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RESEARCH ARTICLE

High performance liquid chromatographic analysis of derivatized saponin content of *Chlorophytum borivilianum*

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Abstract

Among the several *Chlorophytum spp.* present in India, *C. borivilianum L.* is the more commercially exploited species compared to others under the common name 'Safed musli'. It is used in many Ayurvedic vital tonics and aphrodisiac formulations. Triterpenoids Saponins present in the fleshy roots are known to be the active ingredients of Safed musli. In the present study, quantitative determination of saponin content among the different wild accessions was done by HPLC analysis. The 70% methanol extract of a powdered sample of *C. borivilianum* were analyzed by a column – ODS, 4.5 mm × 250mm at a flow rate of 1.0 ml /min and detection wavelength of 203 nm. Well resolved chromatograms are obtained with a gradient elution of acetonitrile - water 40:60 (v/v). HPLC analysis showed that the highest content of saponin was found in V_2 followed by V_8.

Introduction

Chlorophytum borivilianum Santapau & Fernandes (Liliaceae) (Tandon and Shukla, 1995; Kothari, 2004) *is an important medicinal plant commonly known as safed musli,* used in many Ayurvedic vital tonics and aphrodisiac formulations. It has gained prominence as safed musli because on processing it yields milky white tubers which have been in use as nutritive tonic as aphrodisiac agent, employed as Rasayana (Puri, 2003) and also an important constituent of Chyawanprash an important rejuvenator (Handa, 1996). The major constituents of safed musli are carbohydrates, proteins, root fibers and saponins (Tandon and Shukla, 1993). The saponins are considered to be a potent medicinal compound, found in tubers and impart medicinal value. Fasiculated roots of *Chlorophytum borivilianum* are used as tonic and important ingredient of 20 ayurvedic and unnani preparations (Oudhia, 2001). Seventeen species of *Chlorophytum* had been reported in India (Bordia *et al*., 1995). Among all the species of *Chlorophytum* present in India, *C. borivilianum* produces the highest yield of roots and has the highest saponin content (Singh and Samuel 2001). Interestingly, earlier *C. arundinaceum* was the only species to be promoted (Anonymous, 2002) but subsequently, *C. borivilianum* was also included for its promotion (Kothari and Singh, 2001). In India, *C. borivilianum* is mainly found in southern Rajasthan, north Gujarat, and western Madhya Pradesh (Kothari and Singh, 2003). There is shortage of published information on various chemical constituents of therapeutic significance and agro-technology for cultivation of safed musli. Some systematic efforts have recently been made in respect of collection of germplasm, their agronomic and chemical evaluation (Maiti, and Geetha, 2005) and development of agrotechnology. The Medicinal Plant Board of India has documented "*safed moosli*" as the sixth important herb to be protected and propagated as it is kept in the category of an endangered plant.

As it has incredible properties which can be explored for health advancement of human beings, steps should be taken for cultivation of *Chlorophytum borivilianum* and isolation of different phytoconstituents specially saponin, so that true medicinal value of our indigenous medicinal plant can be explored. There is need for commercial cultivation of this species. Thus, subsequently attempts were made to categorize superior germplasm and to develop the cultivation practices (Oudhia, 2001). Therefore the present study aimed to standardize the protocol for the quantative analysis of saponin content in plants of *C. borivilianum* by using HPLC.

Material and Methods

Plant materials: The experimental plant materials of *C. borivilianum* (Figure 1) were collected from different forest regions of Chhattisgarh and its adjoining regions (Table 1).

Standards and Reagents: All the solvents used used in this experiment were HPLC grade. Acetonitrile (HPLC grade, Merck, Darmstadt, Germany) and HPLC-grade water (Milli-Q system, Millipore, Bedford, MA, USA) were used for the mobile phase preparation. A membrane filter (MF3- 13 PTFE, diameter-13 mm, pore size-0.50 µm, Advantec, CA, USA) was used to filterate each sample. Various concentrations of standard saponin (Sigma) was weighed and dissolved in mobile phase i.e. acetonitrile: water 40:60 (v/v). 20µl of the standard solution was injected into the column.

Extraction of saponin using tubers as explants:

Saponin content was estimated by by means of the method approved by Brik *et al.,* 1963). To estimate the saponin content, 10 g of safed musli powder was suspended in 100 ml of 85% ethanol and kept for overnight. The supernatant was collected in a 100 L round bottom flask. The residue was re extracted in 85% ethanol and refluxed for half an hour. The procedure was repeated thrice and ethanol was removed by distillation. The soft extract that remained was extracted using 50ml petroleum ether and refluxed for 30 min. cooled and the solvent poured off. The remaining soft extract was refluxed with 50 ml of ethyl aceatae, followed by 50 ml of chloroform for 30 minutes; successively the organic solvents were removed from the separating funnel.

The soft extract was dissolved in 50ml methanol, filtered and made up to 100ml. This hot extract was added drop by drop to 25 ml of acetone. A white precipitate thus formed was collected in a small beaker, dried in an oven at ± 60°C and finally collected

Thin layer chromatography (TLC) is the most commonly used method for obtaining chemical fingerprints. The colour band of saponin was identified against those of standard by using thin layer prepared by using Silica gel 60 F254. Chloroform: methanol: water (50:45:5) mixture was used as the solvent system for the separation of samples.

The collected light brown powder was purified by using column chromatography. The crude powder was dissolved in 5 ml methanol and passed through silica gel size 60-120 mesh column (2cm x 30cm) for the removal of impurities. Column prepared with benzene → benzene → ethyl acetate → chloroform → acetone → methanol (elution) (30:30:30:30:50) was used as the solvent system (Figure 2).

Sample preparation for HPLC:

Preparation of standards -

Various concentrations of standard saponin was weighed and dissolved in mobile phase i.e.water and acetonitrile (60:40). 20µl of the standard solution was injected into the column.

Purified dried fraction of saponin was diluted with 50ml of HPLC mobile phase i.e. acetonitrile - water 40:60 (v/v). From the diluted samples 20µl was taken for injection in the HPLC and chromatogram was recorded.

HPLC analysis

High performance liquid chromatography (HPLC) was performed using a HP 1100 series Agilant make apparatus. Separations were performed on Column – ODS, 4.5mm × 250mm at a flow rate of 1.0 ml /min and detection wavelength of 203 nm. Well resolved chromatograms were obtained with a gradient elution of acetonitrile: water 40:60 (v/v). Retention time of authentic sample of saponin is 1.753 min. The estimation of saponin was done in three replications and the area under the peak gave the amount (mg/ml) of saponin in each sample.

Results and Discussions:

The result showed that irrespective of saponin content in the genotypes assessed for its lead compound, the percentage conversion in the plant also varied. On the basis of behaviour of the graph obtained using Chemostation Software, values were calculated using external standard graph and the area under the peak gave the amount (mg/ml) of saponin in each sample (Figure 2). HPLC analysis of saponins content showed that the highest content of saponin was found in V_2 followed by V_8. The result given in (Table 2) showed that irrespective of saponin content in the genotypes assessed for its lead compound, the percentage conversion in the plant is also varied. The lowest saponin content was observed in V_4 (2.06%) and the highest in V_2 (12.66%). This might be due to the geographical difference or genotypic variation. While comparing the results within the genotypes, it was observed that the saponin content in the accessions collected from the different locations varied due to genotype, soil type, altitude and geographical environment.

Researchers proved the fact that the herbs collected from nature could be many times superior as compared to cultivated ones in medicinal properties and in the international drug markets, herbs from wild origin fetch three times higher price as compared to the cultivated counterparts (Anonymous, 1991-92).

In the literature different authors was observed large variability within and between various collections with respect to various attributes. The reasons from the existence of variability in different collections for saponin content may be due to different genotypes and also sources of collections belonging to genotypes from different soil types, altitudes, temperature and rainfall (Anonymous. 1990 – 1991).

Further, it was observed that the environment where the genotypes are grown also affects the biochemical content in the roots; be it in forests or cultivated in sandy loam to clay loam soils. Biochemically, when a carbohydrate increases the saponin content decreases and vice versa. In the case of protein there is a direct relation of the saponin content.

It was clearly indicated that the synthesis of saponin during growth of tubers is also affected by other factors. Protein and carbohydrate play an essential role in increasing or decreasing the total saponin content in safed musli (Geetha, and Maiti, 2002).

Data obtained after analysis of saponin content in the various collections by HPLC, values for the saponin content in general were similar in range (2.06 to 12.66%) as reported by Schaneberg *et al*., 2003). The variability in saponin content in the various collections was supported by the factors elaborated above.

Kong Ling Yi *et al.,* (2004) successfully analyzed saponin compounds in *Bupleurum falcatum* using high performance liquid chromatography. A mixture of saponin compounds (saikosaponin c, a and d) in the 70% ethanol extract of a powdered sample was analyzed by an Inertsil ODS-3C 18 Column at a flow rate of 1.0 ml/min and detection wavelength of 203 nm. Well resolved chromatograms of saikosaponin c, a and d were obtained with a gradient elution of acetonitrile - water from 40:60 (V/V) to 50:50 (V/V). The coefficient of variability values for saikosaponins in the extract were below 4% and recoveries for saikosaponin c, a and d are 95.2 $\pm$1.1, 96.5 $\pm$0.9 and 96.2 $\pm$1.0.

Table: 1 Nine accessions of Chlorophytum *borivilianum* collected from wild regions.

S.no.	Collection site	Genotypes
1.	Chhaparawa	V_1
2.	Kawardha	V_2
3.	Kota	V_3
4.	Shivtarai	V_4
5.	Amarkantak	V_5
6.	Achanakmar	V_6
7.	Lamni	V_7
8.	Kewachi	V_8
9.	Raigarh	V_9

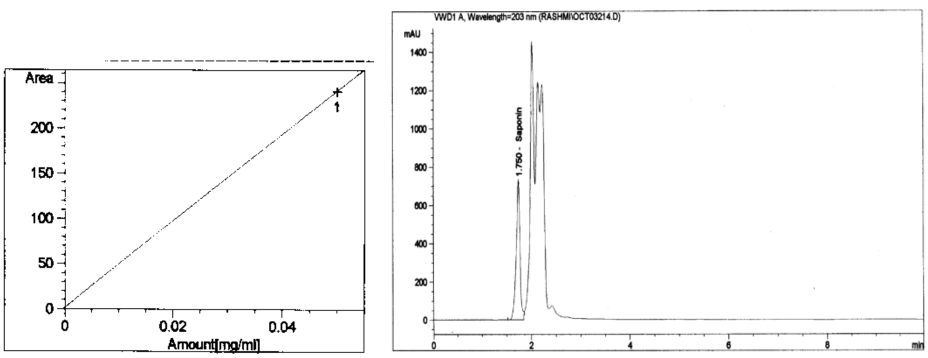
Table 2: Estimation of Saponin content (mg/ml) in 9 genotypes of *Chlorophytum borivilianum* (mean of 3 replications)

Genotypes	Saponin content (mg/ml)	Saponin content (%)
V_1	15.10	2.60
V_2	73.97	12.66
V_3	14.53	2.42
V_4	12.41	2.06
V_5	22.83	3.10
V_6	12.98	2.33
V_7	16.24	2.70
V_8	41.16	6.86
V_9	16.40	2.98

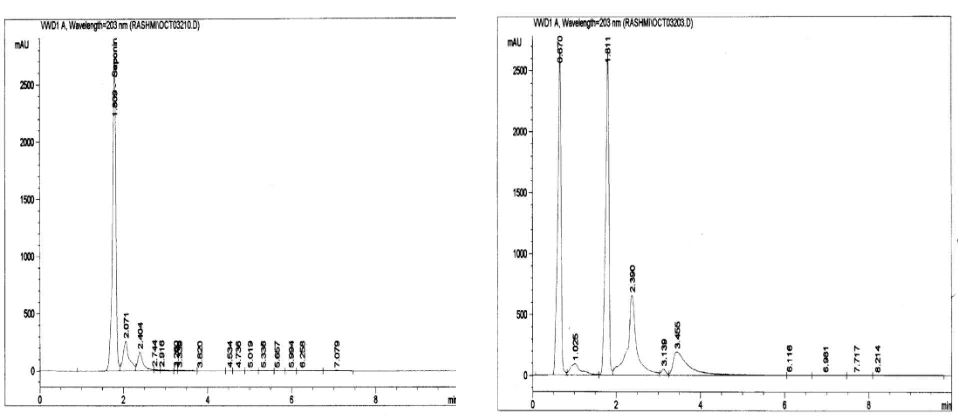
Figure:1 Cultivated *Chlorophytum borivilinaum*

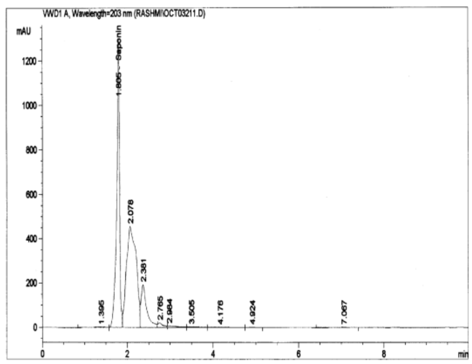
Figure:2 Purified eluted methanol containing sample compared with other eluted solvent

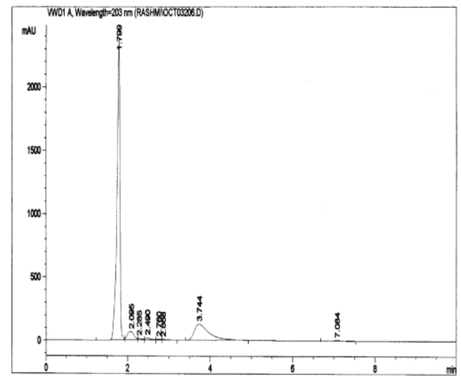
Figure: 3 Chromatograms of (a) Standard, (b) *Chlorophytum borivilianum*

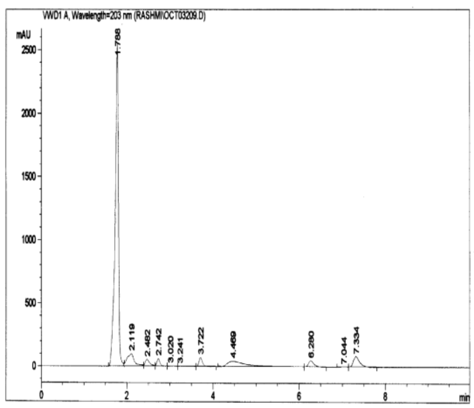


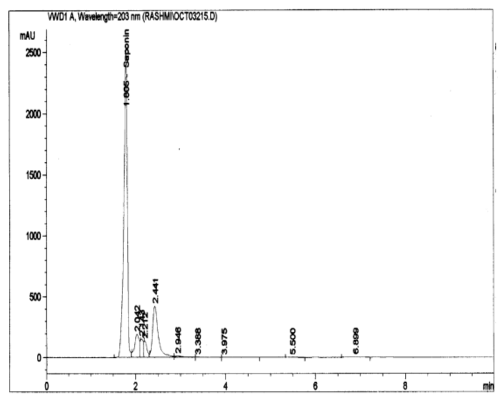
(a) Standard

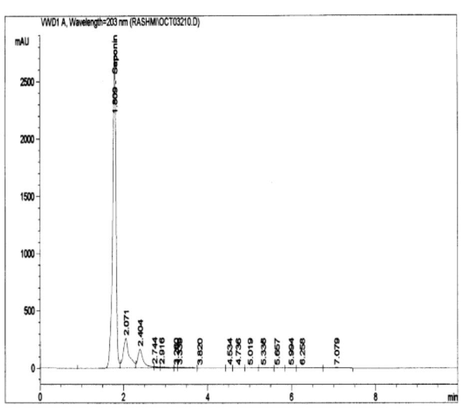


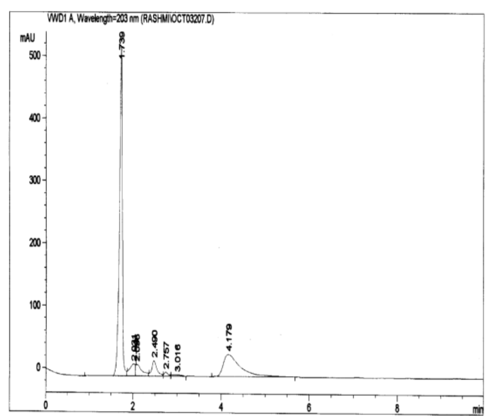




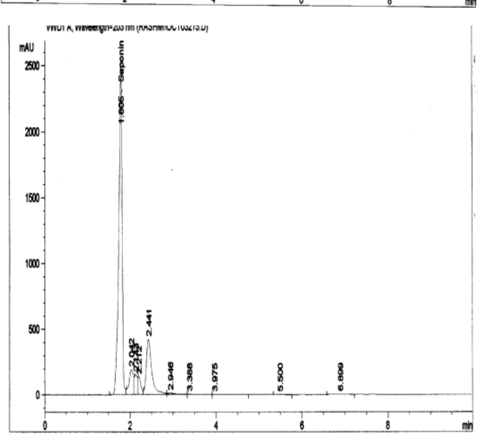








(b) Chromatograms of
***Chlorophytum borivilianum* extract**



Conclusion:

The HPLC method was used to estimate the phytochemical (saponin) variation in each genotype of *Chlorophytum borivilianum.* The highest amount of saponin was detected in the genotype V_2 followed by V_8 and the least amount was found in accession V_4. The present data gave possible information to the effect that the phytochemical character of different genotypes could probably be due to genetic differences. This method is accurate, precise and it successfully analyzed the crude extract of Asparagus species.Thus, it can be suggested that the genotype V_2, the best phenotypic performer and being the highest accumulator of saponin might be potentially useful for cultivation and breeding prospect in future. However, stability of values has to be check through 3 successive generations for validation.

References

(1) Tandon M. and Shukla, Y.N. (1995): Phytoconstituents of *Asparagus adescendens*, *Chlorophytum arundinaceum* and *Curculigo orichoides*: a review. J. Med. Arom. Plant. Sci., 12: 202-204.

(2) Kothari, S.K. (2004): Safed Musli (Chlorophytum borivilianum) revisited. J. Med. Arom. Plant. Sci., 26: 60- 69.

(3) Puri, H.S. (2003): Rasayana , Ayurvedic Herbs for Longevity and Rejuvenation. London: Taylor and Francis, pp. 212-24.

(4) Handa, S.S. (1996): Rasayana drugs. In: Handa SS, Kaul MK (eds). Supplement Cultivation and Utilization of Medicinal Plants, Vol. 1. Jammu Tawi: Regional Research Laboratory, pp. 509-12.

(5) Tandon, M. and Shukla, Y.N. (1993). A bibenzyle xyloside from *C.arundinaceum*, Phytochemistry., 32: 1624-1625.

(6) Oudhia, P. (2001): My experiences with wonder crop Safed Moosli. In: Souvenir. International Seminar on Medicinal Plants and Quality Standardization, VHERDS, Chennai, pp-90-96.

(7) Bordia, P.C., Joshi, A. and Simlot, M.M. (1995): Safed musli. In: Medicinal and Aromatic Plants, (Eds, K.L. Chadha and R. Gupta). Advances in Horticulture, Malhotra Publishing House. New Delhi, pp. 429 – 451.

(8) Singh, H.P. and Samuel, J.C. (2001): National prespective on development of medicinal and aromatic plants. Agri Watch., **1**: 30-40.

(9) Anonymous. (2002). Cultivation Practices of Some Commercially Important Medicinal Plants. National Medicinal Plant Board, New Delhi, pp. 53 – 55.

(10) Kothari, S.K. and Singh, K. (2001): Evaluation of safed musli (*Chlorophytum borivilianum* Santapau and Fernandes) germplasm. J.Spices Aromatic Crops. **10**: 147-149.

(11) Kothari, S.K. and Singh, K. (2003): Production techniques for the cultivation of safed musli (*Chlorophytum borivilianum*). J. Horticultural Science Biotechnology., **78**: 261-264.

(12) Maiti, S. and Geetha, K.A. (2005): Characterization, genetic diversity and cultivation of *Chlorophytum borivilianum* – an important medicinal plant of India. Plant Genet Resour., 3: 264–72.

(13) Oudhia, P. (2001): Problems percieved by *Safed musli* (*Chlorophytum borivilianum*) growers of Chhattisgarh (India) region: A study. Journal of Medicinal and Aromatic Plant Sciences 22/4A and 23/1A: pp. 396-399.

(14) Brik, Y., Gestetner, B. and Ishaaya, I. (1963): A thermostable hemolytic factor in soyabeans. Nature., 197: 1089–1090.

(15) Anonymous. (1991-92): ICAR All India Coordinated Research Project on Medicinal and Aromatic Plants. Progress Report. Rajasthan College of Agricultue, Udaipur. pp. 65-66.

(16) Anonymous. (1990–91). Annual Progress Report of CESS Fund Scheme (ICAR) on Development of Suitable Culture of Safed Musli (*Chlorophytum* species) for Root yield Acceptable Medicinal Quality. Rajasthan College of Agricultue, Udaipur.

(17) Geetha, K. A. and Maiti, S. (2002): Biodiversity in *Chlorophytum borivilianum.* Santapau and Fernandes. Pl Genet. Resour. Newsletter., **129**: 52–53.

(18) Schaneberg, B.T., Mikell, J.R., Bedir, E. and Khan, I.A. (2003): An improved HPLC method for quantitative determination of six triterpenes in *Centella asiatica* extracts and commercial products. Pharmazi., 58:381-4.

(19) Kong, Ling Yi., Wan, JianBo., Shao, Ping., Wang, YiTao. and Kan, Kawing. (2004): Analysis of

saponins from Panax notoginseng using pressurized solvent extraction coupled with high performance thin layer chromatography. Chinese Journal of Natural Medicines., 2: 215 218.