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#### Review

### Safed musli (*Chlorophytum borivilianum*): A review of its botany, ethnopharmacology and phytochemistry



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#### ABSTRACT

Ethnopharmacological relevance: Safed musli (Chlorophytum borivilianum) is an eminent medicinal plant of India and considered as a 'white gold' or 'divya aushad' in Indian systems of medicine. In Ayurveda, Chlorophytum borivilianum belongs to the group of "Vajikaran Rasayana" corroborated to its rejuvenating, aphrodisiac, natural sex tonic properties and effective in alleviating sexual disorders. It is largely used as ethnic medicine by local healers of indigenous communities of India.

Materials and methods: A thorough bibliographic investigation was carried out by analyzing worldwide accepted scientific data base (Pub Med, SciFinder, Scopus and Web of Science), thesis, recognized books, non impact and non indexed journals.

Results: Traditionally, Chlorophytum borivilianum is well known for treating male impotency in India. The multi therapeutic and nutritional importance of Chlorophytum borivilianum is attributed to the rich source of phytochemicals particularly saponins. Recently, Chlorophytum borivilianum has gained a well established domestic (Indian) and international market for being the herbal alternative of "Viagra" without any side effects. Under the trade name 'Nai Chetna', the state government of Gujarat, India, has launched a novel potency drug from Chlorophytum borivilianum. Modern pharmacological studies of Chlorophytum borivilianum have demonstrated a wide range of pharmacological activities, most importantly aphrodisiac, immunomodulatory and anticancer activities.

Conclusion: The increased commercial exploitation of Chlorophytum borivilianum and low productivity of this endangered plant has raised the concern over its conservation. It has been envisaged that efforts should be made to standardize, encourage and popularize the cultivation of Chlorophytum borivilianum as a commercial crop. The analysis of previous pharmacological investigations suggested lack of substantial scientific evidences in various studies and do not stand the test of critical assessment. Due to high economic value, Chlorophytum borivilianum has also encountered a problem of adulteration with closely resembling medicinally inferior species. The studies available on toxicity, safety and quality of Chlorophytum borivilianum are inadequate for providing information on commercial utilization. Thus, the present review summarizes comprehensive information on Chlorophytum borivilianum and possible scope for future research to fill the existing lacunae on its different aspects of the study.

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#### 1. Introduction

Chlorophytum borivilianum Santapau and Fernandes (Liliaceae) is an endangered geophyte with a broad traditional history and medicinal importance. It is commonly known as 'safed musli' in Hindi (meaning "white tubers"). Chlorophytum borivilianum is a widely growing species and an integral part of Ayurvedic, Unani, Homeopathic and Allopathic systems of medicine, where root of the plant holds principal place. Traditionally, safed musli is considered as a general health promotive tonic and has been used to treat various male sexual disorders (Thakur et al., 2009a). The knowledge on indigenous uses of Chlorophytum borivilianum has been mostly passed through oral communication from generation to generation in local spoken languages and left undocumented by various communities. The recent surveys in different states of India revealed that ethnic communities of Aravali Hills, Rajasthan (Meena), Mizorum (Mizo), Maharashtra (Pawra and Thakar) and Madhya Pradesh (Korku and Bharia) have enjoyed the health, vitality and longitivity by incorporating safed musli in their health care system (Patil, 2001; Jagtap et al., 2009; Meena and Rao, 2010; Deshwal and Trivedi, 2011; Rai and Lalramnghinglova, 2011). The indigenous people of Dhule and Nandurbar districts of Maharashtra employ the root tubers of Chlorophytum borivilianum for medicinal purposes and have initiated its cultivation, indicative of a fact that they are aware of its socioeconomic importance (Patil, 2000, 2001).

Chlorophytum borivilianum is now the most commercially exploited species due to its celebrated aphrodisiac as well as immunomodulatory properties. Only in India it has been utilized as a source of medicine and recently, its new found status as the herbal alternative to 'Viagra' raised its popularity even among western countries (Thakur et al., 2009a, 2009b, 2009c). Nowadays, USA and England are making chips/flakes with the tubers to use it as a nutritious meal (Somanath, 2008). Of late, there has been tremendous increase in the demand of this plant in Indian and International drug markets, and it is a vital entity of more than hundred herbal drug formulations (Oudhia, 2000a, 2000b; Oudhia, 2001a). Though the exact static demand in the international market is obscure, it is estimated to be much higher than the present production (Somanath, 2008). To meet the increasing demand and to stop indiscriminate exploitation of this wild plant from its natural habitat, now a farming system has been successfully introduced in some parts of India. The present review is intended to critically analyze the gaps in the scientific studies in terms of phytochemical, pharmacological and toxicological studies and relating them to ethnopharmacological and traditional claims. This review is an attempt to amalgamate the available information concluding the therapeutic utility of *Chlorophytum borivilianum* and the gaps that need research intervention. Thus, the current review is written in order to provide baseline information to the researchers who wish to carry forward the research on this plant. Also, the review will guide practitioners to utilize the holistic approach of combining traditional and modern medicine. The compilation of gaps on quality and safety aspects of the plant will assist in the development of safe and pure pharmaceuticals. Since *Chlorophytum borivilianum* is an endangered plant, the review will provide deeper insight on the importance of its conservation and future economical sustainability.

#### 2. Botany

#### 2.1. Origin and distribution

The centre of origin of the genus *Chlorophytum* is believed to be tropical and subtropical Africa and was introduced in India from South Africa. The species *Chlorophytum borivilianum* came into the prominence around late eighties. The genus *Chlorophytum* is represented by approximately 300 species of rhizomatous plants, distributed predominantly in tropical and subtropical forests of the world up to 1500 m altitude (Nayar and Sastry, 1988; Oudhia, 2001a; Raghavendra et al., 2005; Chakraborthy and Aeri, 2009). It has been reported in *Genera Plantarum* that 40 species of *Chlorophytum* are distributed in Asia, tropical Africa, America and Australia (Bentham and Hooker, 1880). In India, 13 species of *Chlorophytum* have been reported to occur, among which 6–7 species are used in indigenous system of medicine (Sheriff and Chennaveeraiah, 1972; Nair, 1974) (Table 1). While some species of *Chlorophytum* are cultivated for their ornamental values (Bordia et al., 1995).

#### 2.2. Plant description

*Chlorophytum borivilianum* Santapau and Fernandes (family: Asparagaceae; subfamily: Anthericoideae) (Santapau and Fernandes, 1955; Govaerts, 1999) has 6–16, radical, 13–23 cm × 1–2.5 cm in size, spirally

 Table 1

 Distribution of various species of Chlorophytum in India.

Species	Distribution	References
Chlorophytum arundinaceum Baker	Districts of Chhota Nagpur, parts of central India, foothills of northeast Himalaya in Assam, West Bengal and Bihar	Chadha et al. (1980), Haines (1961), Singh (1974)
Chlorophytum attenuatum Baker	Western Ghats from Karnataka southward to Coimbatore	Hooker (1894)
Chlorophytum breviscapum Dalz.	Sikkim Himalaya, Belgaum and West Pennisula	Satija and Singh (2005)
Chlorophytum borivilianum Sant and Fern.	Forest areas of southern Rajasthan, western Madhya Pradesh,	Purohit et al. (1994),
	north Gujarat, subtropical Himalayas from Kumaon, Khasia hills,	Santapau and Fernandes (1955),
	Bengal, Assam, Kokan, Kanara, West peninsula and Chennai extending to Kanyakumari	Sriram et al. (2012)
Chlorophytum glaucum Dalz., Chlorophytum orcbidastrum Lindley	Hilly ranges of Sahyadris in Western India	Naik and Nirgude (1980), Panigrahi (1975)
Chlorophytum kbasianum Hooker, Chlorophytum undulatum Wall. syn. Chlorophytum nepalense (Lindley) Baker	Eastern parts of India	Naik (1977)
Chlorophytum laxum R.Br.	Kakti Hills, Belgaum, Dharwar, Deccan peninsula	Satija and Singh (2005)
Chlorophytum malabaricum Baker	Nilgiris and Western Ghats	Naik (1977)
Chlorophytum tuberosum Baker	Parts of Konkan to Travancore in Kerala, Eastern Himalaya, Bihar and West Bengal	Satija and Singh (2005)

Table 2
Vernacular names of Chlorophytum borivilianum (Singh et al., 2012).

	Country	Language: Vernacular names
-	India Saudia Arabia	Sanskrit: Swetha musli; Hindi: Safed musli, Hazarmuli, Satmuli, Gujrati: Ujlimusli, Dholi musali; Malyalam: Shedeveli, Shedheveli; Marathi: Safed musli, Sufed musli, Kuli; Tamil: Tannirvittang, Tannirvittan-Kizhangu, Vipurutti, Taniravi thang; Telugu: Tsallogadda, Swetha musli; Sinhalese: Hirtha-wariya, Mushali; Garhwali: Jhirna; Bhojpuri: Khairuwa Arabic: Shaqaqule-hindi, Shaqaqule
	England, USA France	English: Indian spider plant, Spider plant, White musale French: Chlorophytum medicinal

imbricate at the base, sessile, linear ovate leaves; small, white, bracteates, pedicellate with joints, zygomorphic flowers arranged in alternate clusters; brown to black skinned, white after peeling, characteristic odor, tasteless, 3–20 in number, fleshy, 8–25 cm long roots; green-yellow colored, loculicidal capsule, triquetrous, bear 3–12 seeds in each fruit and small, black, angular in shape, endospermic seeds (Paturde et al., 2000; Singh et al., 2004a, 2004b, 2012; Mandal and Nandi, 2012).

#### 2.3. Vernacular name

Chlorophytum borivilianum has many common names depending upon the languages used in a particular region. The names used in different languages of India and other countries are given in Table 2.

#### 2.4. Cultivation

The cultivation of *Chlorophytum borivilianum* in India has been carried out in many parts of India *viz.* Chhattisgarh, Gujarat, Haryana, Karnataka, Madhya Pradesh, Maharashtra, Rajasthan and Uttar Pradesh. It is usually grown in an area more than 400 ha for its tuberous roots (Kothari and Singh, 2004). Plants remain in active growth stage or reach maturity at 80–90 days after planting and development of new fleshy roots take place after 40 days. Harvesting is usually carried out during the month of March–April. The lengthy and thick tubers of the harvested crop are removed (50–70%) from disc during October–November, the remaining small and thin tubers (fingers) along with disc are stored for planting in next cropping season. There are various conditions required for optimum growth and development of

Chlorophytum borivilianum for the high yield. The climate and soil conditions, propagation, planting methods, nutrient management, weed and pest control along with intercropping plays a pivotal role in attaining better yield.

#### 2.4.1. Climate and soil

Chlorophytum borivilianum needs warm and humid climate for optimum plant growth and tuber development. The areas receiving 50–150 cm annual rainfall (July–October) are considered as suitable for cultivation (Kharif crop). The very high temperatures (35 °C and above) do not favor the growth and porous soil with high organic matter content helps in development of fleshy tubers (Singh et al., 2003). According to Paturde et al. (2000) soil pH range of 5.0–8.0 is optimum for safed musli. The soil pH above 8 greatly affects the availability of macro and micronutrients of Chlorophytum borivilianum (Bordia et al., 1995; Singh et al., 2001b).

#### 2.4.2. Propagation

Chlorophytum borivilianum is usually propagated by vegetative means and less frequently by seed germination as seeds have poor germination (5-13%) rate, whereas, closely related species Chlorophytum tuberosum showed 28-62% germination (Dalal, 1987; Trivedi and Yadav, 1989; Jat, 1993; Shrivastava et al., 2001; Satyabrata and Geetha, 2005). The seeds collected from natural habitat reported to have 11-24% germination (Bordia and Jat, 1990). Each seedling produces only one unit of planting material and has comparatively higher sprouting and establishment (95-100%) than the tubers. The vegetative propagation of Chlorophytum borivilianum is carried out by splitting of disc from previous year production, with each disc or sprouts containing 1-3 fleshy tubers and weighs about 5 g (Bordia et al., 1995). Since, cultivation using tubers are costly and labor intensive, Kaur et al. (2009) projected the positive influence on germination percentage of Chlorophytum borivilianum seeds by various treatments of plant growth regulators (indole butyric acid, kinetin and 24-epibrassinolide) and steroidal hormones (testosterone and cholesterol). In another study, higher yield of tubers was achieved by pre-treatment of propagule root tubers with potassium salts instead of using expensive hormones (Tyagi and Sharma, 2011).

#### 2.4.3. Planting

Planting of *Chlorophytum borivilianum* is carried out just before or after the onset of rains and optimum time of planting varies from place to place. Bordia et al. (1995); Chandra et al. (2003, 2007)

and Dalal et al. (1987) revealed that different types of beds (flat, ridge and furrow, double row raised bed and triple row raised bed) and plant spacing efficiently contribute to the yield of *Chlorophytum borivilianum*, depending upon the soil texture and amount of rainfall. Chandra et al. (2003) recorded the spacing of  $30~\rm cm \times 10~\rm cm$  with 333,000 plant population/ha for the maximum fresh root yield (3.46 t/ha) under hot semi-arid ecoregion. The other workers suggested planting at a distance of  $30~\rm cm \times 15~\rm cm$  or  $30~\rm cm \times 10~\rm cm$  (Bordia et al., 1995; Dalal, 1987). The optimum weight of planting material was reported as 1 g (350 kg tubers/ha) and 12.5 g/unit (Kothari and Singh, 2001, 2003; Paturde et al., 2000). Recently, Yaseen et al. (2013) reported the maximum dry root yield of safed musli by utilizing 75% PAR (photosynthetically active radiation).

#### 2.4.4. Nutrient management

The studies conducted at CIMAP (Central Institute of Medicinal and Aromatic Plants) demonstrated that the nutrient requirements for the growth of Chlorophytum borivilianum, particularly, nitrogen (N), phosphorus (P) and potassium (K) are very low. The various workers recommended the introduction of N, P2O5 and K2O in the range of 25-75, 60-65 and 2-30 kg/ha, respectively, for higher tuber yield (Paturde et al., 2000; Anonymous, 2002; Kothari and Singh, 2003; Singh et al., 2004a; Wankhade et al., 2004). The studies showed that application of NPK in the ratio of 25:25:25, 50:50:50 and 45:60:60 kg/ha or farmyard manure (FYM) (10–40 t/ha) alone or in combination with NPK greatly enhances the yield of fresh tubers (Singh et al., 2001a; Chouhan and Joshi, 2002; Paturde et al., 2002; Kothari and Singh, 2003; Wankhade et al., 2004; Ingle et al., 2004; Anonymous, 2003, 2005b; Chauhan et al., 2005). The availability of water in the fields also directly effects the uptake of primary (NPK), secondary (S, Mg, Ca, Fe) and micro (Cu, Zn, Mn) nutrients in Chlorophytum borivilianum (Vijaya and Chavan, 2009a).

#### 2.4.5. Intercropping

The various intercrop combination of safed musli includes pigeon pea, cow pea, lablab bean, maize, black gram, bottle gourd, okra, sweet basil, sacred basil and mustard (Bordia et al., 1995; Chouhan and Joshi, 2000). The intercropping of pigeon pea and bottle gourd with safed musli is most advantageous in terms of overall yield, land equivalent ratio (LER), monetary advantage and economic return. These combinations gave an additional yield of 49.82 t/ha of bottle gourd and 6.51 t/ha of pigeon pea without significantly reducing the root yield of *Chlorophytum borivilianum* (Singh et al., 2011).

#### 2.4.6. Weeds

Chlorophytum borivilianum is predominantly invaded by rainy season grasses and Cyperus rotundus L. accounted for 62.0% of the total weeds population; followed by 33.9% of other species. The important grass species includes Dactyloctenium aegyptium (L.) Beauv., Digitaria sanguinalis (L.) Scope, Eleusine indica (L.) Gaertn and Phyllanthus amarus Schum and Thonn, the only dicot

weed present during rainy season. The winter season weeds include *Melitolus indica* and *Gnaphalium indicum* L., and comprise 82.6% and 17.4% of the weed population, respectively. *Parthenium hysterophorous* L. (Parthenium), *Argemone maximana* L. (Satyanasi), *Cyperus scariosus* (Nagarmotha) and *Lantana* are very common and competitive weeds of *Chlorophytum borivilianum* (Oudhia, 1996; Singh et al., 2003). Manual weeding is highly effective in reducing the weed population after 15–20, 25–30 and 50–55 days of planting (Singh et al., 2001b).

#### 2.4.7. Diseases and pests

Chlorophytum borivilianum is usually affected by leaf spots, leaf blight, collar rot, tuber rot and rust diseases (Table 3). The administration of propiconazole (0.1%), tridemorph (0.1%), Zineb+hexaconazole (0.2%), dithane M-45 (0.25%) showed high inhibition in Colletotrichum dematium and Alternaria alternate growth (Tekade et al., 2009).

In summary, efforts should be taken to standardize the cultivation techniques of safed musli. There is a broad scope to study the different parameters of cultivation, depending upon the geographical location; to optimize higher yield. The propagation carried out by treating with hormone is a matter of concern; as there may be likelihood of hormone deposition in roots and analysis might be mandatory before using it as a medicine. There is a call for extensive research to find economical and environmental friendly alternative to enhance propagation by seeds. The reports showed that tuber yield can also be greatly improved by using FYM and hence, organic farming should be encouraged in spite of using synthetic fertilizers. The modern techniques for cultivation including intercropping should be employed to gain maximum benefits of two crops at the same growing season. Timely weeding and pest control management may be implemented to prevent the possible loss of the product.

#### 3. Medicinal aspects of Chlorophytum borivilianum

#### 3.1. Ethnopharmacological uses

The root tubers of *Chlorophytum borivilianum* are used as indigenous drug in India for many ailments and health related problems since 11th century A.D. (Vijaya and Chavan, 2009b). The ancient Indian system of medicine, Ayurveda, holds a very special position for *Chlorophytum borivilianum* and is associated with 'Caitha' as one of the divine plant. It is also considered as 'divya aushad' (divine medicine) in Ayurveda. The Ayurveda describes the properties of *Chlorophytum borivilianum* as sweet, bitter (*Rasa*), moist, unctuous (*Guna*), cold (heavy *Virya*) and sweet post digestive effect (*Vipaka*) (Paques and Boxus, 1987; Toth et al., 1994).

According to the Hindu epic, Srimad Bhagawat, the use of *Chlorophytum borivilianum* dates back to about 4000 years. It is a member of a special group of Ayurvedic plants known as "Vajikaran Rasayana", which are used for rejuvenating, revitalizing properties

**Table 3** Diseases of *Chlorophytum borivilianum*.

Diseases	Causative agents	References
Leaf spots Rust Collar rot Tuber rot Leaf blight Tuber infection by nematodes Tuber infection by insects	Colletotrichum chlorophyti, Colletotrichum dematium Uromyces loculiformis, Uromyces clignii Corticum rolfsii Fusarium solani, Rhizoctonia bataticola Colletotrichum capsici, Colletotrichum dematium, Alternaria alternate Meloidogyne incognita, Tylenchorhynchus brevilineatus, Helicotylenchus indicus Zonabris pustulata (orange banded blister beetle)	Mukherji and Bhasin (1986) Mukherji and Bhasin (1986) Singh et al. (2001a) Raghavendra et al. (2005), Tekade et al. (2009) Sattar et al. (2006), Tekade et al. (2009) Singh et al. (2004a) Oudhia (2000c)
Tuber spoilage during storage	Aspergillus spp., Fusarium spp.	Bordia et al. (1995)

for improving sexual dynamics (Puri, 2003) and alleviating sexual dysfunction (Triveni, 1977). This is also the basis of drugs recommended in the Kamasutra (Hooper, 2002). It is mentioned in the Hindu scriptures, Ashwini Kumars, a divine physician, prepared the 'chyawanprash' for Chyavanrishi, who married at the age of 80 years, from safed musli for improving sexual health. Chlorophytum borivilianum was described as 'Vajikaran,' also in other ancient Ayurvedic literature such as Bhavaprakash Nighantu, Rasendra Sarsangrah and Raja Ballabh Nighantu. The different treatise of Ayurveda and Siddha mentioned 30–35 medicinal plants having 'Rasayana' properties including Chlorophytum borivilianum. Shushruta defined 'Rasayana' as a therapy that arrests ageing (Vayasthapam), increase life span (Ayushkaram), intelligence (Medha) and strength (Bala) (Pushpangadan et al., 2012).

Traditionally, Chlorophytum borivilianum roots are well known for the treatment of male impotency, oligospermia and are a special type of immunomodulator (Kirtikar and Basu, 1956; Triveni, 1977; Tandon and Shukla, 1995; Sharma et al. 1999; Puri, 2003; Kothari and Singh, 2004; Mishra 2005; Singh et al., 2012). They have carminative, anti-pyretic, diuretic and astringent effects (Kirtikar and Basu, 1975). The roots are also used as lactogogue and appetizing agent (Bhandary et al., 1995; Chetty and Rao, 1989). In some regions of Mewar, India, tubers have been used to treat erectile dysfunction (Deshwal and Trivedi, 2011). The action of Chlorophytum borivilianum tubers on the central nervous system is similar to Ginseng and is popularly considered as the 'Indian Ginseng' because of its miraculous therapeutic potential (Singhania, 2003). Apart from rejuvenating the reproductive system, Chlorophytum borivilianum prevents premature ejaculation and strengthens the general immune system of the body. It is known for improving strength and physical endurance, fighting general body weakness, building muscle mass and aid in recovering from the physical exhaustion and fatigue. The roots are regarded as an energy booster also in asthmatic patients. The root powder along with other herbal materials has been utilized in the preparation of several Ayurvedic and Unani health tonics (Haque

In western part of India, especially Gujarat, consuming a spoon of safed musli, twice a day with milk is a part of the daily health care regime. The leaves are eaten by the indigenous people of Western Ghats of India as an expectorant (Sebastian and Bhandari, 1988; Anonymous, 1992). The roots are utilized as a nutritive tonic for both expecting women and the fetus during pregnancy and are used to replenish the body fluids in the post-partum stage (Singh et al., 2013). The fleshy tubers of safed musli are one of the ingredients in the preparation of special type of laddoos (ball shaped sweet), consumed by women in their diets as an energizing food after delivery. The dried root powder also increases lactation amongst the nursing mothers and lactating cows, and is considered as remedial for various gynecological disorders, gonorrhoea, leucorrhoea, pre- and post-natal symptoms (Deshwal and Trivedi, 2011). Besides, Chlorophytum borivilianum tubers are used in the treatment of joint pains, rheumatism and arthritis (Singh et al., 2013). It is recognized for removing the knee pain within a week if consumed daily with milk (Elizabeth, 2001; Singhania, 2003; Singh and Chauhan, 2003). The tubers of Chlorophytum borivilianum have been effectively used by Meena community of Rajasthan, India, against bone fracture, leucorrhea as well as a male tonic (Meena and Rao, 2010).

Rai and Lalramnghinglova (2011) recently presented a study of 302 plants from 96 families and used as medicine by indigenous Mizo and other communities in North East Indian region over last decade. Among these plants, *Chlorophytum borivilianum* has been placed as a main source for metabolic enhancer and aphrodisiac. The Bharia and other communities of Patalkot valley near Chindwara district of Madhya Pradesh employ safed musli as a native therapy for health care, particularly to improve immunity. The local healers

known as Bhumkas or some older people know the system of healing. The ethnobotanical survey conducted in Korku dominated forest areas of Madhya Pradesh revealed that 38 plant species were used by Korku population for food, fodder, oil and medicine including Chlorophytum borivilianum (Anonymous, 2005a). The traditional uses of Chlorophytum borivilianum tubers against other diseases like diabetes mellitus, dysuria, diarrhoea and dysentery are well documented in the literature (Negi et al., 1993; Oudhia, 2001a; Purohit and Prajapati, 2003; Dabur et al., 2007). The root powder fried in clarified butter is chewed in case of aphthae of mouth and throat and also taken orally as supplementary therapy for blood purification and delaying ageing process. It is also considered as an effective drug to control and prevent obesity and its side effects. The roots are also reported to have cosmetic applications. The paste of the root with goat milk or honey can be applied locally over the face to brighten the skin complexion (Singh et al., 2013). Even though Chlorophytum borivilianum shows no adverse effect but in case of over dosing it may lead to gastrointestinal disorders. Apart from folklore, the leaves of Chlorophytum borivilianum are used as vegetable in various culinary preparations in Maharashtra and Goa (Vartak, 1981; Patil, 2000).

#### 3.2. Phytochemistry

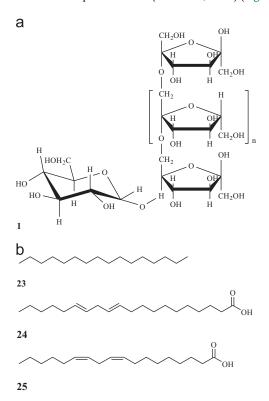
Chlorophytum borivilianum contains wide range of phytochemicals such as saponins, alkaloids, flavonoids and phenolic acids (Visavadiya et al., 2010). The roots of Chlorophytum borivilianum have 42% carbohydrate, 8–9% protein, 3–4% fibers and 2–17% saponin (Bordia et al., 1995; Wagle et al., 2000). The other workers reported that the root constitutes about 30% alkaloids, 10–20% saponins, 40–45% polysaccharide (mucilage) and 5–7% protein (Tandon et al., 1992; Haseeb et al., 2007; Mayank and Dixit, 2008; Deore and Khadabadi, 2009a). In addition, Chlorophytum borivilianum also contains steroid, triterpenoids, gallo-tannins, vitamins, potassium, calcium, magnesium, rare elements such as Zn, Cu, P, resins and high quantity of simple sugars mainly sucrose, glucose, fructose, galactose, mannose and xylose (Kokate et al., 2004; Thakur and Dixit, 2005; Singh et al., 2012).

Although there is a lack of extensive chemical investigation on Chlorophytum species, still several saponins, sapogenins, furostane and spirostane glycosides have been isolated in recent years (Kaushik, 2005). However, studies on secondary metabolites of Chlorophytum borivilianum are scanty. Nevertheless, there are some reports on qualitative analyses of phytochemicals in Chlorophytum borivilianum. The researchers from National Research Centre for Medicinal and Aromatic Plants (NRCMAP), Boriavi, India, have studied chemical composition of Chlorophytum borivilianum fasciculated root extracts, qualitatively, by comparing high performance thin layer chromatography (HPTLC) chromatogram of related species, Chlorophytum aurundinaceum. Both species exhibited similarity in majority of their peaks (Phurailatpam et al., 2009). Kothari and Singh (2004) were the first to report the inulin type  $2 \rightarrow 1$  linked fructans by comparative reverse phase high-pressure anion exchange (RP-HPAE) chromatography. Later Narasimhan et al. (2006) have successfully isolated for the first time, the same fructo-oligosaccharide from Chlorophytum borivilianum and identified as  $O-\beta-D$ -fructofuranosyl- $(2 \rightarrow 1)$ - $(\beta-D$ -fructofuranosyl)<sub>n</sub>-(2 $\rightarrow$ 1)- $\alpha$ -D-glucopyranoside (n=5-30) (1) using HPAE chromatography (Fig. 1a). The extract and inulin type  $2 \rightarrow 1$  linked fructans showed significant antidiabetic activity in streptozotocin-induced diabetic animals. A moderate decrease in blood sugar level (118.32  $\pm$ 3.56,  $110.21 \pm 4.22$ ) was observed on 21st day of extract and compound (10 mg/kg) administration in male albino rats; as compared to the disease control group (231.25  $\pm$  3.03).

Chlorophytum borivilianum is extensively used as raw drug and there are numerous studies merely on the extracts of the plant. These important biological activities owe to their high saponin content (Estrada et al., 2000; Liu and Henkel, 2002), chiefly present in underground tubers (Marker et al., 1943; Pullaiah, 2002), like ginseng root (Panax ginseng), well known as immunomodulator and aphrodisiac in traditional oriental medicines and worldwide (Fukuda et al., 2000). Among all the species of Chlorophytum present in India, Chlorophytum borivilianum roots produces highest saponin content and is the most popular species against male sexual problems (Bordia et al. 1995). In the course of the search for novel saponins from Chlorophytum borivilianum Acharya et al. (2008a, 2008b, 2009) have performed significant work, which is discussed in the proceeding text.

#### 3.2.1. Saponins of Chlorophytum borivilianum

The saponins are reported to form the main constituents of many herbal drugs and folk medicines, and are accountable for numerous pharmacological properties (Estrada et al., 2000; Sparg et al., 2004). The surface-active properties of saponins differentiate them from other glycosides (Tyler et al., 1981). In *Chlorophytum borivilianum* steroidal spirostane and steroidal furostane type of saponins have been reported so far (Bruneton, 1995) (Fig. 2).



**Fig. 1.** Miscellaneous compounds isolated from *Chlorophytum borivilianum*: (a) Fructans (n=5-30) (compound 1). (b) Long chain hydrocarbon (compound 23) and fatty acids (compounds 24–25).

The saponins, stigmasterol (2) and hecogenin (3) (Fig. 3), are major secondary metabolites of Chlorophytum borivilianum, responsible for aphrodisiac, antioxidant, anticancer activities and immune boosters (Kumar et al., 2011a; Patel et al., 2011). Stigmasterol is similar to the structure of testosterone and primarily contributes to the aphrodisiac activity of Chlorophytum borivilianum. In parallel way, hacogenin helps in the production of anabolic hormones. There are reports on improved production of medicinally important stigmasterol and hacogenin through in vitro cultures of Chlorophytum borivilianum (Bathoju and Giri, 2012a and b). The HPTLC (high performance thin layer chromatography) and HPLC (high performance liquid chromatography) analyses were performed by various researchers for qualitative analysis of saponins and to standardize the saponin and sapogenin of Chlorophytum borivilianum (Joshi et al., 2000; Govindarajan et al., 2005; Narasimhan et al., 2006; Thakur et al., 2009a). Recently, Sharma et al. (2012) reported an improved method for detection of saponins. The suspension of sheep erythrocytes was used to develop TLC plates in which saponin appeared as white spots against pink background. Barve et al. (2010) gave optimum parameters for the effective extraction of saponins from Chlorophytum borivilianum tubers. According to this study, material of extraction with mesh size 30/60 in water or methanol at 60 °C attained appreciable yield of saponins after 4 h.

Till date only a few studies have been reported on the systematic isolation of saponins. Lately, Acharya et al. (2008a, 2008b, 2009) have reported more than 20 steroidal saponins from butanol (n-BuOH) layer of ethanol extract of roots (Table 4). These saponins were isolated and identified through several chromatographic steps (VLV, FLASH, and MPLC) over silica gel and RP-18 column and various spectral techniques, respectively. Acharya et al. (2007) isolated for the first time four new insecticidal and cytotoxic furostane type saponins. The further investigation of n-BuOH layer of ethanolic root extract of Chlorophytum borivilianum led to the isolation of six new steroidal saponins (Acharya et al., 2008a). Among the six new steroidal saponins, two were furostanol (4, 5) and four were spirostanol (6–9) steroid saponins. The isolated furostanol saponins were structural analogues with the same sugar sequence. MTT (methylthiazolyldiphenyl-tetrazolium bromide) cytotoxic assay of these compounds showed moderate cytotoxic activity in human colorectal cancer cell lines (HCT 116 and HT 29), in range of 9-85 μg/ml against paclitaxel as positive control. These compounds also exhibited moderate insecticidal activity against Helicoverpa armigera. In another study performed by Acharya et al. (2008b) in the same year, four new furastanol steroid saponin, borivillianoside A, B, C and D (10-13) along with two known compounds, has been obtained (14,15). The sugar moieties present in borivillianoside A and B were identified as glucose, galactose, and rhamnose, while borivillianoside C and D showed the presence of glucose, galactose, and xylose. The GC analyses revealed the absolute configurations of the sugars and were determined to be D for glucose, galactose, and xylose and L for rhamnose. Recently, Acharya et al. (2009) obtained another four new spirostane-type saponin, borivillianoside E, F, G and H (16-19), from ethanol extract of roots together with two known steroid saponins (20, 21). The borivillianoside H exhibited significant toxicity against HCT 116

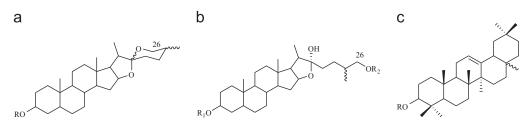


Fig. 2. Algycone skeletons of saponins. (a) Steroidal spirostane, (b) steroidal furostanes, (c) triterpenoid saponins, R=sugar moiety (Sparg et al., 2004).

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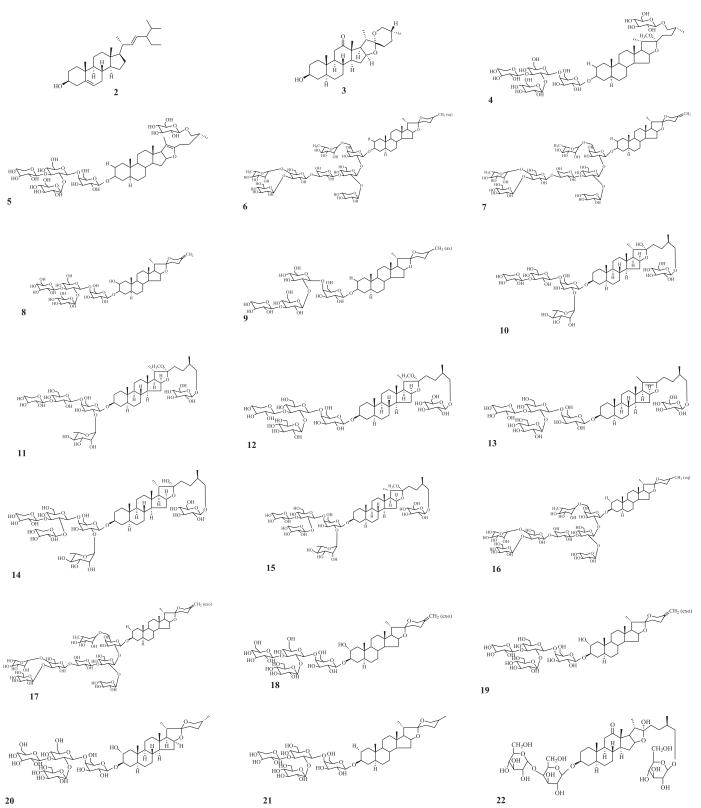


Fig. 3. Chemical structure of saponins from Chlorophytum borivilianum (compounds 2–22).

 $(0.38~\mu M)$  and HT 29  $(2.6~\mu M)$  cell lines in MTT assay. In the study paclitaxel was taken as positive control which exhibited IC50 values of 1.1 nM (HCT 116) and 3.6 nM (HT 29).

More recently, Deore and Khadabadi (2010a) isolated a novel furostanol saponin, chlorophytoside-I, from methanolic extract of *Chlorophytum borivilianum* roots (22). In addition stigmasterol (2)

(Fig. 3), a long chain hydrocarbon, hexadecane (23), and two long chain fatty acids, 11, 14 eicosadienoic acid (24) and linoleic acid (25), were obtained from methanolic root extracts (Fig. 1b).

Briefly summarizing, phytochemical examinations carried out on *Chlorophytum borivilianum* are limited and most of the compounds (saponins) have been isolated merely from butanol fraction of

Table 4
Saponins isolated from *Chlorophytum borivilianum* roots.

UPAC name/trivial name	Skeleton type	Activity (in vitro)	References
25R)-3 $\beta$ ,5 $\alpha$ ,22 $\alpha$ -22-methoxyfurostan-3,22,26-triol 3-0- $\beta$ -D-xylopyranosyl-(1 $\rightarrow$ 3)-[ $\beta$ -D-glucopyranosyl-(1 $\rightarrow$ 4)- $\beta$ -D-galactopyranosyl-26-0- $\beta$ -D-glucopyranoside (4)	Furostane	Cytotoxic activity (9–85 µg/ml), insecticidal activity (moderate)	Acharya et al. (2008a)
$^{1.5}$ R)-3β,5α,furost-en-20(22)-3,26-diol 3-O-β-b-xylopyranosyl-(1 $\rightarrow$ 3)-[β-b-glucopyranosyl-(1 $\rightarrow$ 2)]-β-b-glucopyranosyl-(1 $\rightarrow$ 4)-β-b-galactopyranosyl-26-O-β-b-glucopyranoside (5)	Furostane	Cytotoxic activity (9–85 µg/ml), insecticidal activity (moderate)	Acharya et al. (2008a)
5R)-3 $\beta$ -5 $\alpha$ -spirostan-3-ol 3- $\Omega$ - $\alpha$ -t-rhamnopyranosyl (1 $\rightarrow$ 4)-[ $\beta$ -D-glucopyranosyl-(1 $\rightarrow$ 3)]- $\beta$ -D-glucopyranosyl-(1 $\rightarrow$ 2)]- $\alpha$ -t-arabinopyranosyl-(1 $\rightarrow$ 3)]- $\beta$ -D-glucopyranosyl-(1 $\rightarrow$ 2)-[ $\alpha$ -t-rhamnopyranosyl-(1 $\rightarrow$ 6)]- $\beta$ -D-galactopyranoside (6)	Spirostane	Cytotoxic activity (9–85 µg/ml), insecticidal activity (moderate)	Acharya et al. (2008a)
-5α spirost-ene-25(27) -3-ol 3-0-α-i-rhamnopyranosyl(1 – 4)-[β-ρ-glucopyranosyl-(1 → 3)  β-ρ-glucopyranosyl-(1 → 3)-α-i-arabinopyranosyl-(1 → 3)[β-ρ-yx)lopyranosyl-(1 → 2)[β-ρ-glucopyranosyl-(1 → 2)[α-i-rhamnopyranosyl-(1 – 6)]β-ρ-galactopyranoside (7)	Spirostane	Cytotoxic activity (9–85 µg/ml), insecticidal activity (moderate)	Acharya et al. (2008a)
-5α- spirost-ene-25(27) -2,3-diol 3-0-β-p-glucopyranosyl-(1→3)-[β-D-glucopyranosyl-(1→2)] -β-p-glucopyranosyl-(1→4) -β-D-galactopyranoside (8)	Spirostane	Cytotoxic activity (9-85 µg/ml), insecticidal activity (moderate)	Acharya et al. (2008a)
SS)-3β-5α-spirostane-3-ol 3-0-β-D-xylopyranosyl-(1→3)-β-D-glucopyranosyl-(1→2)-β-D-glucopyranosyl-(1→4)-β-D-galactopyranoside (9)	Spirostane	Cytotoxic activity (9-85 µg/ml), insecticidal activity (moderate)	Acharya et al. (2008a)
$\beta$ ,5 $\beta$ ,22 $R$ ,25 $R$ )-26-( $\beta$ -D-glucopyranosyloxy)-22-hydroxyfurostan-3-yl O- $\beta$ -D-xylopyranosyl-(1 $\rightarrow$ 3)-O- $\beta$ -D-glucopyranosyl-(1 $\rightarrow$ 4)-O-[ $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 2)]- $\beta$ -D-galactopyranoside/Borivilianoside A (10)	Furostane	Negative cytotoxic activity against HCT 116 and HT 29 cell lines (Acharya et al., 2009)	Acharya et al. (2008b)
$3,5\alpha,22R,25R$ )- $26-(\beta-p-glucopyranosyloxy)-22-methoxyfurostan-3-yl O-\beta-p-xylopyranosyl-(1 \rightarrow 3)-O-\beta-p-glucopyranosyl-(1 \rightarrow 4)-O-(\alpha-\beta-rhamnopyranosyl-(1 \rightarrow 2)-\beta-p-galactopyranoside/Borivilianoside B (11)$	Furostane		Acharya et al. (2008b)
$3.5\alpha$ ,22R,25R)-26-( $\beta$ -0-glucopyranosyloxy)-22-methoxyfurostan-3-yl O- $\beta$ -0-xylopyranosyl-(1 $\rightarrow$ 3)-O- $\beta$ -0-xylopyranosyl-(1 $\rightarrow$ 2)-O- $\beta$ -0-glucopyranosyl-(1 $\rightarrow$ 4)- $\beta$ -0-galactopyranoside/Borivilianoside (12)	Furostane	Negative cytotoxic activity against HCT 116 and HT 29 cell lines (Acharya et al., 2009)	Acharya et al. (2008 b)
$\beta$ ,5α,25 R)-26-(β-D-glucopyranosyloxy)furost-20(22)-en-3-yl O-β-D-xylopyranosyl-(1 $\rightarrow$ 3)-O-[β-D-glucopyranosyl-(1 $\rightarrow$ 2)]-O-β-D-glucopyranosyl-(1 $\rightarrow$ 4)-β-D-galactopyranoside/Borivilianoside D (13)	Furostane		Acharya et al. (2008 b)
$3.5\alpha_*22R_*25R_*)-26-(\beta-o-glucopyranosyl-(1-2)-0-[\beta-o-xylopyranosyl-(1-2)-0-[\beta-o-xylopyranosyl-(1-2)]-\beta-o-glucopyranosyl-(1-4)-0-[\alpha-t-rhamnopyranosyl-(1-2)]-\beta-o-glactopyranoside/[Tribuluside A (14)]$	Furostane (known), earlier reported by Xu et al. (2007)		Acharya et al. (2008 b)
$1,5\alpha,22R,25R$ )- $26-(\beta-b-glucopyranosyl-(1\rightarrow 2)-0-p-b-xylopyranosyl-(1\rightarrow 2)-0-p-b-xylopyranosyl-(1\rightarrow 3)]-0-p-b-glucopyranosyl-(1\rightarrow 4)-0-[\alpha-i-rhamnopyranosyl-(1\rightarrow 2)]-p-b-alactopyranoside (15)$	Furostane (known), earlier reported by Achenbach et al. (1994) from <i>Tribulus cistoides</i>		Acharya et al. (2008 b)
(R)- $5\alpha$ -spirostan- $3\beta$ -ol $3$ - $0$ - $\alpha$ -t-rhamnopyranosyl-(1 $\rightarrow$ $4$ )- $[\beta$ - $0$ -glucopyranosyl-(1 $\rightarrow$ $3$ )- $[\beta$ - $0$ -glucopyranosyl-(1 $\rightarrow$	Spirostane		Acharya et al. (2009)
-spirost-25(27)-ene-3 $\beta$ -ol 3-O- $\alpha$ -t-rhamnopyranosyl (1 $\rightarrow$ 4)-[ $\beta$ -D-glucopyranosyl-(1 $\rightarrow$ 3)]- $\beta$ -D-glucopyranosyl-(1 $\rightarrow$ 3)]- $\beta$ -D-glucopyranosyl-(1 $\rightarrow$ 3)]- $\beta$ -D-glucopyranosyl-(1 $\rightarrow$ 2)]- $\beta$ -D-glucopyranosyl-(1 $\rightarrow$ 2)]- $\beta$ -D-glucopyranosyl-(1 $\rightarrow$ 3)]- $\beta$ -D-glucopyranosyl-(1 $\rightarrow$	Spirostane	Negative cytotoxic activity against HCT 116 and HT 29 cell lines	Acharya et al. (2009)
-spirost-25(27)-ene-2 $\alpha$ ,3 $\beta$ -diol 3-O- $\beta$ -D-glucopyranosyl-(1 $\rightarrow$ 3)-[ $\beta$ -D-glucopyranosyl-(1 $\rightarrow$ 2)]- $\beta$ -D-glucopyranosyl-(1 $\rightarrow$ 4)- $\beta$ -D-galactopyranoside/Borivilianoside G (18)	Spirostane	Negative cytotoxic activity against HCT 116 and HT 29 cell lines	Acharya et al. (2009)
-spirost-25(27)-ene-2 $\alpha$ ,3 $\beta$ -diol 3- $\theta$ - $\beta$ -D-xylopyranosyl-(1 $\rightarrow$ 3)-[ $\beta$ -D-glucopyranosyl-(1 $\rightarrow$ 2)]- $\beta$ -D-glucopyranosyl-(1 $\rightarrow$ 4)- $\beta$ -D-galactopyranoside/Borivilianoside H (19)	Spirostane	Cytotoxic activity (0.38 µM in HCT 116 cell lines and 2.6 µM in HT 29 cell lines)	Acharya et al. (2009)
ogenin 3-0- $\beta$ -D-glucopyranosyl- $(1 \rightarrow 3)$ - $[\beta$ -D-glucopyranosyl- $(1 \rightarrow 2)]$ - $\beta$ -D-glucopyranosyl- $(1 \rightarrow 4)$ - $\beta$ -D-glacopyranoside (20)	Spirostane (known), earlier reported by Mimaki et al. (1996) from Chlorophytum comosum		Acharya et al. (2009)
R,S)- $5\alpha$ -spirostan- $3\beta$ -ol $3$ -O- $\beta$ -D-xylopyranosyl- $(1\to 3)$ - $[\beta$ -D-glucopyranosyl- $(1\to 2)]$ - $\beta$ -D-glucopyranosyl- $(1\to 4)$ - $\beta$ -D-galactopyranoside (21)	Spirostane (known), first report from genus <i>Chlorophytum</i> Previously reported by Ikeda et al. (2003) from <i>Solanum</i> plants	Negative cytotoxic activity against HCT 116 and HT 29 cell lines	Acharya et al. (2009)
$\beta$ , $5\alpha$ , $22R$ , $25R$ )- $26$ - $(\beta$ -D-glucopyranosyloxy)- $22$ -hydroxyfurostan- $12$ -one- $3$ yl $0$ - $\beta$ -D-galactopyranosyl- $(1 \rightarrow 4)$ glucopyranoside/Chlorophytoside-I ( <b>22</b> )	Furostane		Deore and Khadabadi (2010a)
β-hydroxy-24-ethyl 5,22-cholestadiene/stigmasterol (2) x-Spirostan-3β-ol-12-one/Hacogenin (3)	Sterol (known) Spirostane (known)		Deore and Khadabadi (2010a) Bathoju and Giri (2012a, 2012b)

**Table 5**Recent pharmacological activities of genus *Chlorophytum*.

Extract/active compound	Activity	Species	References
Root extract	Anticancer, antitumor	Chlorophytum comosum	Matsushita et al. (2005), Mimaki et al. (1996)
Chloromaloside-A	Anticancer	Chlorophytum malayense	Qiu et al. (2000)
Root extract	Immunomodulatory,	Chlorophytum arundinaceum	Kumar et al. (2011a),
	antiulcer, antimicrobial		Rachchh et al. (2004), Valya et al. (2009)
Homoisoflavonoid	Antimycobacterial	Chlorophytum inornatum	O'Donnell et al. (2006)
Shoot extract	Antifungal	Chlorophytum nimonii	Lakshmi et al. (2007)
Chloragin (shoot)	Antihyperglycaemic, antidyslipidaemic	Chlorophytum nimonii	Lakshmi et al. (2009)
Root extract	Anticancer	Chlorophytum orchidastrum	Acharya et al. (2010)

ethanol root extract. There is a scope to investigate different extracts and the other parts of this plant such as leaves and flowers. The studies conducted by researchers revealed that the saponins can be extracted efficiently by simple techniques in combination with improved methods and also *in vitro* techniques can be utilized for large scale production of saponins for medicinal application. Further, bioassay guided isolation of the compounds should be encouraged which might lead to the discovery of more active secondary metabolites. The preliminary phytochemical studies evidenced the presence of various classes of beneficial compounds, so their isolation and biological studies need more attention.

#### 3.3. Pharmacology

Even though several traditional uses of Chlorophytum borivilianum are recognized, however, a scientific validity and supporting evidence is a pre-requisite for commercial exploitation. In the genus Chlorophytum, many species are known to possess medicinal value in traditional system and their supporting pharmacological importance was also identified (Table 5). In contrast Chlorophytum borivilianum also represented a wide range of pharmacological properties including antimicrobial (Sriram et al., 2012), analgesic (Panda et al., 2007), anti-inflammatory (Panda et al., 2011c), antipyretic (Panda et al., 2011d), hepatoprotective (Sharma and Kumar, 2011), antioxidant (Kaur et al., 2010), hypolipidemic (Visavadiya and Narasimhacharya, 2011), antistress (Deore and Khadabadi, 2009a), antiarthritic (Deore and Khadabadi, 2010c), antidiabetic (Mujeeb et al., 2009), aphrodisiac (Thakur et al., 2009, 2011b; Thakur and Dixit, 2007), immunomodulatory (Kumar et al., 2011b; Thakur et al., 2007; 2011a), antiulcer (Panda et al., 2011a), anticancer (Deore and Khadabadi, 2010b), anthelmintic (Panda et al., 2011b) and larvicidal activities (Deore and Khadabadi, 2009b). Tables 4, 6 and 7 provide an overview on in vivo and in vitro pharmacological properties of Chlorophytum borivilianum extracts as well as compounds isolated from it. In the proceeding text some of the available reports pertaining towards the important pharmacological potential of Chlorophytum borivilianum extracts are being discussed.

#### 3.3.1. Aphrodisiac activity

The first study conducted on animals related to aphrodisiac activity of *Chlorophytum borivilianum* was reported by Thakur and Dixit (2006) (Table 6). This study was turned out to be one of the initial supporting scientific evidence towards the traditional use of *Chlorophytum borivilianum* as an aphrodisiac. The effect of ethanolic and sapogenin extract from the *Chlorophytum borivilianum* roots were evaluated; on sexual behavior and spermatogenesis in albino rats, at two different doses, *i.e.* 100 mg/kg and 200 mg/kg

body weight (b.w.) (Thakur and Dixit, 2006). The increase in weight of body and reproductive organs including histological activities indicated the pronounced anabolic and spermatogenic effect in treated animals. The marked reduction in mount, ejaculation, post ejaculatory and intromission latency, increase in mount frequency and attraction towards female were observed, signifying enhanced sexual behavior. Thakur and Dixit (2007) studied the pedunculation activities and sperm count of Wistar strain male albino rat under the influence of 'Vajikaran' plants such as Asparagus racemosus Willd., Curculigo orchioides Gaertn., Dactylorhiza hatagirea (D. Don) Soo, Orchis latifolia Linn and Chlorophytum borivilianum (200 mg/kg b.w.). According to their observations, these 'Vajikaran' plants have significantly improved the *in vitro* sperm count and pedenculatory activity after 14 days of drug administration.

In the following years, Thakur et al. (2008, 2009b, 2009c, 2011a, 2011b) extensively studied different aspects of sexual behavior and parameters to assess aphrodisiac potential of Chlorophytum borivilianum roots. Thakur et al. (2008) further explored their previous studies on 'Vajikaran' plants and reported the preventive effect of these plants on testicular damage induced through heat by dipping scrotal sacs of males in water bath (40  $\pm$  2 °C, 15 min daily for 14 days). The mount, intromission and ejaculatory latencies were found to be reduced, while the frequencies for the same parameter were significantly restored in rats exposed to heat followed by treatment with extracts; as compared to heat exposed control group alone. As a part of continuing research on 'Vajikaran Rasayana' plants, Thakur et al. (2009c) reported comparative aphrodisiac activities of these plants (Asparagus racemosus and Curculigo orchioides) including Chlorophytum borivilianum. The administration of 200 mg/kg b.w. aqueous root extracts exhibited marked anabolic effect in treated male albino rats as evidenced by weight gain in the body and reproductive organs. There was a significant variation in the sexual behavior of animals attributed to the testosterone-like effects. Recently. Thakur et al. (2011b) examined the aphrodisiac effect of the same 'Vajikaran Rasayana' plants (Asparagus racemosus, Curculigo orchioides, Dactylorhiza hatagirea and Chlorophytum borivilianum) on different other sexual parameters at 100 mg/kg b.w. The penile erection index and sperm count were determined by visual observation; the seminal fructose concentration and NO (nitric oxide) release in a mouse macrophage cell line (RAW264) was assessed spectrophotometrically by using resorcinol reagent; and a commercial Griess reagent kit, respectively. Chlorophytum borivilianum showed highly significant response for all parameters measured in both in vivo and in vitro studies, which is directly correlated with the erectile function.

Another group, Kenjale et al. (2008) demonstrated aphrodisiac and spermatogenic potential of *Chlorophytum borivilianum* aqueous root extract against standard aphrodisiac drug, sildenafil

 Table 6

 Important pre-clinical studies of Chlorophytum borivilianum roots.

Extract	Dose	Activity	Observations/effects	In vivo models/control/standard/induction	References
Sapogenin and ethanolic extract	100 and 200 mg/kg b.w., p.o.	Androgenic activity and enhanced sexual behavior	Weight gain in the body and reproductive organs, reduction of mount, ejaculation, post ejaculatory and intromission latency. Increase in mount frequency and attraction towards female	Male albino rats	Thakur and Dixit (2006)
Methanolic extract	100 and 200 mg/kg b.w., p.o.	Hypoglycaemic and analgesic activity	Significant reduction of blood glucose after 2– 4 h of drug administration. Analgesic effect was observed after 45 and 30 min	Normoglycaemic and alloxan induced hyperglycemic rats, tail flick and tail immersion method	Panda et al. (2007)
Powder	0.75 and 1.5 g root powder/rat/day for	Hypolipidemic and antioxidant activity	Increase in HDL cholesterol levels and decrease in plasma and hepatic lipid profiles.	Hyperlipaemic and hypercholesteraemic male albino rats, basal diet as normal	Visavadiya and Narasimhacharya (2007)
Aqueous extract	4 weeks, p.o. 125 and 250 mg/kg b.w. for 7 days, p.o. (dose-dependent manner)	Anti-stress activity	Increase in SOD and ascorbic acid levels Reverted the elevated levels of plasma glucose, triglycerides, cholesterol and serum corticosterone. Reduction in ulcer index, adrenal gland weight	control Chronic cold restrain stress model of Wistar albino rats, diazepam (1 mg/kg b. w.) as standard	Kenjale et al. (2007)
Aqueous extract	200 mg/kg b.w., p.o. $(P < 0.05)$	Pendiculatory activity and in vitro sperm counts	Significant improvement in the pendiculatory activity and <i>in vitro</i> sperm count as compared to control group after 30 min of incubation	Wistar strain male albino rats	Thakur and Dixit (2007)
Ethanolic and sapogenin extract	200 mg/kg (ethanolic) and 100 mg/kg b.w. (sapogenin extract), p.o. (P < 0.05)	Immunomodulatory activity (Balasubramani et al., 2011)	Improved survival against Candida albicans infection, increase in SRBC induced delayed- type hypersensitivity response (DTH), % neutrophil adhesion and in vivo phagocytosis	Male albino rats, carbon clearance method, administered vehicle as control, azathioprine (100 mg/kg b.w.) induced myelosuppresion	Thakur et al. (2007)
Methanolic, hydroalcoholic and aqueous extract	500 mg/kg and 700 mg/kg b.w., p.o. ( <i>P</i> < 0.01)	Anti-inflammatory activity	After 3 h of treatment aqueous (700 mg/kg) and methanolic extract (500 mg/kg) exhibited maximum activity with 83.33% and 80.55% inhibition, respectively. Whereas hydroalcoholic extract (500 mg/kg) showed inhibition of 50.55% against standard (91.11%)	Carragenan induced rat paw oedema model, 2% v/v aqueous Tween 80 as control, diclofenac (5 mg/kg b.w.) as standard.	Deore and Khadabadi (2008)
Aqueous extract	125 and 250 mg/kg b.w., (in a dose dependent manner), p.o.	Aphrodisiac and spermatogenic activity	Enhanced aphrodisiac action, increased libido, sperm count, sexual vigour and sexual arousal	Male Wistar albino rats, Viagra® (sildenafil citrate, 4 mg/kg b.w.) as standard	Kenjale et al. (2008)
Aqueous extract	200 mg/kg b.w., p.o.	Prevent sexual dysfunction against heat induced testicular damage	Prevented the decrease in sperm count in rats and protected the genital organs against physical stress <i>i.e.</i> heat	Male albino rats, administered vehicle as control <i>i.e.</i> double distilled water (DDW)	Thakur et al. (2008)
Alcoholic extract	200 mg/kg b.w., p.o. (P < 0.001)	Anti-stress and anti-ulcer activity	Alcoholic extract exhibited significant increase in swimming time and reduces the ulcer index compared to that of control	Swim endurance stress, anorexic test, despair swim test and cold stress induced gastric ulceration model in albino rats	Deore and Khadabadi (2009a)
Aqueous extract	200 mg/kg b.w., p.o. (P < 0.05) (P < 0.01)	Prevent sexual dysfunction in hyperglycemic rats	Very low weight loss, low latency time, mount and intromission as well as high ejaculation frequencies in Chlorophytum borivilianum tread animals as compared to the diabetic control	Hyperglycemic Wistar strain male albino rats induced with streptozotocin (50 mg/kg b.w.) or alloxan (100 mg/kg b.w.), polyvinylpyrollidone solution (2%) as control	Thakur et al. (2009b)
Aqueous extracts	200 mg/kg b.w., p.o.	Aphrodisiac activity (Patel et al. 2011)	Weight gain in the body and reproductive organs, reduction of mount, ejaculation, post ejaculatory and intromission latency. Increase in mount frequency, enhanced penile erection and reduced hesitation time	Male albino rats	Thakur et al. (2009c)
Aqueous extract	250 and 500 mg/kg b.w., p.o. (P < 0.01)	Anti-diabetic activity	Dose dependant reduction of blood glucose levels particularly 6 h after treatment. Reduction of the elevated level of blood glucose from 285.56 to 221.79 at 250 mg/kg b.w. and 281.62 to 206.82 g/dl at 500 mg/kg b.w. of extract	Streptozotocin induced hyperglycaemic Wistar rats of either sex, glibenclamide (3 mg/kg b.w.) as standard	Mujeeb et al. (2009)

Aqueous extract	800 mg/kg b.w./day orally in double distilled water ( $P < 0.05 - 0.001$ )	Anti-tumour, anti-mutagenic and chemomodulatory activity at pre-, peri- and post-initiation stages of carcinogenesis	Increase in activity of GSH, CAT and SOD, decrease in the hepatic MDA level, decrease in cumulative numbers of papilloma, tumour incidence, tumour burden, tumour size and tumour weight. Increase in average latent period, decreased chromosomal aberration and micronuclei	Albino mice, lipid peroxidation, SOD, CAT assay, chromosomal aberration assay, micronuclei assay, DMBA (100 µg/50 µl acetone per animal) followed by croton oil for papilloma induction	Kumar et al. (2010)
Aqueous and alcoholic	300 and 100 mg/kg b.w., p.o. (P < 0.001)	Anti-arthritic activity	Aqueous extract (300 mg/kg b.w.) inhibited the rat paw oedema by 43.61% and alcoholic extract (300 mg/kg b.w.) by 46.29% as compared to methotrexate (48.50%) after 21 days	Freund's adjuvant induced arthritis in Wistar albino rats, DDW as control, methotrexate (0.75 mg/kg b.w.) as standard	Deore and Khadabadi (2010c)
Polysaccharide fraction derived from hot water extraction	50 and 100 mg/kg b.w., p.o.	Immunomodulatory activity (Kumar et al. 2012)	Extract exhibited non-toxicity against P388 cells, showed stimulation of NK cell cytotoxic activity toward K562 cells, humoral response to SRBCs and enhancement of IgG level	Wistar strain albino rats of either sex (in vivo), cellular cytotoxicity against P388 cell lines and ELISA (in vitro), administered vehicle as control, chlorambucil and curcumin were used as positive controls	Thakur et al. (2011a)
Mehanolic extract	200 and 400 mg/kg b.w., p.o. (dose dependent manner)	Anti-ulcer activity	Exhibited free radical scavenging property, inhibition of acid secretory parameters and strengthening of gastric mucosa barrier	Pyloric ligation and aspirin induced ulceration of albino rats	Panda et al. (2011a)
Aqueous extracts	100 mg/kg b.w., p.o.	Aphrodisiac activity	Improved penile erection, sperm count, seminal fructose level (in vivo) and NO release in a mouse macrophage cell line (RAW264) (in vitro)	Wistar strain male albino rats	Thakur et al. (2011b)
Composite extracts of Cfycyrrhiza glabra, Withania somnifera, Asparagus racemosus, Chlorophytum borivilianum and seeds of Sesamum indicum	5 g, administration for 4 weeks, p.o.	Hypolipidemic and antioxidant activity	Reduced plasma and hepatic lipid profiles. Increased faecal excretion of cholesterol, neutral sterol and bile acid. Increased hepatic HMG-CoA reductase activity, improved hepatic antioxidant status (CAT, SOD, ascorbic acid levels) and plasma antioxidant capacity with reduced hepatic lipid peroxidation	Hypercholesterolemic rats	Visavadiya and Narasimhacharya (2011)
Methanolic extract	200 mg/kg and 400 mg/kg b.w., p.o. ( $P < 0.05$ )	Anti-inflammatory activity	Administration of extract inhibited the oedema starting from the first hour, showed a significant inhibitory effect on granuloma formation. Reduction of oedema up to 35.06% and 42.8% (200 mg/kg b.w.) and 34.6% and 42.3% (400 mg/kg b.w.) after 2 and 3 h, respectively was observed. The standard drug exhibited 58.4% and 55.1% of inhibition respectively at 2 h and 3 h as compared to the control group	Carrageenan induced rat paw oedema (acute test model) and cotton pellet induced granuloma (chronic test model) in Wistar albino rats of either sex, indomethacin (10 mg/kg b.w.) as standard	Panda et al. (2011c)
Methanolic extract	200 and 400 mg/kg b.w., p.o. (P < 0.05)	Anti-pyretic activity	Significant dose dependent reduction in the yeast elevated rectal temperature was observed	Fever induced Wistar strain of albino rats of either sex, paracetamol (150 mg/kg b. w.) as standard	Panda et al. (2011d)
Aqueous extract	800 mg/kg b.w., p.o.	Hepatoprotective activity	Increased body and liver weight, increased ATPase levels and almost normal hepatoarchitecture were observed in combination group as compared to arsenic treated group	Arsenic (NaAsO <sub>2</sub> ) intoxicated (4 mg/kg b. w.) male Swiss albino mice, reducing power assay, total ATPase assay, DDW as control	Sharma and Kumar (2011)
Mixture (powder form) of Mucuna pruriens (Linn), Chlorophytum	40 days and 90 days treatment (in oligozoospermic males), o.p.	Improved semen quality and motility	Increased body weight, testis, epididymes weight, increased sperm motility, sperm density (40 days). Increased sperm density and motility in oligozoospermic patients as a result of elevation in serum testosterone levels	Neem oil treated male albino rats (infertile male)	Mahajan et al. (2012)

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ktract	Dose	Activity	Observations/effects	In vivo models/control/standard/ induction	References
borivilianum and Eulophia campestris (Wall) sietry supplement containing Chlorophytum borivilianum and velvet bean Aqueous extract	Subjects has consumed 1–3 capsule per day (each capsule 750 mg), for 28 days 800 mg/kg b.w/ day for 30 days	Improves sleep quality Antioxidant and modulatory activity	(90 days). When compared with neem oil treated males there was significant reduction in almost all the parameters Improved sleep quality as evidenced by PSQI questionnaire (P < 0.05). No adverse outcomes reported as indicated by heart beats, blood pressure and blood borne parameter.  Arsenic treatment of animals has increased LPO, cholesterol, acidic and alkaline phosphatase level, significantly and decreased sperm count, sperm motility, GSH and serum testosterone level. Reverse trend was observed in combined treatment	Sleep quality of Men (n=9) and women (n=9) were assessed by Pittsburg sleep quality index (PSQI)  Sodium arsenite treated (4 mg/kg b.w.)  Swiss albino mice (toxicity induced by arsenic), radical scavenging activity measured by DPPH assay	McCarthy et al. (2012) Sharma and Kumar (2012)

citrate, commercially known as Viagra<sup>®</sup>. This aphrodisiac study was first of its kind involving comparison with positive control. The lower dose (125 mg/kg) of *Chlorophytum borivilianum* showed increased libido, sexual vigor and sexual arousal (1, 7, 14, 21 and 28 days of treatment). Whereas at higher dose (250 mg/kg) all the parameters of sexual behavior though enhanced on days 1, 7 and 14 exhibited saturation after 14 days of treatment. The sperm count was also increased considerably at both doses, after 60 days long drug administration. Therefore, on the basis of these results it was inferred that *Chlorophytum borivilianum* can be an alternative for the treatment of certain forms of sexual inadequacies, such as premature ejaculation and oligospermia.

In another report, Thakur et al. (2009b) investigated the sexual dysfunction in hyperglycaemic male albino rats, induced by streptozotocin (50 mg/kg) or alloxan (100 mg/kg). The diabetic male rats were treated with Chlorophytum borivilianum extract (200 mg/kg) and tested for sexual behavior in the presence of female rats and other sexual parameters were recorded. It was observed that Chlorophytum borivilianum extract treated animals exhibited improved diabetes-induced sexual dysfunction. However, streptozotocin and alloxan treated groups had a significantly lowered sexual behavior (P < 0.05) compared to the normoglycemic control group. More recently, efficacy of herbal composition of medicinal plants, traditionally known to have aphrodisiac potential; was investigated on infertile males (Mahajan et al., 2012). The herbal formulation, consisting of Mucuna pruriens (Linn), Chlorophytum borivilianum and Eulophia campestris (Wall), significantly improved the semen quality and motility after 40 days and 90 days of treatment in oligozoospermic patients. In a new study, methanolic and successive aqueous extracts of 30 plants together with Chlorophytum borivilianum were screened for Rho-kinase 2 (ROCK-II) inhibitory potential (Goswami et al., 2012). ROCK-II inhibition activity of extracts was performed using HTRFKinEASE™ STK S2 Kit (Cisbio Bioassays) and Y-27632 was used as the standard inhibitor. The methanolic extract of Chlorophytum borivilianum exhibited low inhibition (4.11%) while successive aqueous extract showed considerable inhibition percentage (40.50%). Hence, plant extracts capable of inhibiting ROCK-II enzyme can be utilized in the management of erectile dysfunction (ED) by relaxing corpus cavernosum. The authors concluded that the moderate activity of the Chlorophytum borivilianum aqueous extract was due to steroidal saponin content. The dual mechanism, i.e., androgen synthesis and ROCK-II inhibition of Chlorophytum borivilianum indicate its importance as 'Vajikaran' plant (Goswami et al., 2012; Thakur et al. 2009b).

From the above mentioned studies it may be concluded that the obtained findings on *Chlorophytum borivilianum* are endowed with a scientific basis for its purported traditional usage as 'Vajikaran Rasayana' plant. Thus, the observed results confirmed that the plant can act as an aphrodisiac and their utilization in traditional Indian medicine is justified. The *in vivo* research carried out so far revealed that only ethanolic and aqueous extracts of the root has been examined and there is scope to explore various other extracts of the plant for aphrodisiac potential. The limited tested dosages of crude extracts and fractions on animal model at 100, 125, 200 and 250 mg/kg b.w. is of major concern.

#### 3.3.2. Immunomodulatory activity

Immunomodulators are biological or synthetic substances that can stimulate, suppress or modulate any aspect of the immune system including both adaptive and innate arms (Kumar et al., 2012). The plants belong to the category of 'Rasayanas' are rejuvenators, nutritional supplements and possess strong antioxidant activities. They exert opposite effect on oxidative stressors, giving rise to the formation of different free radicals. They are used mainly to

 Table 7

 In vitro pharmacological activities of Chlorophytum borivilianum.

Plant part	Extract	Dose	Activity	Observations/effects	In vitro models/ control/standard	References
Roots	Ethanolic extract	100 μg/ml	Antioxidant activity	Scavenge DPPH (84.51%), hydroxyl radical (48.95%), ferryl bi-pyridyl complex (84.53%) along with the inhibition of lipid peroxidation (67.17%)	DPPH, ferryl bi-pyridyl complex and lipid peroxidation assay	Govindarajan et al. (2005)
Roots	Acetone and methanol extract	75–1200 μg/ml	Antimicrobial activity	peroxidation (9.17/s) Strong activity with minimum inhibition concentration (MIC) was observed at 1200 µg/ml and 600 µg/ml of acetone and methanol extract, respectively against Staphylococcus aureus. MIC of methanol extract reported at 600 µg/ml concentration against Escherichia coli, Pseudomonas aerugenosa	Microbroth dilution assay	Dabur et al. (2007)
Roots	Aqueous extract	500–2500 mcg/ml and 100–600 mcg/ml (dose-dependent manner) (P < 0.05)	Antioxidant activity	Low levels of DPPH free radicals (maximum 92% inhibition, IC <sub>50</sub> =1200 mcg) and thiobarbituric acid reactive substances (maximum 66.91% inhibition, IC <sub>50</sub> =300 mcg)	DPPH and lipid peroxidation assay	Kenjale et al. (2007)
Roots	Hydro alcoholic, methanolic and chloroform extract		Antimicrobial activity	Moderate activity against Staphylococcus aureus, Proteus vulgaris, Escherichia coli, Pseudomonas aerugenosa, Shigella sonni, Aspergillus niger, Candida albicans	Well diffusion method	Deore and Khadabadi (2007)
Roots	Methanolic, hydroalcoholic and aqueous extract	100, 250, 500, 1000 µg/ml	Antioxidant activity	Methanolic extract showed good activity, while hydroalcoholic extract exhibited moderate and aqueous extract showed poor activity in both types of method	DPPH and nitric oxide assay, without test sample as control, butylated hydroxyl anisole (BHA) as standard	Deore and Khadabadi (2008)
Leaves	Petroleum ether, ethyl acetate, methanol and water extract		Antioxidant activity	<b>3</b>	DPPH and nitric oxide radical inhibition assays, ascorbic acid and quercetin as standard	Chakraborthy (2008)
Leaves, nodes and flowers	n-hexane, ethyl acetate and n-butanol fractions of methanolic extract		Antimicrobial activity	Ethyl acetate extract of leaves showed strong activity against Staphylococcus urreus, Staphylococcus epidermidis, Aspergillus niger and Candida albicans. Hexane and ethyl acetate extract of nodes exhibited activity against Klebsiella pneumoniae and Microsporium gypseum	Disc diffusion method, DMSO as control, amphicillin (1 mg /ml) and voriconazole (1% w/v) as positive control	Chakraborthy (2009)
Leaves and stem	Methanolic extract	250, 500, 1000 mg/ml	Antimicrobial activity	Leaves and stem extract displayed concentration dependent antimicrobial properties far above amphicillin, zone of inhibition ranges from 10 to > 20 mm. The extracts showed activity against Staphylococcus aureus, Escherichia coli, Bacillus cereus, Aspergillus niger and Candida dibicans	Disc diffusion method, DMSO as control, amphicillin (1 mg /ml) and voriconazole (1% w/v) as positive control	Chakraborthy and Aeri (2009)
Roots	Methanolic extract, crude saponin extract and purified saponin fractions		Larvicidal activity	All extracts are found to be larvicidal but purified saponin fractions was found more effective with LE <sub>50</sub> 4000–3850 ppm and EC <sub>50</sub> 4872–2290 ppm. Standard exhibited LC <sub>50</sub> 0.00547–0.00477 ppm and EC <sub>50</sub> 0.00547–0.0022 ppm	Against mosquito species Anopheles stephensi, Culex quinquefasciatus and Aedes aegypti, malathion as standard	Deore and Khadabadi (2009b)

Table 7 (	continued)
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Plant part	Extract	Dose	Activity	Observations/effects	In vitro models/ control/standard	References
Peels of tubers	Aqueous extracts		Antioxidant activity	Radical scavenging activity of aqueous extract was found to be dose dependent in all the assays	DPPH, deoxyribose degradation, reducing power, ferrous ion chelation and lipid peroxidation assays	Kaur et al. (2010)
Roots	Mehanolic extract and crude saponin fraction		Antiproliferative activity	Crude saponin extract of Chlorophytum borivilianum has potential anticancer activity	MTT and SRB assay, DNA fragmentation assay	Deore and Khadabadi (2010b)
Root	Aqueous and ethanolic extracts	25–1000 μg/ml	Antioxidant activity	Aqueous extract exhibited high antioxidant activity evidenced by nitric oxide, superoxide, hydroxyl, DPPH, ABTS radical scavenging activity, reducing capacity, metal chelating ability, and suppressed lipid peroxidation in mitochondrial fractions as compared to ethanolic extract. Coppermediated human serum and kinetics of LDL oxidation has been significantly decreased $(P \sim 0.05)$ by aqueous extract	DPPH, ABTS, FRAP assays	Visavadiya et al. (2010)
Root	Methanolic extract	10, 25, 50 and 100 mg/ml (dose dependent manner)	Anthelmintic activity	Causes paralysis as well as death	Adult earthworms, Pherentima posthuma, 2% w/v gum acacia in distilled water as contol, piperazine citrate as standard	Panda et al. (2011b)
Plantlets	Water, ethanol, acetone, glacial acetic acid extract	DMSO stock concentration of 100 mg/ml	Antibacterial activity	Zone of inhibition ranges from 3–24 mm against human pathogenic bacteria, Staphylococcus aureus, Bacillus subtilis, Escherichia coli. and Pseudomonas aeruginosa	Agar cup diffusion method, DMSO as control	Sundaram et al. (2011)
Root	Ethanolic extract	50–200 μg/disc	Antibacterial activity	Maximum antibacterial inhibition was obtained against Staphylococcus aureus (12 nm) followed by Escherichia coli, Pseudomonas aeruginosa and Bacillus cereus	Disc-diffusion method, streptomycin (50–200 $\mu g/disc$ ) as positive control	Sriram et al. (2012)
Root	Aqueous, Tris-HCl, phosphate buffer, isopropanol, butanol- water and acetone extract	100 µl and 200 µl	Antibacterial activity	The extracts were tested against Staphylococcus aureus, Escherichia coli, Klebsiella, Proteus, and Pseudomonas aeruginosa Butanol—water extract showed maximum antibacterial activity against all the organisms (10–28 nm at 100 µl and 16–34 nm at 200 µl)	Agar-diffusion method, compared with positive and negative control	Roselin and Raj (2012)
Root	Methanol/Aqueous extract	50 μg/ml	Rho-kinase 2 inhibitory activity (used in management of erectile dysfunction)	Mean percent inhibition of ROCK-II was found to be 4.11% and 40.54% for methanolic and successive aqueous extract, respectively	ROCK-II inhibition assay by HTRF method, Y-27632 (4 ml of 375 nM) was used as the standard Inhibitor	Goswami et al. (2012)
Leaves and roots	Saponin fraction from methanol extracts	1% w/v	Transdermal enhancer activity	Increased flux of the drug, permeability coefficient (Kp), cumulative amount release (Q24) and enhancement ratio (ER) against control	Human cadaver skin, 3 ml saturated solution of pramipexole as standard	Pawankumar and Shiradkar (2012)

neutralize the effects of ageing, atherosclerosis, cancer, diabetes, rheumatoid arthritis, autoimmune disease and Parkinson's disease. The 'Rasayana' plants seem to operate through immunostimulant, immunoadjuvant, and immunosuppressant activities. The mechanism of immunomodulation activity occur mainly via phagocytosis stimulation, macrophages activation, immunostimulatory effect on peritoneal macrophages, lymphoid cells stimulation, cellular immune function enhancement and nonspecific cellular immune system effect, antigen-specific immunoglobulin (IgG) production, increased nonspecific immunity mediators and natural killer cell numbers, reducing chemotherapy-induced leukopenia, and increasing circulating total white cell counts and interleukin-2 levels (Chulet and Pradhan, 2010; Kumar et al., 2012).

Thakur et al. (2007) evaluated the immunomodulatory effect of ethanolic and sapogenin extract of Chlorophytum borivilianum roots (Table 6). The immunomodulatory activity was assessed by inducing myelosuppresion through azathioprine and determining hematological and serological parameters upon administration of extracts. The ethanolic extract exhibited more prominent activity as compared to the sapogenins. The treatment of extracts appreciably improved survival against Candida albicans infection with  $94 \pm 5.56\%$  and  $88.86 \pm 5.6\%$  survival for ethanolic and sapogenin extracts, respectively. The enhanced sheep red blood cell (SRBC) induced delayedtype hypersensitivity response (DTH), %neutrophil adhesion and in vivo phagocytosis having phagocytic index approximately 20%  $(1.2 \pm 0.03)$  and 25%  $(1.36 \pm 0.51)$  in sapogenins and ethanolic extract-treated groups, respectively, were observed. The aqueous extract, polysaccharide fraction as well as nonpolysaccharide fraction obtained from hot water extraction of Chlorophytum borivilianum were tested for immunomodulatory activity (Thakur et al., 2011a). The extensive phytochemical evaluation of the hot water soluble root extracts of Chlorophytum borivilianum leads to the quantification of nearly 60% w/w of polysaccharides, which were mainly 31% inulintype fructans and 25% acetylated mannans (Thakur et al., 2009b). These extracts were evaluated for its effect on natural killer (NK) cell activity (in vitro). Human peripheral blood mononuclear cells (PBMCs) isolated from whole blood on a Ficoll-Hypaque density gradient were tested in the presence or absence of varying concentrations of each Chlorophytum borivilianum fractions, for modulation of NK cell cytotoxic activity towards K562 cells. In comparison to the control, a significantly higher effect of polysaccharide fraction (P < 0.01) in augmenting the NK cell activity was observed  $(98 \pm 2.5\%)$ at 5 µg/ml). Although the aqueous extract stimulated NK cells significantly (P < 0.05), most of the effect appeared to be contributed by polysaccharide fraction alone. The reported study confirmed the nontoxic nature of aqueous extract ( $IC_{50}=1219.67 \mu g/ml$ ) and polysaccharide fraction (IC $_{50}$ =722.31  $\mu g/ml$ ) against sensitive mouse leukemic P388 cell lines. The significant higher HA titer values of  $169.2 \pm 6.1$ ,  $174.3 \pm 3.1$ , and  $168.6 \pm 2.6$  were observed in the aqueous extract and polysaccharide fraction at 100 µg/ml and 50  $\mu$ g/ml, respectively, against the control (147.6  $\pm$  11.9). Furthermore, in vivo study carried out on Wistar strain albino rats to determine humoral response to SRBCs and IgG-level through enzyme-linked immunosorbent assay (ELISA) exhibited the efficacy of Chlorophytum borivilianum aqueous extract in improving immune function. The administration of aqueous extract showed a significant increase (P < 0.01) in IgG level (1.93  $\pm$  0.02), followed by polysaccharide fraction at 100 mg/kg b.w. (1.85  $\pm\,0.01)$  and polysaccharide fraction at 50 mg/kg b.w. (1.81  $\pm$  0.04) (Thakur et al. 2011a).

In conclusion, the available knowledge holds promise for *Chlorophytum borivilianum* being used as an immunostimulating agent and an in-depth study on various fractions of different extracts is warranted to determine the most potent immunostimulating fraction. So far, ethanolic and aqueous extracts at 50, 100 and 200 mg/kg b.w. have been investigated and need further investigation on the aspect of effective dosage. Besides the studies

carried out so far validate the traditional use of the plant as a 'Rasayana' in Ayurvedic system of medicine.

#### 3.3.3. Anticancer activity

The effect of Chlorophytum borivilianum on cell kinetics and apoptosis in human breast cancer cell lines was reported by Jamal (2005). The methanolic extract along with crude saponin fraction of Chlorophytum borivilianum has been tested for antiproliferative activity by Deore and Khadabadi (2010b). The study was conducted by MTT and SRB assay followed by DNA fragmentation assay. The findings of the study suggested that the crude saponin extract of Chlorophytum borivilianum has potential anticancer activity with very high percentage inhibitions. Kumar et al. (2010) observed antitumor, antimutagenic and chemomodulatory activity of aqueous root extracts of Chlorophytum borivilianum (Table 6). In preliminary studies; no toxicity was observed in mice treated at various doses (100, 400 and 800 mg/kg b.w./day) of root extract. The highest significant decrease in LPO (lipid per oxidation) level and significant increase in GSH (reduced glutathione), SOD (superoxide dismutase) and CAT (catalases) level as compared to control was considered as a basis for dose selection for anti-tumor study. The oral administration of 100 µl of root extract (800 mg/kg b.w.) at pre-, peri- and post-initiation stages of papillomagenesis per day for 7 days was employed. Papilloma in animals was induced by application of a carcinogen, 7, 12dimethylbenz (a) anthracene (DMBA) followed by a promoter (croton oil). The extract showed a significant reduction in the tumor incidence (56.7  $\pm$  3.3%, P < 0.001) and cumulative number of papillomas (22  $\pm$  1.15, P < 0.001) as compared to the control group  $(100 \pm 0.0\%, 43 \pm 0.94)$ . In antimutagenic study, animals treated with Chlorophytum borivilianum root extract at pre-, peri- and postinitiation stages of papillomagenesis exhibited significant reduction in the total chromosomal aberrations (46.11  $\pm$  0.57, P < 0.001) in the form of chromatid and chromosome breaks, centric rings, dicentrics, exchanges, acentric fragments, pulverized cells and polyploids as compared to control (153.14  $\pm$  1.05). Also, micronuclei assay showed a significant decrease in the number of micronuclei (14.91  $\pm$  0.57, P < 0.001) as compared to control (25.43  $\pm$  0.89).

Summarizing the anticancer activities reported in literature, it is observed that the work carried out is quite preliminary and narrow. There are some in vitro cytotoxic studies conducted by Acharya et al. (2008a, 2009) on human colorectal cancer lines and there is a possibility to carry forward these analyses against different cancerous cell lines. The in vitro studies are scanty as was carried out only on methanolic extract and a few pure saponins isolated from roots that warrant at least preliminary screening against various extracts of the plant. Overall, before performing in vivo experiments, the positive leads of in vitro tests should be chosen, rather than directly evaluating in the animal models. So far, a single in vivo study has been published in which the effective dosage reported at 800 mg/kg b.w. and toxicity reports indicated the safety of tested aqueous extract at this concentration. Since, not much information is available on in vivo anticancer studies; further detailed studies might be a subject of future investigations.

#### 4. Quality and safety

#### 4.1. Adulterants

The increase in demand of the commercial herbal medicine leads to the indiscriminate and unscientific collection without much consideration on the quality of the material. The immature collection of fruits and tubers are one of the main reasons of reduced quality as well as quantity of the raw product to be below the critical standard

level. The adulteration in market samples is a greatest drawback in promotion of herbal products (Dubey et al., 2004). A treatise published two centuries ago in 1820 on adulteration in food and culinary materials is a proof of this old practice (Accum, 1820). The means of adulteration and substitution may be deliberate or sometimes unintentional. Broadly, adulteration is considered as an intentional malpractice and due to this faith in herbal drugs has been declining (Gupta et al., 2003).

Indian Ayurvedic industries generally face the problem of adulteration at raw material stage, and generally procured through market channels. In the herbal markets of the country, not only various species of the particular genus but entirely different taxa are being sold under the same vernacular name (Rawat et al., 1996; Rawat, 2005) akin to genus Chlorophytum. The various species of Chlorophytum found in India are different in their medicinal properties, but due to the lack of appropriate information, all the species are known as 'safed musli' in the Indian drug market because of their white tuberous roots (Sheriff and Chennaveeraiah, 1972; Nair, 1974). In spite of the species from the same genus e.g. Chlorophytum arundinaceum, Chlorophytum tuberosum and Chlorophytum laxum, a plant from different genus, Asparagus adscendens is also among the adulterants of Chlorophytum borivilianum. All these species are used as aphrodisiac but Chlorophytum borivilianum claimed as a better source. A lot of confusion prevails in the herbal drug market regarding the identification of true 'safed musli' and rampant adulteration of the drug with inferior plant material is reported.

The study conducted by Mishra et al. (2009) on Nagpur market of India revealed that primary collectors mix similar looking species (*Chlorophytum tuberosum* and *Chlorophytum arundinaceum*) in the samples of *Chlorophytum borivilianum*. The traders and middlemen also add diseased musli and chaff matter to increase the weight. The laboratory analysis showed that in one kilogram of market sample, more than 20% raw material was found adulterated. The dry samples of *Chlorophytum borivilianum* collected from traders was found inferior as compared to samples of natural forest in terms of quality (790 g/kg vs 970 g/kg), infection by fungus or insects (80 g/kg vs 15 g/kg), adulterant (110 g/kg vs 00 g/kg) and chaff matter (soil, seed, stone and sand, 20 g/kg vs 25 g/kg), respectively. The data suggested that high degree of adulteration made by traders to gain more monitory profit compromises the quality of raw material and safety of consumers.

Thus, to prevent adulteration and assure quality of drug, pharmacognostic studies play a critical role to discriminate right and desirable species. WHO (World Health Organization) laid much emphasis on testing of medicinal plants for authenticity and to control the purity of prepared medicine (Anonymous, 1996, 1999). The two congeneric species of Chlorophytum i.e. Chlorophytum borivilianum and Chlorophytum tuberosum (Roxb.) Baker, have quite similar morphological features, causing problem to ready identification in the field and also in dry forms. Mandal and Nandi (2012) observed that Chlorophytum tuberosum is slightly taller and have undulated leaf margins while, in Chlorophytum borivilianum leaf margins are plane. Moreover, floral morphology is also one of the distinguishing features among these species. However, it is difficult to identify the species if present in crude drugs; Joshi et al. (2000) developed a HPLC method to detect adulteration and identification of closely resembling plants. The characteristic spectral pattern of each plant is the basis for identification of these species.

The biochemical markers used in the authentication of herbal drugs have many limitations due to the impact of environmental conditions. To resolve this problem development of molecular marker is also one of the solutions for plant authentication purpose. Misra et al. (2007) carried out experiment to develop molecular marker for the differentiation of different species and

genus in "safed musli" complex by AFLP (amplified fragment length polymorphisms) analysis based on the proximity of DNA fragment-match. Recently, species-specific RAPD (random amplified polymorphic DNA) markers and comparisons of plastid ribulose bisphosphate carboxylase (rbcL) region sequences together with the gene rpl16 and rpl16-rpl14 spacer region allowed the identification of five species of *Chlorophytum (Chlorophytum borivilianum, Chlorophytum arundinaceum, Chlorophytum laxum, Chlorophytum capense* and *Chlorophytum comosum*) (Katoch et al., 2010). Furthermore, there are various findings that confirmed that the RAPD and AFLP markers have potential for identification and characterization of genetic relationship between the species of *Chlorophytum* and genotypes of *Chlorophytum borivilianum* present in different geographical locations (Dwivedi and Sharma, 2011; Samantaray et al., 2011; Tripathi et al., 2012).

From the above knowledge it may be concluded that monitoring of adulteration at raw material stage is very important in order to maintain the quality of *Chlorophytum borivilianum* products. The development of processed products should be carried out under strict rules and regulations and every stage of production may be supervised by authentic vigilance officials to avoid contamination. In addition, to maintain quality and safety, the available AFLP and RAPD markers are effective tools but there is still scope for the development of better genetic based technique for identification and authentication of *Chlorophytum borivilianum* drugs. Also, there is need to create awareness among traders, producers and consumers about life threatening implications of adulteration to check this heinous malpractice.

#### 4.2. Toxicology

The insufficient scientific evidences on the level of safety, quality and toxicity are the major shortcoming of employing traditional medicines in the society. Despite the paucity of scientific information on safety or efficacy of traditional medicines, there is widespread promotion of complementary and alternative medicine (CAM) in the popular media. The unsubstantiated health care claims of CAM seem to be driving their demand and forcing even conventional medical practitioners to incorporate CAM therapies into their practices. Although traditional medicines are perceived as being natural and safe, many have adverse effects that can sometimes produce lifethreatening consequences. Therefore, millions of people are exposed to the risk of these potential adverse interactions, especially with products that contain several herbs.

Deore and Khadabadi (2009a) demonstrated an acute toxicity test as per the guidelines of AOT-421 using albino mice. The test drug (alcoholic/aqueous extract of Chlorophytum borivilianum roots) was found to be nontoxic up to the dose of 2000 mg/kg b.w. The analysis were made by inspecting changes in skin, fur, eyes, mucous membrane, respiratory rate, heart rate, blood pressure, salivation, lacrimation, perspiration, piloerection, urinary incontinence, defecation, ptosis, drowsiness, gait, tremors and convulsion for 14 days. Similar results were also obtained by Thakur et al. (2009b). Kumar et al. (2010) have observed nontoxic effects in terms of sickness, mortality, morbidity and behavior in mice treated with different doses (100, 400 and 800 mg/kg b.w./day) of Chlorophytum borivilianum root extract, for 7 consecutive days. The results of study suggested that tolerance level of mice was 800 mg/kg b.w./day, indicated by highly significant decrease in LPO level (2.7651  $\pm$  0.0575 nmol/mg protein P < 0.001), increase in GSH (0.1553  $\pm$  0.0074  $\mu$ mol/g tissue, P < 0.001), SOD (3.9610  $\pm$  0.0254  $\mu$ mol/mg protein, P < 0.001) and P < 0.001) level as compared to control.

Since roots are the main part of therapeutic use and are capable of absorbing and accumulating toxic chemicals from the soil, therefore research in this regard is of grave concern (Bhat and Karim, 2010). A notification was issued by AYUSH (Department of Ayurveda, Yoga, and Naturopathy, Unani, Siddha and Homeopathy), India, under the Drugs and Cosmetics Act 1940 to maintain the quality of medicinal products as well as testing and display of heavy metals in permissible limits has been made mandatory. Alim et al. (2010) collected samples of Chlorophytum borivilianum roots from herbal plant material suppliers of the local market and complimentary packet from minor forest produce to examine their purity and heavy metal content. The trace elements, Cu and Zn, present in forest sample were observed in lower range (0.0820-0.0790 and 0.0983-0.0861 ppm) as compared to local market sample (0.3907-0.1895 and 0.2590-0.1632 ppm), respectively, while heavy metals (Ni and As) in both samples were found below detectible limits (0.001 ppm). Dev et al. (2009) recently examined the Pb and Cd content of 35 medicinal plants collected from same source used by traditional healers and commercial drug manufacturers. The observations revealed that 54.29% and 77.14% of medicinal plants exceeded the maximum permissible level of Pb and Cd concentration, respectively, as designated by the WHO. The leaves of Chlorophytum borivilianum contaminated with  $14.444\,\mu g/g$  of Pb and  $1.415\,\mu g/g$  of Cd as compared to maximum permissible limit of 10 μg/g (Pb) and 0.30 ppm (Cd), respectively (Anonymous, 1999).

Summarizing the data on toxicological studies, it may be observed that investigations on relative systemic toxicity and safety of Chlorophytum borivilianum crude extracts are insufficient. Only preliminary studies have been conducted focusing on acute toxicity. There are only few in vivo reports available on drug tolerance level and detailed safety studies of active extracts, fractions, herbal formulations and pure compounds concerning various organs are necessary before commercialization. Chlorophytum borivilianum has been part of the Indian traditional system since centuries but the studies on exact dosage; long term use, adverse drug reaction (ADR) and safety especially on humans are missing. Since roots reported to possess significant aphrodisiac activity due to elevation in serum testosterone levels, there is a wide gap on safety of employing these extracts, their appropriate dosage on person with special medical conditions like pregnant women, nursing women and person suffering from heart diseases, which is an alarming question. Moreover, there are no reliable data available on microbial contaminants after harvest or during storage that may produce aflatoxins. In addition, there is scanty of data present on heavy metal contamination of herbal products. These issues raised the concern over the need of thorough toxicological investigations before employing it as a drug.

Table 8
List of some commercial *Chlorophytum borivilianum* containing medicinal products (http://www.allayurveda.com, Thakur et al., 2009a).

Product name	Manufacturers/procured from
Musli Safed Capsules	Dehlvi Naturals, New Delhi, India
Eterna Safed Musli Granule, Foreva Safed Musli	Prakruti Bio Farms, Borivali,
Powder, Naturest Safed Musli Tablets	West Mumbai, India
Musli Power Xtra	World Viewer Dot Com (India)
	Pvt. Ltd., Kerala, India
Safed Musli Power	Vadamalai Enterprises, India
Tribulus Power Planet	Ayurveda, Chandigarh, India
Dabur Shilajit Gold	Dabur, India
Safed Musli powder, Safed Musli Veg Capsules,	http://www.allayurveda.com
Vigor-100 stamina, Vita- ex Gold Plus, Musli	

pak, Musli X-Capsules

#### 5. Economic potential

Chlorophytum borivilianum is a cash crop with low risk, and high returns can be fetched within a short period of 1 year. In genus Chlorophytum, the Chlorophytum borivilianum possess the highest economic value owing to its highest saponin content (17%) (Manjunatha et al., 2008). The profit generated from Chlorophytum borivilianum farming is tax free, since, it is an agricultural income. The right mode of cultivation in a scientific way and careful selection of planting material will yield repeated benefits year after year. On an average, 2000-3000 kg of wet musli can be harvested from Chlorophytum borivilianum per acre; and after drying up to 20% (400-450 kg) dry musli is finally obtained. The market rate of dry musli is around Rs 800 to Rs 1800 per kg in indigenous Indian market and more than Rs 3000 per kg in the international market. For an acre of land Chlorophytum borivilianum generates about Rs. 4–6 lakh (More than \$10,000) net income. The processed product of Chlorophytum borivilianum in the form of Ayurvedic formulations and supplements commands an exorbitant price both in indigenous and global markets. According to one published report which claims the existence of 7200 formulations of safed musli, suggesting its huge economic potential (http://www.agricare.org/safed\_musli.htm). It is well known that "Pfizer's Viagra" has been a sensation all over the world for its aphrodisiac qualities. Being a synthetic drug its prescription is limited depending upon medical conditions of person. On contrary, Chlorophytum borivilianum exhibits similar phenomenon without any adverse reaction and is considered as "herbal Viagra". The potency drug having trade name 'Nai Chetna' (new sensation) was launched by Gujarat State Forest Development Corporation, India, in the year 1999 and was published in The Indian Express Newspaper (Bombay) on 1 December, 1999 (Thakur et al. 2009a). The drug has Chlorophytum borivilianum as a chief component and gained widespread acceptance as an alternative to Viagra (Anonymous, 1997).

To date, the market demand of the tuberous roots on a commercial scale exceeds the supply and in India, the demand of *Chlorophytum borivilianum* root is over 2500 t/annum (Somanath, 2008). In another report, the demand of safed musli roots in India is estimated as 3500 t against supply of 500–600 t per annum (Kothari and Singh, 2001). The demand in foreign countries has been calculated as 300–700 t annually (Bordia et al., 1995). The largest global markets of *Chlorophytum borivilianum* are China, India, France, Germany, Italy, Japan, Spain, UK and USA. Some countries in the Gulf, Europe, including USA have been major importers of the dry roots of *Chlorophytum borivilianum* for preparation of various herbal products and drinks. Currently, several pharmaceuticals and herbal companies are selling products of *Chlorophytum borivilianum* with commercial name such as Eterna, Foreva, Musli Power Xtra and Indian Herbal Viagra (Table 8).

At present, the estimated global market demand and production is approximately 35,000 t/annum and 5000 t/annum respectively which fulfil less than 15% of the required demand. According to another report, the world demand of roots is close to 50,000 t/annum (Somanath, 2008). Of the available quantum of *Chlorophytum borivilianum*, over 95% of it comes through wild harvesting; an act expressly prohibited by the various state governments. The current global trades in medicinal plants is more than \$60 billion which is expected to increase around \$5 trillion by 2050 with growth rate of 7% per annum. India's current potential in global trade is Rs. 4.36 billion which is expected to rise to Rs. 222 billion by 2050 (http://www.farmwealth.com/safed musli, http://www.krdmuslifarm.com/safed musli).

The analysis of above mentioned data indicates the high economic importance of safed musli in both domestic and international market; hence, its trade may be promoted by the government to fetch high foreign returns. There is a need of wide recognition among

growers, traders, producers and medicine experts who could work in harmony to realize its full commercial benefit.

#### 6. Conservation

The disturbances of the natural habitats of *Chlorophytum borivilianum*, as a result of anthropogenic activities and invasion of the exotic species have resulted in the drastic decline in the population. Until few years back, the forests of India were very rich in *Chlorophytum borivilianum*, however, large scale indiscriminate collection, inadequate attempts have been made either to allow its replenishment or promote cultivation enlisted this species as 'endangered' by government of India in 2000 (Kothari and Singh, 2004; Mishra, 2011). Now it is widely felt that immediate steps for the conservation of safed musli are warranted, or else Indian forests will soon lose this wonder plant (Oudhia, 2001b, 2001c; Maiti and Geetha, 2005; Phurailatpam et al., 2009).

There are several reasons reported for the fast depletion of Chlorophytum borivilianum population from natural habitats. Chlorophytum borivilianum has very shy flowering behavior; formation of few boles on each inflorescence; less number of seeds in each bole, low germination of seeds, slow rate of multiplication through vegetative means and long seed and tuber dormancy (6-7 months) (Jat and Bordia, 1990). To avoid the long dormancy period and low germination of seeds, cultivators usually choose vegetative cultivation via tubers. The dual agricultural function of tubers as planting material as well as organ of economic importance has created a severe shortage. Thus, to meet the demand and for gaining large profit, collection of roots from the forest are usually done before seed maturity, thus hampering natural regeneration and further depleting the wild population (Nayar and Sastry, 1988). Moreover, plants cultivated by vegetative means are prone to bacterial and fungal attack, causing loss of raw material. Apart from this, obnoxious weeds like Parthenium and Lantana are competing with Chlorophytum borivilianum and taking its place (Oudhia, 1996).

Summing up the above information, it may be inferred that, to fulfil the requirements and shun undue pressure on natural forests, attempts should be made to implement the systematic cultivation practices, identify and preserve superior germplasm of Chlorophytum borivilianum. Recently, farming system has been introduced in Madhya Pradesh, Andhra Pradesh and Maharashtra and there is a possibility to encourage cultivation in other parts of the country. Furthermore, to fill the gap amid demand and supply and to provide genetically uniform planting material from a selected elite source, micropropagation is one of the most desirable options. This biotechnological technique can help to undertake large scale cultivation and co-cultivation program and at the same time can provide large number of disease free plants in shorter time frame. Also, cultivation of closely related species, Chlorophytum aurandinaceum, should be promoted as a substitute of Chlorophytum borivilianum due to their similar chemical composition, which thereby cut the collection load from the wild habitats. So, it is the need of present, to either look for an alternative of Chlorophytum borivilianum or produce genetically modified fast growing variety to cope with the demands for sustainable future of this vulnerable crop.

#### 7. Conclusion

It is evident from the available literature that *Chlorophytum* borivilianum roots are the most investigated part of the plant. Since *Chlorophytum* borivilianum is predominantly utilized as aphrodisiac, further studies concerning its extent of efficacy are warranted as compared to its commercial counterparts, as well as

clinical studies on this plant have to be encouraged to evaluate effects on human model. Besides, several in vitro and in vivo pharmacological studies lack scientific strength and necessitate more authentic research approach. Hitherto some of the medicinal knowledge on safed musli has not been validated, as claimed in the traditional system of medicine like antipyretic, anti-dysuria, anti-diarrhoea, anti-dysentery, anti-ageing, anti-obesity and treat gynaecological disorders, which require attention. The interest in scientific relation between pharmacological effects and traditional uses of Chlorophytum borivilianum has considerably increased; hence, the detailed study of pharmacology, bioassay guided isolation of active principles and mechanism of action may help to understand this connection and possible side effects or toxicities. Furthermore, to meet the international standards of commercial safed musli products, GAP, GMP, GSP should be implemented with HACCP approach. Also, there is call for strict implementation of the existing laws (Indian Forest Act 1927) to check and regulate the illegal movement of the forest produce. Thus, it is mandatory to fill the huge gap of insufficient knowledge and awareness among commoner as well as researchers to hold the position of this plant in providing better medicinal values to the society. There are many areas to work in this plant for its full recognition; these can be only fulfilled with interest generated by research community through writing reviews and carrying out research on different uses of this plant.

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