or regulatory steps have been isolated. Nevertheless, there are examples of pathway engineering leading to improvements of potential value in the breeding of medicinal plants[25,26,27]. A recent article illustrating the challenges and opportunities of this approach[27] describes a nine fold enhancement in production of the sedative compound scopolamine in hairy root cultures of *Hyoscyamus niger* (black henbane), brought about by simultaneously overexpressing two genes encoding the rate-limiting upstream and downstream biosynthetic enzymes. Yun *et al.*[28] increased the production of scopolamine in *A. belladonna*, from the naturally occurring chemical precursor hyoscyamine, by transformation with the enzyme hyoscyamine 6 *b*-hydroxylase from *Hyoscyamus*. Preliminary progress has been made towards engineering alkaloid production in *P. somniferum*[29]. A threefold enhancement in production of the putative anti-malarial, anti-cancer agent artemisinin has been reported in transgenic *Artemisia* plants overexpressing farnesyl diphosphate synthase, the enzyme immediately preceding the first committed biosynthetic step[30]. As an alternative to targeting an individual rate-limiting enzyme reaction, exploiting transcription factors that turn whole secondary pathways on or off shows great promise as a metabolic engineering strategy[31]. New genomic approaches and efficient gene isolation methods applied to difficult secondary pathways in *C. borivillianum* metabolism will undoubtedly expand the range and precision of manipulations via transgenesis, providing potentially superior material for the breeder.

Selection assisted by genetic markers is an extension of traditional crop breeding, which has been used extensively in food crop improvement. It is a way to recognize desirable genotypes at an early stage to speed up the selection process.

It relies on detecting specific DNA sequences that are either gene alleles directly concerned with the trait in question or that are closely linked to such genes. Identifying functional genes and useful marker sequences linked to them is a lengthy and expensive technical process but progress in this area of molecular biology has been facilitated and accelerated by the results of whole genome sequencing of model species, such as *Oryza sativa* (rice) and *Arabidopsis thaliana*. There is a high degree of similarity in the DNA sequences of functional genes between different plant species; therefore, DNA probes from one species can often be used to identify homologous sequences in another closely related species.

8. Future Prospects

Inulin type fructans have latterly received a special denomination in scientific community[29]. The polymer has been conferred with number of prebiotic and medicinal benevolence[30]. A few reported medicinal benefits of inulin

containing herbs are antitussive activity[31] prevention of post gastrectomy anemia and osteopenia[32] antidiabetic activity[33], immunomodulatory activity[34]. They have also been found useful for targeting drugs to colon[35] and prevention of colon cancer[36]. Since Safed Musli contains appreciable quantity of fructans so there is an ample possibility for exploration of mentioned medical attributes in the herb. 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 sale 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[37]. 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[38]. 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[33].

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[33].

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 lose 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 on the plant is the need of the hour.

9. Medicinal aspects of *Chlorophytum Borivillianum*

9.1 Immunomodulatory Activity

The term "immunomodulation" denotes a change, a strengthening of suppression, of the indicators of cellular and humoral immunity and nonspecific defense factors. The essence of immunomodulation is that a pharmacological agent acting under various dose and time regimens displays an immunomodulating effect. The immunomodulating action is reversible and requires maintaining the dose of a preparation. The extreme manifestations of immunomodulating action of biologically active substances are immunosuppression (depression of the immune response) and immunostimulation

(immunopotential or strengthening of the immune reactions). Hence both immunostimulating agents and immunosuppressing agents have their own standing and search for better agents exerting these activities is becoming the field of major interest all over the world. Ethanolic extract of the tubers of *C. borivilianum* and its sapogenin were evaluated for their immunomodulatory activity. The assessment of immunomodulatory activity was carried out by determining the effect of azathioprine induced myelosuppression and administration of extracts on haematological and serological parameters. Administration of extract greatly improved survival against *Candida albicans* infection. An increase in delayed type hypersensitivity response, % neutrophil adhesion and *in-vivo* phagocytosis by carbon clearance method was observed after treatment with extracts. Results suggested a potent activity of ethanolic extract when compared to sapogenin fraction of *C. borivilianum*.

9.2 Aphrodisiac Activity

The term aphrodisiac originated from the Greek word Aphrodite, eulogizing the Greek goddess of love and romance. In modern times, this term has been used for substances that enhance sexual activity and are helpful in treating sexual dysfunction. *C. borivilianum* is generally found in forests and are members of a special group of Ayurvedic herbs known as Vajikaran Rasayana, which are used for improving potency and alleviating sexual dysfunction. Kamasutra of Vatsyayan a treatise on the art and science of love making discuss the use of numerous herbs for Spermatogenic potential of the aqueous extract of dried roots of *C. borivilianum* (CB) was also observed by Kenjale *et al.* in rats. Rats were orally treated with dried roots powder of *C. borivilianum* 125 mg/kg/day and 250 mg/kg/day and their sexual behavior was monitored 3 hour later using a receptive female. Their sexual behavior was evaluated on days 1, 7, 14, 21 and 28 of treatment by pairing with a pro-oestrous female rat. For sperm count the treatment was continued further for 60 days. At 125 mg/kg, CB 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 behaviour were enhanced, but showed a saturation effect after day 14. On day 60 the sperm count increased significantly in both the CB groups, 125 mg/kg and 250 mg/kg, in a dose dependent manner. Thus, it is evident that roots of *C. borivilianum* can be useful in the treatment of certain forms of sexual inadequacies, such as premature ejaculation and oligospermia.

9.3 Antidiabetic and Antioxidant Activity

Diabetes, long considered a disease of minor significance to world health, is now emerging as one of the main threats to human health in the 21st century not only in developed nations but also in developing countries where non-communicable diseases are rapidly overtaking communicable diseases as the commonest cause of death. Recent World Health Organization (WHO) projections suggest that in the next two decades, the largest increase of diabetes will be seen in the economically productive age group i.e. 20–45 year old individuals in developing countries. With its population over 1 billion, India leads the world with its largest number of diabetic subjects (over 35 million) and this number is predicted to increase to around 80 million by the year 2030.

Chlorophytum borivilianum 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. The aqueous extract of roots of *C. borivilianum* L. at a dose of 250 mg/kg and 500 mg/kg body weight respectively was tested for antidiabetic activity in streptozotocin (STZ)-induced hyperglycemic rats. The blood glucose levels were measured at 0, 2h, 4h and 6h after the treatment. The aqueous extract reduced the blood glucose in STZ- induced diabetic rats from 285.56 to 206.82 mg/dl, 6h after oral administration of extract ($P < 0.01$). It has been reported that using medicinal plant extract to treat STZ induced diabetic rats results in activation of β -cells and insulinogenic effects. The antihyperglycemic activity of the aqueous extract of *C. borivilianum* roots was comparable with glibenclamide, a standard hypoglycemic drug. Govindrajan *et al.* also reported antidiabetic activity of *C. borivilianum* against streptozotocin induced diabetes. The study thus provides evidence for the effectiveness of drug in managing diabetic stress. 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, the presence of fructans in the herb may have a major role to play in reducing glucose level in diabetic individuals. The aqueous extract of *C. Borivilianum* rich in polysaccharides could ameliorate the sexual dysfunction induced by streptozotocin and alloxan induced oxidative stress. Thus, the study did not only validate the concept of Vajikaran but also substantiated the role of the plant as a Rasayana herb.

Anti-stress and anti-oxidant effects of roots of *C. Borivilianum* was also determined by Kenjale *et al.*. The aqueous extract of *C. borivilianum* 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 in rats. At 125 mg/kg, it showed a mild anti-stress activity. By *in vitro* 1, 1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging assay, and lipid peroxidation assay, the extract considerably inhibited, in a dose-dependent manner, the levels of DPPH free radicals and thiobarbituric acid reactive substances, suggesting that it could be used for the treatment of oxidative stress-induced disorders.

9.4 Effectiveness against Lipid Metabolism

Visavadiya and Narsimhcharya reported the efficacy of *C. borivilianum* tuber powder in modulating the hyperlipaemic/hypercholesterolaemic conditions in male albino rats. The whole root powder of *C. borivilianum* was administered in two doses i.e. 0.75 and 1.5 g root powder/rat per day for 4 weeks to hypercholesterolaemic rats. The administration significantly increased high-density lipoprotein-cholesterol levels and decreased plasma and hepatic lipid profiles. An increase 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 hypercholesterolaemic rats treated with both doses of *C. borivilianum* also exhibited increases in superoxide dismutase and ascorbic acid levels. There was no evident variation in lipid or anti-oxidant profiles in control normocholesteremic animals. Therefore the herb was significantly effective in

ameliorating the lipid metabolism in hypercholesteremic animals which remained normal and unaltered in untreated animals. Also the presence of fructans as reported by Sreevidya *et al.* could also be considered as the major contributing factor in better management of hypercholesteremia. It is well known that increased HDL-cholesterol levels have a protective role in cardiovascular diseases.

9.5 Antistress Property

Tuber extracts of *C. borivillianum* have been proved to be as antistress agent. This property is assessed by swim endurance stress, anorexic test in rats and despair swim test. Cold stress induced Gastric ulceration model was also selected to evaluate antiulcer activity. The effect of single oral dose of the extracts was evaluated at 30, 100 and 300 mg/ kg. It was found that alcoholic extract significantly increases swimming time and reduces the ulcer index compared to that of control group. A significant effect ($p < 0.001$) for both the alcoholic and aqueous extracts was observed in all four models. Anti-stress effects of roots of *C. borivillianum* was also determined by Kenjale *et al.*. The aqueous extract of *C. borivillianum* (250 mg/kg for 7 days) significantly reduced the ulcer index, adrenal gland weight as effectively as the standard drug in rats. The results suggested that *C. Borivillianum* extract possess significant antistress and adaptogenic activity.

9.6 Analgesic Activity

An analgesic (also known as a painkiller) is any member of the diverse group of drugs used to relieve pain (achieve *analgesia*). Analgesic drugs act in various ways on the peripheral and central nervous systems; they include paracetamol (acetaminophen), the non-steroidal anti-inflammatory drugs (NSAIDs) such as the salicylates, narcotic drugs such as morphine, synthetic drugs with narcotic

properties such as tramadol, and various others. Panda *et al.* have reported the effectiveness of methanolic extract of *C. borivillianum* in treating pain. 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.

9.7 Anti-tumour and anti-mutagenic activity

The roots of *C. borivillianum* contain cytotoxic steroidal glycoside saponinchloromaloside-A and spirostanolpentaglycosides embracing beta-Dapiofuranose which are responsible chemicals for anticancer property. Antitumour and anti-mutagenic property of aqueous extract of roots of *C. borivillianum* were also established when he reported that skin papillomagenesis studies demonstrated a significant ($p < 0.001$) decrease in cumulative numbers of papilloma, tumour incidence, tumour burden, tumour size and tumour weight and significant ($p < 0.01$) increase in average latent period when the animals received *C. borivillianum* root extract at a dose level of 800 mg/kg body weight/day orally in double distilled water at pre, peri and post initiation stages of carcinogenesis. A significant reduction in the frequency of

chromosomal aberration and micronuclei was observed in the treated animals as compared to carcinogen controls. The present investigation suggests that *C. borivillianum* has anti-tumour, anti-mutagenic and chemomodulatory effects.

9.8 Antiulcer activity

Alcoholic extract of *C. borivillianum* show ulcer healing property. Here cold stress induced gastric ulceration model was selected to evaluate antiulcer activity. The effect of single oral dose of the alcoholic extracts at the dose of 200 mg/ kg reduces the ulcer index significantly ($p < 0.001$) compared to that of control group.

9.9 Anthelmintic activity

Saponin extract of *C. borivillianum* has Anthelmintic property when checked against *Pheretima posthuma* and *Ascaridia galli*. He used methanolic extract, crude saponin extract and purified saponin extract, Piperazine as standard drug and dist Water as control. Parameters used were time of paralysis and time of death of the worm. All extracts showed significant anthelmintic activity on selected worms. Purified saponin extract was found more active than other extracts.

9.10 Antimicrobial activity

The antimicrobial potential of *C. Borivillianum* was screened against eight bacteria and four pathogenic fungi, using microbroth dilution assay. Lowest concentration of the extract, which inhibits any visual microbial growth after treatment with p-iodo-nitrotetrazolium violet, was considered to be minimum inhibitory concentration. Water extracts of *Chlorophytum borivillianum* showed antimicrobial activity in a range of 75-1200 µg/ml.

9.11 Larvicidal activity

The larvicidal properties of *C. Borivillianum* saponin extracts (Methanolic extract, crude saponin extract, purified saponin extracts) was examined for the mosquito species *Anopheles stephensi*, *Culex quinquefasciatus* and *Aedes aegypti* on the basis of LC50 and EC50 values. All extracts found to be larvicidal activity and among them purified saponin fraction was found more effective. Recently some other activity reported includes antiviral activity of *C. borivillianum* extract which shows a potent antiviral activity BHV-1 virus29.

Antibacterial property of different extracts of *C. borivillianum* was carried out against 4 bacteria, *Staphylococcus aureus*, *E. coli*, *Pseudomonas aeruginosa* and *Bacillus subtilis*, using cup diffusion method. Acetic acid extract shows antibacterial activity against all these 4 bacteria in the order of sensitivity as *Staphylococcus aureus* *Pseudomonas aeruginosa* *E. coli* *Bacillus subtilis*.

10. Biochemistry of Safed Musli

- Carbohydrates (35-45%)
- Proteins (5-10%)
- Fibre (25-35%)
- Saponins (2-20%)

- Alkaloids (15-25%)

The Saponins and Alkaloids present in the plant are the primary source of its significant medicinal properties.

11. Other Benefits of *Chlorophytum borivilianum*

Apart from its aphrodisiac property, *Chlorophytum borivilianum* has following other health benefits -

- **Strengthens immunity** – In asthma patients it boosts the energy. Roots are used for strengthening the body's immune system.
- **Pregnancy** – The herb is used as nutritive tonic for fetus and mother both. The herb replenishes the fluids of body during parturition.
- **Obesity** – It controls and prevents obesity. It also helps in prevention of side-effects related to obesity.
- **Diabetes** – The herb is an effective cure for arthritis and diabetes.
- **Leucorrhea** – The herb is used in treatment of chronic leucorrhea and prevents premature ejaculation.
- Consumption of this herb increases HDL (or production of good cholesterol).
- Regular intake of the herb reduces hepatic lipid profiles and plasma.

11.1 Applications for Male

- a) Controls erectile dysfunction due to any reason whether psychological reasons or health problems.
- b) Stops and cures premature ejaculation.
- c) Increases sexual desire and overcome frustration and embarrassment over a sexual dysfunction, especially erectile problems.
- d) Improves energy.
- e) Provides ability to maintain the erection throughout the sexual act.
- f) Improves physical power and stamina.
- g) Improves the semen quality.
- h) Increases sperm count substantially (Plays a vital role in Fertility).
- i) Increases frequency of orgasm.
- j) Solves erectile problems.
- k) Increases the volume of ejaculation.
- l) Used for faster recovery for second orgasm.
- m) Provides extra time, extra pleasure, and extra satisfaction in sexual act.
- n) Controls premature ejaculation.
- o) Improves and promotes general well being and vitality.
- p) Helpful in prolong performance.
- q) Increases libido.
- r) Equally good for male and female.
- s) Increases sexual confidence.
- t) Yearlong action with the same intensity.

11.2 Applications for Female

- a) Controls Erectile dysfunction due to any reason whether psychological reasons or health problems.
- b) Increases sexual desire and overcome frustration and embarrassment over a sexual dysfunction.
- c) Improves energy.
- d) Checks veganism's (tightness of Vagina).
- e) Checks for vaginal dryness.
- f) Improves physical power and stamina.
- g) Improves the vaginal lubrication.
- h) Increases frequency of desire.
- i) Checks the menopause.
- j) Maintains youthfulness.
- k) Checks urinary tract infections.
- l) Checks excessive menstrual bleeding.
- m) Checks hormonal shifts and imbalances.
- n) Provides full sexual satisfaction throughout the sexual act.
- o) Checks lack of willingness.
- p) Able to respond to natural sexual urges which is a leading cause of Nervous disorders in women.
- q) Improves and promotes general well being and vitality.
- r) Develops curiosity towards the sexual act.
- s) Increases libido.
- t) Checks the loss of sensation in sexual organs.

12. Conclusion

Medicinal Plants are the source of valuable drugs of natural origin. Many active principles are still obtained in large quantities through medicinal herbs and plants till date. The drugs may effective against cancer, aging, hypercholesterolemia, hyper-lipidemia, diabetes, stress etc. Without side effects, it is the focal point of the research through-out the world. Many studies have been conducted so far on the medicinal and nutritional importance of *C. borivilianum*, the plant with potential therapeutic and nutritional uses. Traditional medicine system provided effective platform for the discovery of many new drugs. The increased cost of health care and side effects of allopathic drugs has become a driving force in the shift towards greater recognition between diet and health care. *C. borivilianum* is a better option in the current context which can be taken up as a medicinal and nutritional diet in the form of powders, chips and kind of sweets.

References

- [1]. Loo JV., Clune Y., Bennett M., Collins JK., The specificity of interaction with intestinal bacterial fermentation by prebiotics determines their physiological efficacy, Br. J. Nutr. 2005;1:91-98.
- [2]. Kothari SK and Singh K., Production technique for the cultivation of safed musli (*Chlorophytum borivilianum*), J Horticulture Sci Biotech. 2003;78:2:261-264.
- [3]. Altman A. and Ziv M., Horticultural biotechnology: a historical perspective and future prospects. In: Proceedings of the Third International ISHS Symposium

- on *In Vitro* Culture and Horticultural Breeding. (ed. A. Altman & M. Ziv). Acta Hort. 1997;446:31-49.
- [4]. Thorpe T., Morphogenesis and regeneration. In: Plant cell and tissue culture (ed. L.K. Vasil & T.A. Thorpe), Kluwer Academic Publishers, Dordrecht, Netherlands 1994; pp. 17-36.
- [5]. Steward FC., Mapes MO., Kent AE., Holsten RD., Growth and development of cultured plant cells, Biochemical and morphogenic studies with cells yield new evidence on their metabolism and totipotency Science 1964;113:20-27.
- [6]. Skoog F. and Miller CO, Chemical regulation of growth and organ formation in plant tissues cultured *in vitro*, Symp. Soc. Exp. Biol. 1957;11:118-140.
- [7]. Sharpe WR., Sondhal MR., Calder RS., Maraffa SB., The physiology of *in vitro* asexual embryogenesis. Horticultural Reviews 1980;2:268-310.
- [8]. Kozai T., Kubota C. Jeong B.R., Environmental control for the large scale production of plants through *in vitro* techniques, Plant Cell, Tissue Organ Cult. 1997;51:49-56.
- [9]. Kumar A., Palni LMS., Sood A., Sharma M., Palni UT., Gupta AK., *In vitro* propagation of Bulgarian Rose from selected mature bushes. Journal of Medicinal and Aromatic Plant Sciences 2000;22:593-603.
- [10]. Kumar A., Studies on vitro propagation, biochemistry and field evaluation of two economically important plants: *Rosa damascena* Mill. and *Gladiolus* spp. Ph.D. Thesis 1996; Kumaun University, Nainital, India.
- [11]. Purohit SD., Dave A., Kukda G., Micropropagation of safed musli (*Chlorophytum borivilianum*), a rare Indian medicinal herb, Plant Cell Tissue Organ Cult. 1994;39: 93-96.
- [12]. Joshi N., Purohit SD., Dave A., Bilochi G., Factors influencing *In vitro* culture of *Chlorophytum borivilianum*, Oikoassay 2003;15:1&2):19-27.8.
- [13]. Dave A., Bilochi G., Purohit SD., Scaling-up production and field performance of micropropagated medicinal herb 'safed musli' (*Chlorophytum borivilianum*), *In-Vitro* Cell. Dev. Biol., 2003;39:419-424.
- [14]. Kim EK., Hahn EJ., Murthy HN., Paek KY., Enhanced shoot and bulblet proliferation of garlic (*Allium sativum* L.) in bioreactor systems, J. Hortic. Sci. 2004;79:818-822.
- [15]. Akita M., Shigeoka T., Koizumi Y., Kawamura M., Mass propagation of shoots of *Stevia rebaudiana* using a large-scale bioreactor, Plant Cell Rep. 1994;13:180-183.
- [16]. Paek KY., Chakrabarty D., Hahn EJ., Application of bioreactor systems for large scale production of horticultural and medicinal plants, Plant Cell Tissue Organ Cult. 2005;81:287-300
- [17]. Kohlenbach HW., Wernicke W., Investigations on the inhibitory effect of agar and the function of active carbon in another culture, Z. Pflanzenphysiol. 1978;86: 463-472.
- [18]. Paque M., Bercetche J., Dumas E., Liquid media to improve and reduce the cost of *in vitro* conifer propagation, Acta Hort. 1992;319:95-100.
- [19]. Chu CY., Knight SL., Smith MAL., Effect of liquid culture on the growth and development of miniature rose (*Rosa chinensis* Jacq. 'Minima'), Plant Cell Tissue Organ Cult. 1993;32:329-334.
- [20]. Sandal I., Bhattacharya A., Ahuja PS., An efficient liquid culture system for tea shoot proliferation, Plant Cell Tissue Organ Cult. 2001;65:75-80.
- [21]. Kim EK., Hahn EJ., Murthy HN., Paek KY., High frequency of shoot multiplication and bulblet formation of garlic in liquid cultures, Plant Cell Tissue Organ Cult. 2003;73:231-236
- [22]. Shah Taj-Uddin MS., Qureshi NA., Chasti AR., Wani AH., Ramboo AR., Performance of *Chlorophytum borivilianum* Linn. in Kashmir., National Seminar on Organic Products and their Future Prospects, SKUAST 2003;(K), Srinagar, 98.
- [23]. Williams JGK., Kubelik AR., Livak JK., Rafalski JA., Tingey SV., DNA polymorphisms amplified by arbitrary primers are useful as genetic markers 1990;18:22:6531-6535.
- [24]. Welsh J., McClelland M., Fingerprinting genomes using PCR with arbitrary primers, Nucleic Acids Res. 1990;18:7213-7218.
- [25]. Thakur M., Dixit VK., Effect of *Chlorophytum borivilianum* root extracts on androgenic and sexual behavior in male rats, Indian Drugs 2006; In Press.
- [26]. Charlwood BV. and Pletsch M., Manipulation of natural product accumulation in plants through genetic engineering, J. Herbs Spices Med Plants 2002;9:139-151.
- [27]. Zhang L., Engineering tropane biosynthetic pathway in *Hyoscyamus niger* hairy root cultures, Proc. Natl. Acad. Sci. U. S. A. 2002;101:6786-6791.
- [29]. Facchini PJ., Toward the metabolic engineering of benzyloisoquinoline alkaloid biosynthesis in opium poppy and related species, Rec. Res. Dev. Phytochem. 2002;4: 31-47.
- [30]. Chen DH. et al. Expression of a chimeric farnesyl diphosphate synthase gene in *Artemisia annua* L. transgenic plants via *Agrobacterium tumefaciens*-mediated transformation. Plant Sci. 2002;155:179-185.
- [31]. Robbins, M.P., Sn, a maize bHLH gene, modulates anthocyanin and condensed tannin pathways in *Lotus corniculatus*, Exp. Bot. 2003;54:239-248.

- [28]. Yun DJ., Metabolic engineering of medicinal plants: transgenic *Atropa belladonna* with an improved alkaloid composition, *Proc. Natl. Acad. Sci. U. S. A.* 1992;89: 11799– 11803.
- [29]. Loo JV., Clune Y., Bennett M., Collins JK., The specificity of interaction with intestinal bacterial fermentation by prebiotics determines their physiological efficacy, *Nutrition Research Reviews* 2005;17:89-98.
- [30]. Roberfroid MB., Introducing inulin type fructans, *Br. Journal of Nutrition* 2005;93:8:1:813- 825.
- [31]. Kardosova A., Ebringerova A., Alfoldi A., Nosalova G., Franova V., Hribalova S., A biologically active fructan from the roots of *Articum lappa* L. var. *Herkules*. *Int.J. Biological Macromolecules* 2003;33:135-140.
- [32]. Ritsema T., Smeeckens S., Fructans beneficial for plants and humans, *Current opinion in Plant Biology* 2003;6: 223-230
- [33]. Thakur M., Dixit VK., Fructan; The Polymer with unexplored potential, *Indian Pharmacist* 2005;4:40:7-12.
- [34]. Nergard CS., Diallo D., Michaelsen TE., Malterud KE., Kiyohara H., Matsumoto T., Yamada H., Paulsen BS., Isolation, partial characterization and immunostimulating activity of polysaccharides from *Vernonia kotschyana* Sch. Bip. Ex Walp., *Journal of Ethnopharmacology* 2004;91:141-152.
- [35]. Vandamme Th.F., Lenourry A., Charrueau C., Chaumeil JC., The use of polysaccharides to target drug to colon, *Carbohydrate Polymers* 2002;48:219-231.
- [36]. Kaur N., Gupta AK., Applications of Inulin and Oligofructose in health and nutrition, *Journal of Biosciences* 2002;27:7:703-714.
- [37]. Mukherjee PK., Phytopharmacology in the evaluation of herbal drugs, *Journal of Pharmaceutical Research* 2002;2:45-54.
- [38]. World Health Organization (WHO), *Quality Control Methods for medicinal Plant Materials* 2000, WHO, Geneva, Switzerland, 115-116.

Source of support: Nil, Conflict of interest: None Declared