Determination of the Saponin Content in Chlorophytum Genotype

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Abstract

The success of the drug discovery process is often a function of the diversity of chemotypes examined. Natural products screening represents a potential source of organic chemicals of unparallel diversity. A medicinal herb can be compared with a chemical factory due to presence of number of chemical constituents like alkaloids, glycosides, saponins, resins, oleoresins, sesquiterpene lactones and oils (essential and fixed). In the present study we analyzed and quantified the saponin from musli (*Chlorophytum)* respectively for identification of best quality genotype. In order to determine the quantity of lead compound i.e. saponin in different genotypes, HPLC based protocol was standardized. Saponin extracted by 85% ethanol. Extracted material purified through column chromatography. After identification of saponin fraction by thin layer chromatography, extracted and purified sample subjected to HPLC for the estimation of saponin.

Introduction

The screening of natural products is one of the earliest steps in drug discovery-Lead identification. A lead compound, also frequently referred to as a chemical template, is a compound with many of the characteristics of a desired new drug which will be used as a model for chemical modification, but which lacks either the potency or specificity expected of a product candidate. Historically, medicinal plants and microorganisms have been extraordinarily rich sources of medicinally and agriculturally useful compounds. Interest in these sources of new bioactive molecules continues to present time.

Medicinal plants comprise a group of large number of plant species that produce raw material for pharmaceuticals and phyto-chemicals for manufacturing drugs. In the commercial market, medicinal herbs are used as raw drugs, extracts or tinctures. The World Health Organization (WHO) estimates that up to 80% of the world populations rely on plants for their primary health care. The international medicinal plants market is worth US $60 billion per year, and growing at the rate of 7% per annum (Bhojvaid, 2003). Plants have contributed more than 7,000 different

compounds in use as heart drugs, laxatives, diuretics, antibiotics, decongestants, analgesics, anesthetics, ulcer treatments anti-parasitic compounds and so on (Ved *et al.,* 1998). With introduction of sophisticated techniques, the scientists started exploring the plant flora for active constituents. The concept of standardization has great impact on quality of herbal products. Standardization helps in adjusting the herbal drug formulation to a defined content of a constituent or constituents with therapeutic activity. Safed Musli (*Chlorophytum*) is a medicinal plant that grows in dense forests. It belongs to Liliaceae, a family of 175 species distributed all over the world. Among the 13 species found in India, Safed Musli (*Chlorophytum biovirilianum*) has market potential (Borodia *et al.*, 1995). Safed Musli is a medicinal plant used to overcome general and sexual weakness. It is a well-known ayurvedic medicine rich in alkaloids, vitamins, minerals, protein, carbohydrate, steroids and polysaccharides. Owing to its enormous uses, its worldwide demand is estimated to be 35000 tones annually as compared to current annual production of 5000 ton (Udhyamita samachar patra, 1998). Dried roots of *Chlorophytum* contain 42% carbohydrate, 8–9% protein, 3–4% fiber and 2–17% saponin (Borodia *et al.*, 1995). Research studies on *Chlorophytum* conducted in India and elsewhere indicate that saponins are responsible for medicinal properties (Arora *et al.*, 1999). The plant yields a flavonone glycoside, which is a powerful uterine stimulant. Mainly, Saponins are natural surfactants, or detergents, found in many plants. With the increasing population, coupled with the shrinking of genetic diversity in traditional farming systems and reduction in the area of prime land available for agri-horticultural crops, there is emergent need for better utilization of plant genetic resources including lesser known or underutilized plant species like *Chlorophytum* through critical characterization and evaluation of existing biodiversity. Considering above facts, the present investigation was designed to analyzed and quantified the saponin from *Chlorophytum* for identification of best quality genotype. Magalhães *et al.*, (2003) investigated the presence of saponins and the molluscicidal activity of the roots, leaves, seeds and fruits of *Swartzia langsdorffii* Raddi (Leguminosae) against *Biomphalaria glabrata* adults and eggs. The roots, seeds and fruits were macerated in 95% ethanol. These extracts exerted a significant molluscicidal activity against *B. glabrata*, up to a dilution of 100 mg/l. Four mixtures of triterpenoid oleanane type saponins were chromatographically isolated from the seed and fruit extracts. Two known saponins were identified as â-D-glucopyranosyl-[á-L-rhamnopyranosyl-(1.3)-â-D-glucurono-pyranosyl-(1.3)]-3 â-hydroxyolean-12-ene-28-oate, and â-D-glucopyranosyl-(1.3)- â-D-glucuronopyranosyl-(1.3)]-3 â-hydroxyolean-12-ene-28-

oate, respectively. These two saponins were present in all the mixtures, together with other triterpenoid oleane type saponins, which were shown to be less polar, by reversed-phase HPLC.

Materials and Methods

1.1 Experimental materials: Genotype of *Chlorophytum* (M1) of was collected and maintained at Department of Biotechnology, Indira Gandhi Agricultural University, Raipur, India. The field experiment was conducted and the crop was harvested in the first week of November, when leaves became yellow and ultimately dried.

1.2 Estimation of lead compound (Saponin):500 mg. of dried root musli (*Chlorophytum*) powder was used for extraction of saponin. The extarction was done by Soxhlet apparatus with 85 % ethanol and purified through silica gel column chromatography. Purification was done using Hexane (20 ml), Ethyl acetate (20 ml), Chloroform (20 ml), Acetone (20 ml) and finally extracted through methanol. The fractions were analyzed and identified through thin layer chromatography. The samples were spotted on Silica gel coated glass plates with standard saponin. The spots were developed in the mixture of chloroform, methanol and water (60:30:10). Plates were examined by UV fluorescence and sprayed with conc. H_2SO_4, followed by heating at 105^0 C for 1-2 min. Extracted saponin separated and estimated by HPLC (Park *et al*., 2000).

1.3 HPLC Conditions

The HPLC method was used to estimate the saponin. The area under the major peak was measured for quantitative analysis of the saponin.

1.3.1 Chromatographic system

HPLC Pump: LC 10 AD - Agilent make

HPLC Detector SPD 10 A - Agilent makes

Syringe: Hamilton 100 µl Syringe

Injector: 7725I manual Injector

Column: 250 x 4.8 mm SS column containing amino packing 5 micron particle size.

1.3.2 Instrument conditions

Mobile Phase: Water and acetonitrile are mixed in the ratio 60: 40 and the mixture is degassed and filtered.

Injection size: 20 u l

Flow rate: 1 ml per minute

Detector: UV

Wavelength: 203 nm

1.3.3 Preparation of standards

Various concentrations of standard saponin was accurately weighed and dissolved in mobile phase i.e. water and acetonitrile (60:40).

1.3.4 Preparation of Sample

Purified saponin was evaporated and diluted with the mobile phase i.e. water and acetonitrile (60:40).

1.3.5 Procedure

Each standard preparation and sample preparation was injected, separately into the chromatograph. The responses of the sample preparation in terms of areas under the major peak corresponding standard saponin were measured.

Results

Estimation of saponin content

A mixture of saponin in the ethanol extract of powdered samples of *Chlorophytum* were analyzed by an Agilent make C18 column at a flow rate of 1.0 ml/min and detection wavelength of 203 nm. Well-resolved chromatogram of saponin was obtained with a gradient elution of water–acetonitrile 60:40 (v/v). The total time required for a single analysis was approximately 20 min.

The occurrence of saponin in the root extract analyzed by reverse phased HPLC. The retention time found for saponin standard was 1.6 min. in 20 min total runtime The saponin content percentage was found in M1 was 28.2%.

Discussion

Research studies on *Chlorophytum* indicated that saponin is responsible for medicinal properties. Matured finger root used for the extraction. After peeling of skin root fingers were dried at 60 degree followed by Sun drying for 3-4 days. Saponin extracted by 85% ethanol. Extracted material purified through column chromatography. After identification of saponin fraction by thin layer chromatography, extracted and purified sample subjected to HPLC for the estimation of saponin. Saponin content reported in accession number M1. High heritability (79.87%) in sapoginine content confirmed less influence of environment, further suggesting the presence of additive gene effect in expression of this economically important trait with aphrodisiac property was reported by Bhagat and Jadeja (2003). Studies on saponin indicated that content in *Chlorophytum* were significantly affected by the environment. Over the year analysis indicated that saponin content

can be improved by sacrificing the root yield through selection. (Jat and Sharma, 1996). Saponin compound present in *Bupleurum falcatum*, the coefficient of variability values for silksaponin in the extract are below 4%. (Park *et. al.*, 2000).

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