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Extraction and detection of saponin-enriched fractions from different plants of North-western Himalayas, India

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Abstract
The different plant species throughout the world have been used in traditional medicines, and some of their bioactive constituents including saponins have excellent biological activities for new drug development. The present study was designed to explore four plants of North-western Himalayas, *Asparagus adscendens* Roxb. (AA) (fruits), *Silene inflata* Sm. (SI) (whole plant), *Sapindus mukorossi* Gaertn. (SM) (pericarp of fruit) and *Chlorophytum borivilianum* (CB) (leaves) for saponin content. The total crude saponin-rich fraction yields were 8.0±2.564%, 4.0±1.486 %, 20.0±2.845%, and 9.8±1.439%, respectively. The presence of saponins in plants was confirmed by foam test and spectrophotometry. The foam forming capacity of AAS, SI, SM and CB was significantly lower than standard saponin. The concentration of saponins was found to be 560 ±2.426 µg/ml (AA), 240 ±0.536 µg/ml (SI), 820±1.846 µg/ml (SM) and 110 ±1.664 µg/ml (CB) in the samples. The saponins from these plants could be used in formulation of various drugs, nutraceuticals or other uses, thereby avoiding overexploitation the plant(s) currently in use for the purpose.

Keywords: Plant(s), North-western Himalayas, methanol extract, saponins, concentration

1. Introduction
Saponins are a class of natural products which structurally consist of polycyclic aglycones (steroidal C27 or triperpene C30) and one or more sugar side chains. Saponins are widely distributed in several plants and are fairly widespread in our foodstuffs and herbal preparations. Current focus of research on nutraceuticals and functional foods is identification and depiction of their bioactive constituents. Saponins are remarkably stable to heat processing, and their biological activity is not reduced by normal cooking [1]. Saponins are essential constituents of nutraceuticals and useful foods [2] and are recognized with a number of bioactivities like anti-inflammatory, antimicrobial, immunostimulant, hypocholesterolaemic, anticarcinogenic and antioxidant [3]. In addition, saponins form complexes with cholesterol of erythrocytes membrane forming pits and holes, which leads to increase in permeability and haemolysis [4]. It is used also in food ingredients, beverages, shampoos, toothpastes, liquid detergents and extinguishers as an emulsifier and long-lasting foaming agent. Recently, the saponin mixture has shown to possess immunoadjuvant property and has pharmaceutical application as suspension stabilizer [5]. Overexploitation of *Quillaja saponaria* bark has caused concern about the ecological damage and a consequent shortage of the available supplies. Therefore, during the last decade, other natural products have been screened for saponin content and have shown to possess different pharmacological activities. Based on above information, the objective of the present study was to explore the new plant-derived saponins from fruits of *Asparagus adscendens* Roxb., whole plant of *Silene inflata* Sm., pericarp of fruits (seed coat) of *Sapindus mukorossi* Gaertn. (SM), and leaves of *Chlorophytum borivilianum*.

2. Materials and methods
2.1 Collection of plant material
Selected plant parts mentioned in Table 1 were collected in fruiting season (winter) months, October-January from areas adjoining Palampur (1200 m above msl) and Kangra (733 m above msl) towns except *C. borivilianum* leaves which were procured from CSIR- Institute of Himalayan Bioresource Technology (IHBT), Palampur, Himachal Pradesh (H.P.), India. The plants were identified in the Biodiversity Division of CSIR- IHBT, Palampur, H.P.

2.2 Preparation and extraction of crude saponins

Collected plant parts were dried at 50^{0}C in hot air oven and ground to a fine powder using on electric grinder. Saponins extraction was carried out using standard protocol of Sharma and co-workers [6] with minor modifications. For this, the plant parts were dried at 70^{0}C and 100 g, dried sample was defatted with 1 L n-hexane using Soxhlet apparatus (5 h). The defatted sample (10 g) was extracted with methanol:water (1:1) thrice using 100 ml for each extraction. Methanolic extracts were pooled, the solvent was removed *in vacuo* and the remaining aqueous portion was partitioned three times with equal volume of n-butanol. The butanol layers were pooled, the solvent was removed *in vacuo* at 40^{0}C and the residue was used as saponin-enriched extract. The saponins enriched total content was calculated as a percentage:

Total yield of saponins (%) = (Weight of saponins / Weight of sample) x100

2.3 Saponin estimation

2.3.1 Foam test

About 12.5 mg standard Quil-A® saponin (≥95% purity, InvivoGen, USA) and the test samples each were taken in 250 ml measuring cylinders, separately in triplicate. Then, distilled water (87.5 ml) was added in all the measuring cylinders. After that, the measuring cylinders were shaken vigorously for about 30 times by closing the mouth of cylinder with stopper. After shaking, stopper was removed and mouth of the cylinder was covered with aluminum foil. Three observations were recorded, immediately after shaking, after 30 min and after overnight standing.

2.3.2 Spectrophotometric analysis of saponins

The quantification of the saponin-enriched fractions was carried out by spectrophotometry as described previously [7] with minor alterations. Quil-A was used as the standard. Briefly, two reagents, i.e., (A) p-anisaldehyde (Sigma-Aldrich) in ethyl acetate (0.5:99.5) and (B) H_2SO_4: Ethyl acetate in equal parts, were mixed in equal proportions. The methanolic extracts of all the plants and standard (QA) were dissolved in 2 ml ethyl acetate. Then, 1 ml of reagent A and 1 ml of reagent B was added. The mixture was stirred and incubated at 60^{0}C for 10 min in a water bath. The solutions were cooled at room temperature for 10 min and the absorbance of the color-developed solution was recorded at 430 nm. Ethyl acetate was used as a control for the measurement of absorbance. Solutions containing 75-175 µg standard saponin in 2 ml ethyl acetate were used to obtain a calibration curve. The total saponins in extract were calculated from the regression equation obtained from the calibration curve of standard saponin.

$y = 0.0029x + 0.0599$, $R^2 = 0.9987$

where y is absorbance at 430 nm and x is total saponin content in the extracts and expressed in µg/ml (Fig. 1).

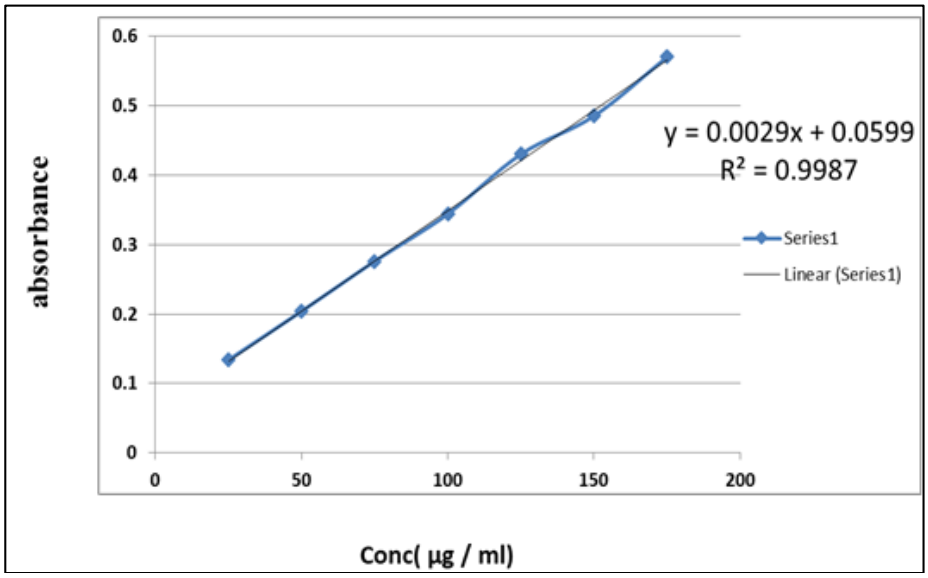

Fig 1: Calibration curve of standard saponin (Quil-A)

3. Results and discussion

The present study was designed to explore the saponin content of herbal plant parts, *A. adscendens* Roxb. (AAS) fruits, *S. inflata* Sm. (SI) whole plant, *S. mukorossi* Gaertn. (SM) pericarp of fruit and *C. borivilianum* (CB) leaves collected from different regions of Northern-western Himalayas. The qualitative estimation was done using foam test (Table 1). The foam forming capacity of AAS, SI, SM and CB was significantly lower than standard (QA) saponin at all the time intervals (Table 1, Fig. 2). It has been reported that saponins have strong surface-active property and can form stable foam in aqueous solution [8, 9]. This detergent property leads to considerable foaming of aqueous saponins solutions. The characteristic honey-comb froth produced is often employed in plant screening work as presumptive evidence of the presence of saponins. The stability and strength of saponin foams are closely dependent on pH. Due to this property, they are being used in shampoos, liquid detergents, toothpastes and beverages as emulsifier and long-lasting foaming agents [10].

Excessive feeding of saponin-rich plants to the ruminants lowers the surface tension of ruminal contents leading to a condition known as bloat. In this condition, huge accumulation of gas in the digesta and distension of the rumen occurs which impedes blood flow and eventually the animal develops anorexia and respiratory failure. Hence, high foam forming saponin-rich forages should be fed in limited quantity to the livestock. In the present study, the foam forming capacity of the saponins of selected plant parts was less than the standard saponin. This could be partially attributed to the lesser purified extracts as compared to standard purified saponin.

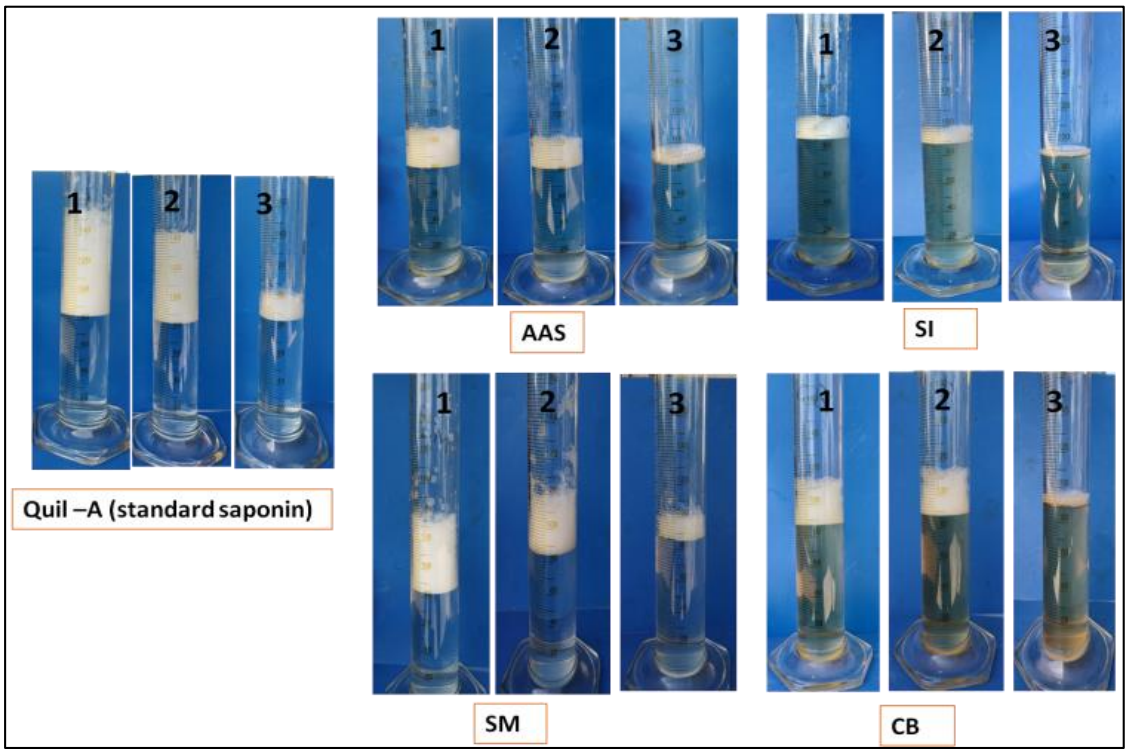

Fig 2: Foam test of saponin-enriched fractions of AAS, SI, SM and CB plants in comparison to standard Quil A saponin, at different time intervals. 1) Immediately after shaking 2) After 30 min 3) After overnight standing of respective plants.

Table 1: Amount of foam formed (ml) at different time intervals and percentage of variation in foam production in saponin-enriched fractions of different plants in comparison to Quil-A (Standard) saponin.

Amount of foam formed (ml)	Quil-A (Std.)	AAS	% variation in foam production in AAS saponins from std.	SI	% variation in foam production SI saponins from std.	SM	% variation in foam production SM saponins from std.	CB	% variation in foam production CB saponins from std.
Immediately after shaking	77.00	28.00	63.63	17.00	77.92	53.00	31.17	29.00	76.62
After 30 min	56.00	23.00	58.93	10.00	82.14	36.00	35.71	26.00	53.57
After overnight standing	21.00	8.00	61.90	3.00	85.71	14.00	33.33	6.00	71.42

In the present study, the total crude saponin-enriched fraction yields were 8.0±2.564%, 4.0±1.486%, 20.0±2.845% and 9.8±1.439%, respectively (Table 2). These results showed that appreciable amount of saponin was found in the selected plant parts. Saponin protects against microbial attack in plants; it is also used in various food ingredients, beverages, shampoos, toothpastes, liquid detergents, pharmaceutical industries to formulate different drugs to treat human and animal diseases, extinguishers, as an emulsifier and long-lasting foaming agent [4].

Table 2: Details of the plants, plant parts and percent yield of extracted saponin-enriched fractions.

Plants	Common name	Plant part	% Yield
Asparagus adscendens Roxb.	Sansban, Saunspali	Fruit	8.0±2.564
Silene inflata Sm.	Bigru	Whole plant	4.0±1.486
Sapindus mukorossi Gaertn.	Soapnut, Reetha	Pericarp of fruit (seed coat)	20.0±2.845
Chlorophytum borivilianum	Safedmusli	Leaf	9.8±1.439

The spectrophotometric analysis of the methanolic extracts of plants confirmed the presence of saponins at concentration of 560 ±2.426 µg/ml in AAS, 240 ±0.536 µg/ml in SI, 820±1.846 µg/ml in SM and 110 ±1.664 µg/ml in CB (Table 3).

Table 3: Spectrophotometric quantification (µg/ml) of the saponin-enriched fractions in different plant parts.

Sl. No	Plants	Quantification of saponins (µg /ml)
1.	*S. mukorossi* Gaertn.	820±1.846*
2.	*A. adscendens* Roxb.	560 ±2.426
3.	*C. borivilianum*	110 ±1.664
4.	*S. inflata* Sm.	240 ±0.536

*Each value is presented as Mean±SE, (n=3)

Spectrophotometry methods are practical, simple and very less expensive than HPLC, making them preferable for specification testing. Because of the weak absorbance of saponins, a colorimetric determination is used for their

evaluation. The result showed that the seed coats of *S. mukorossi* contained highest saponin content followed by fruits of *A. adscendens*, whole plants of *S. inflata* and leaves of *C. borivilianum*. The amount of saponin in the aqueous and methanolic extracts of different plants by spectrophotometry method using p-anisaldehyde and sulphuric acid has been studied earlier as well [12].

The foam test and spectrophotometry method confirmed the presence of saponin-enriched fractions in *A. adscendens* Roxb., *S. inflata* Sm., *S. mukorossi* Gaertn., and *C. borivilianum* plant extracts. The saponins from these plants could be used in formulation of various drugs, nutraceuticals or other uses, thereby avoiding overexploitation the plant(s) currently in use for the purpose.

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Conflicts of interest

There are no conflicts of interest.

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