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Quantification for Saponin from a Soapberry (*Sapindus mukorossi* Gaertn) in Cleaning Products by a Chromatographic and two Colorimetric Assays

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The extract from the pericarp of the Chinese Soapberry (*Sapindus mukorossi* Gaertn.) is increasingly being added into a variety of cleaning products. To establish a standardized quantification method, the accuracy of the reverse phase high performance liquid chromatography (HPLC), vanillin–sulfuric acid and antimony pentachloride colorimetric assays were compared. The chemical structural features of saponin of *S. mukorossi* for the major peak in the HPLC chromatograms were identified by LC/MS/MS, ¹H and ¹³C NMR spectroscopy. Organically extracted crude soapnut saponin was employed as the standard. Linearity for the calibration graphs was found for the range of organic extracts of crude saponin down to 0.2 g/L aqueous solutions, for all three assays studied. The area of the main peak in the HPLC chromatograms and the absorbance values of two colorimetric assays of the commercial glycerin clear soap base with predetermined amount of soapnut extract were recorded and compared with calibration graphs constructed with an aqueous saponin solution. It was found that HPLC analysis provided more precise values than the two colorimetric assays. Discrepancies were found between the saponin contents for 12 commercial cleaning products in Taiwan as analyzed by HPLC and the values estimated by antimony pentachloride and vanillin–sulfuric colorimetric assays.

Keywords: *Sapindus mukorossi*, Soapnut, Saponin, Cleaning Products, Colorimetric Assay

INTRODUCTION

The Chinese soapberry tree (*Sapindus mukorossi* Gaertn.) is a small deciduous tree, widely distributed in the lowland area of Taiwan, as well as in the forests below 1000 m of China, India and Japan (*Flora of Taiwan*, 2003). Extract of the soapnut pericarps used to be popular ingredients for hair and body cleaning purposes in traditional Taiwanese culture. Extracts were also employed as a folk medicine as an expectorant, for cough relief, for detoxification and defervescence (*Flora Yunnanica*, 1997). In addition, since Chinese Soapberry trees are an endemic species in Taiwan, the Taiwan Forest

Bureau has designated the Chinese soapberry tree as one of the primary species for the flat-lands reforestation program. It is believed that the high value-added soapnuts and their derivatives may encourage more farmers and land-owners to plant Chinese Soapberry trees.

There are many existing biological and phytochemical saponin screening methods available (Farnsworth, 1966), including the anillin–sulfuric acid (Hiai *et al.*, 1976) and antimony pentachloride colorimetric assays (Hanzas and Barr, 1969). Saponin contents in beverages can also be determined by the antimony pentachloride colorimetric assay (Chinese National Standards, 1986). Based on the review of the chromatographic methodologies for determining plant saponins (Oleszek, 2002; Oleszek and Biely, 2006), was employed the reverse phase HPLC with acetonitrile–water gradient as a mobile phase to quantify the saponin contents.

The major active ingredient of the Chinese Soapberry extract is saponin. Saponins are generally known as non-volatile, surface-active compounds that are widely distributed in nature, occurring primarily in the plant kingdom (Oleszek, 2002). Saponins have a diverse range of properties, including foaming and emulsifying (Price *et al.*, 1987), pharmacological and medicinal properties (Attele *et al.*, 1999), haemolytic properties (Oda *et al.*, 2000; Sparg *et al.*, 2004), as well as antimicrobial, insecticidal, spermicidal and molluscicidal activities (Huang *et al.*, 2003; Sparg *et al.*, 2004; Saxena *et al.*, 2004). Saponins have found wide applications in beverages and confectionery, as well as in cosmetics (Price *et al.*, 1987;

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Petit *et al.*, 1995; Uematsu *et al.*, 2000).

Currently, natural detergents derived from soapberries are becoming increasingly popular in Taiwan and around the globe (Kimata *et al.*, 1983). More and more saponin-based household products derived from *Sapindus mukorossi* Gaertn. are being marketed. Many of these commercial products are advertised as "with the addition of natural soapnut saponin" although their saponin content is questionable. Hence, better and easier verification methods for determining the amount of natural soapberry saponin ingredients in household cleaning products are needed.

Although there are many existing assays, accurate quantification methods for determining the saponin content in household cleaning products are yet to be established. In this work, the accuracy of high performance liquid chromatography (HPLC), vanillin-sulfuric acid and antimony pentachloride colorimetric assays for measuring commercial glycerin clear soap base spiked with pre-determined soapberry extract was compared. Calibration curves were constructed with aqueous and organic extract saponin solution, as well as with commercial Quillaja saponin. Chemical structural features of *S. mukorossi* saponin for the major peak in HPLC chromatograms were also identified by LC/MS/MS, ^1H and ^{13}C NMR spectroscopy.

MATERIALS AND METHODS

Plant Material

Batches of the pericarp of *S. mukorossi* (soapberry) were collected from Da-Wu township, Taitung, Taiwan. Quillaja saponin was purchased from Sigma Chemical Company (St. Louis, MO).

Organic extract of crude saponin

Air-dried powdered pericarp of *S. mukorossi* (5 g) was extracted by maceration in methanol at 50 °C for 12 hours at a solid/liquid ratio of 1/20 (w/v). After filtration and evaporation of the methanol extract, the gummy residue was suspended in 50 mL H_2O and extracted successively with 100 mL ethyl ether three times. After removing the remaining ethyl ether in the aqueous layer by evaporation, the solution was further extracted successively with 100 mL, and 50 mL *n*-butanol respectively. Then, the *n*-butanol fraction was dried by evaporation. The resulting product (1.3 g) is referred to as the organic extract of crude saponin. The aqueous solutions of the organic extract of crude saponin were then prepared for further analysis.

HPLC analysis

As reported (Hamburger *et al.*, 1992), linear gradient acetonitrile: water (4:6 to 6:4) over 30 minutes was used to analyze triterpenoid saponin from *Sapindus rarak*.

In this work, a BDS Hypersil C18 column (4.6 mm ID \times 250 mm, 5 μm) column was employed with a linear gradient mobile phase acetonitrile/water = 40/60 to 60/40 in 30 minutes, with a flow rate of 1 mL/min delivered by

two Jasco PU-980 Intelligent HPLC pumps. A TSP UV 6000 photo diode array detector was used to analyze at a wavelength of 206 nm. Saponins were expected to be eluted at retention times of 10–20 minutes, as reported by Hamburger *et al.*, (1992) in order to analyze triterpenoid saponin from *Sapindus rarak*. Peak areas of selected peaks at retention times of 10–20 minutes were employed to quantify sample saponin contents.

Structural identification of the major saponin component

To identify structure of peaks in chromatograms, a Hitachi AS-4000 intelligent auto sampler was employed to repetitively inject 20 μl methanol solutions organic extracts of saponin samples, with the gradient HPLC elution procedure as same as the one specified in Section 2.3. Peaks among 10–20 minutes of chromatograms were continually collected in ten second intervals in glass test tubes by means of an Advantec 2120 fraction collector. The injection process was repeated 100 times. Then the collected samples were concentrated by evaporation, and dissolved by 1 mL methanol. The samples then underwent ^1H and ^{13}C NMR and spectroscopy analysis. ^1H (500 MHz), ^{13}C (125 MHz) NMR spectra were recorded on a Varian Unity INOVA-500 spectrometer (Varian Inc., Palo Alto, California). Mass spectra were obtained using a nanoelectrospray ionization source on a hybrid quadrupole-trap instrument (ABI 4000 Q Trap LC/MS/MS System, Applied Biosystems, Foster City, CA).

Antimony Pentachloride Colorimetric Assay

According to the reported method (CNS 9429, 1986; Hanzas and Barr, 1969), the sample solution (125 mL) was placed in a 250 mL Erlenmeyer flask. Then the solution was diluted with distilled water to 250 mL with 2.5 mL of concentrated HCl. The sample solution was then filtered through a Buchner funnel. Before filtration, 2.75 g of filter aid was added in the solution, and filter aid (1.25 g) was spread in the funnel. Celite® 545 coarse filter aid from Fluka (Buchs, Switzerland) was employed. After filtration, the precipitate was washed by HCl (pH 2) solution and dried for 20 minutes in an oven at 105 °C. The residue was dissolved with 8 mL of hot acetic acid. Then the volume of the mixture was added, up to 10 mL. One mL of the solution was placed in a colorimetric tube, and 7 mL of 10% (w/v) antimony pentachloride solution was added and mixed thoroughly. The mixture was stilled for 10 minutes to allow maximum color development. Absorbance of the mixture at 535 nm was recorded against the blank with the reagents using a Jasco V-530 UV/VIS spectrophotometer (Jasco, Tokyo Japan).

Vanillin-Sulfuric acid assay

The procedure reported by Hiai *et al.* (1976) was adopted. To 0.5 mL of aqueous solution of the sample, 0.5 mL vanillin solution of 8% (w/v) and then 5.0 mL of sulfuric acid of 72% (w/v) were added and thoroughly mixed in an ice water bath. The mixture was then warmed in a bath at 60 °C for 10 minutes then cooled in ice-cold water. Absorbance at 535 nm was recorded

against the blank with the reagents using a Jasco V-530 UV/VIS spectrophotometer (Jasco, Tokyo Japan).

Preparation of organic extracted crude saponin on commercial soap base

Saponin soap base solution was prepared by dissolving solid commercial glycerin clear soap base (South King Co., Ltd., Pingtung, Taiwan) with DI water at 5% (w/w). Commercial glycerin clear soap base contains following ingredients (% by weight): neo-decanoic acid 15.2%, stearic acid 19.1%, mystic acid 9.3%, lauric acid 2.5%, sodium hydrosulfide 0.2%, ortho-tolyl biguanide 0.3%, glycerin 11.3%, triethanolamine 31.3%, sodium hydroxide 5.3% water 5.3%, pentasodium diethylenetriamine pentaacetic acid 0.2%. Then spiked organic extracted crude saponin was added to the soap solution at concentrations of 0.5%, 1%, 2%, 3%, 4%, 5% (w/w), respectively. Other commercial products were directly analyzed without further purification.

RESULTS AND DISCUSSION

HPLC analyses

The HPLC chromatograms for 2 and 10 g/L aqueous solutions of organic extracted *S. mukurossi* saponin and Quillaja saponin (Sigma) are shown in Fig. 1. The optimal separation was obtained on a reversed phase C-18 column, as reported by Hamburger *et al.* (1992). The use of acetonitrile enabled the reduction of the baseline drift during gradient elution when detected at a wavelength of 206 nm. The peaks eluted with retention times of 10–20 min are indicative of saponins. Since Quillaja saponin is bisdesmodic, its elution times are different from the values of monodesmodic *S. mukurossi* saponin. The lower peak heights of Quillaja saponin (Sigma) correspond to approx. 25% of the saponin content specified by the manufacturer. For *S. mukurossi* saponin, there were eight distinct peaks eluted from 10 to 20 min, as shown in Fig. 1. The number of peaks was close to seven and six hedragenin saponins were reported by Huang *et al.* (2003) and Saxena *et al.* (2004) for Indian *S. mukurossi*.

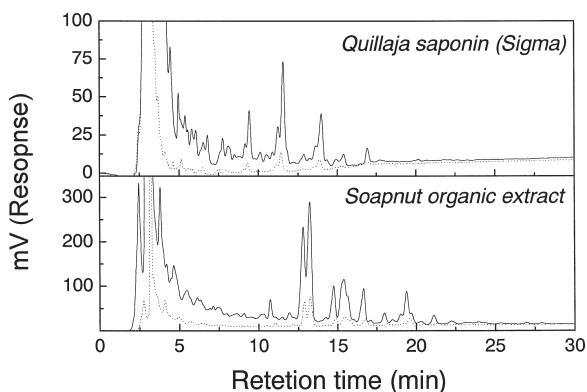


Fig. 1. HPLC chromatograms of 2 and 10 g/l aqueous solutions of organic extracted *S. mukurossi* saponin and Quillaja saponin (Sigma). Column, BDS Hypersil C18 (4.6 mm i.d. \times 250 mm, 5 μ m) column; eluent, linear gradient acetonitrile: water (4:6 to 6:4) in 30 min; flow-rate, 1 ml/min; detection at 206 nm.

rossi. The peak eluted at 12.5 minutes was selected for further isolation and structural identification. Although there are two compounds shown in the peak at 12.5 minutes, the former part of the peak was selected. The objective was to ensure that the feature of the triterpenoid glycoside was presented in the peak. The peak was utilized to quantify the contents of *S. mukurossi* saponin in cleaning products.

Structural identification of the major saponin peak

A single compound from the 15th fraction from 100 collections by HPLC was isolated. The compound corresponded to the earlier portion of the peak retention time of 12.5 minutes in Fig. 2. The signal at δ 5.10 indicated an olefinic moiety. The 13 C NMR spectra revealed a signal for a carboxylate group (δ 182.11) but not for an acetyl group due to a lack of resonance at δ 2.1 (a typical value for a methyl group next to a carbonyl carbon). It was found that the 1 H and 13 C-NMR data were all in accordance with the reported values by Lavaud *et al.* (2001), as shown in Tables 1 and 2. The retention time of the HPLC analysis is fully matched for the data reported by Hamburger *et al.* (1992). Therefore, the structure is assigned as in Fig. 2.

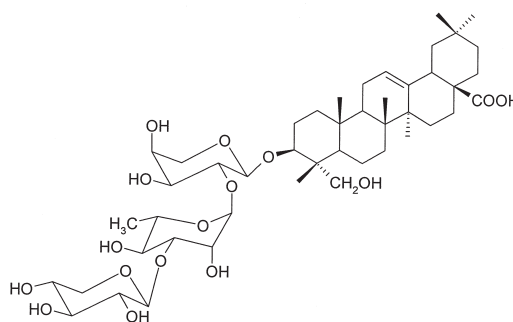


Fig. 2. The structure of the 15th fraction.

The LC/MS/MS data of the 15th fraction is shown in Fig. 3. The molecular weight for this compound should be 863. No expected value was observed for the corresponding molecular weight by LC/MS/MS analysis. However, the fragment patterns of 606 [M+H–arabinose–rhamnose], 474 [M+H–arabinose–rhamnose–xylose] and 456 [M+H–arabinose–rhamnose–xylose–H₂O] were in

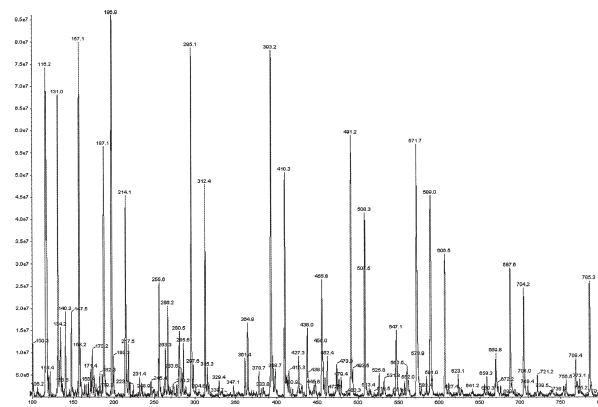


Fig. 3. LC/MS/MS spectroscopy results of the isolate No. 15.

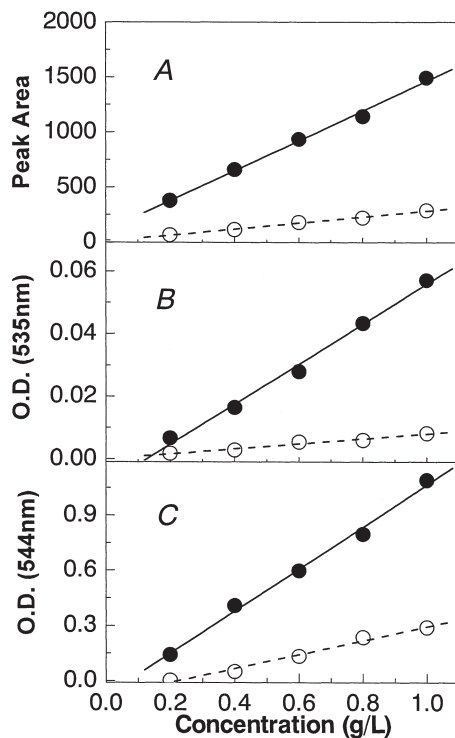


Fig. 4. Saponin content calibration curves analyzed by HPLC (A), antimony pentachloride (B) and vanillin–sulfuric acid (C) colorimetric assays of 0.2 to 1.0 g/L aqueous solutions of organic extracted *S. mukurossi* saponin (solid symbols) and Quillaja saponin (Sigma) (open symbols).

Table 1. ¹H NMR data for reference (Lavaud *et al.*, 2001) and the 14th, 15th, 16th fractions

position	Reference	No.14	No.15	No.16
Ara-1	4.54	4.49	4.49	4.48
Ara-2	3.72	3.70	3.70	3.70
Ara-3	3.72	3.72	3.72	3.71
Ara-4	3.77	3.79	3.79	3.79
Ara-5	3.54	3.54	3.54	3.53
Ara-5	3.86	3.84	3.83	3.83
H-2	1.76	1.75	1.75	1.75
H-2	1.85	1.872	1.87	1.87
H-3	3.63	3.62	3.60	3.60
H-11	1.85–1.98	1.98	1.98	1.98
H-12	5.23	5.10	5.10	5.10
H-18	2.93	2.83	2.83	2.83
H-19	1.11	1.14	1.14	1.14
H-19'	1.64	1.66	1.66	1.66
Me-24	0.72	0.67	0.67	0.68
Me-25	0.99	0.95	0.95	0.95
Me-26	0.89	0.88	0.88	0.88
Me-27	1.17	1.17	1.17	1.17
Me-29	0.9	0.91	0.91	0.91
Me-30	0.97	0.93	0.93	0.93
Rha-1	5.27	5.21	5.22	5.22
Rha-2	4.11	3.99	3.98	3.98
Rha-3	3.87	3.86	3.85	3.86
Rha-4	3.58	3.57	3.56	3.57
Rha-5	3.94	3.91	3.91	3.90
Rha-6	1.27	1.26	1.26	1.27
Xyl-1	4.51	4.47	4.47	4.48
Xyl-2	3.31	3.30	3.30	3.30
Xyl-3	3.35	3.36	3.36	3.36
Xyl-4	3.51	3.52	3.52	3.52
Xyl-5ax	3.24	3.27	3.27	3.27
Xyl-5eq	3.88	3.87	3.88	3.87

accordance with the expected molecular weight. Therefore, the structure of these samples is likely the structure shown in Fig. 3.

Comparison of quantification methods on aqueous solution

Linearity of the calibration curves of an aqueous solution for Quillaja saponin (Sigma) and organic extracted *S. mukurossi* saponin analyzed by HPLC, antimony pentachloride and vanillin–sulfuric acid colorimetric assays were demonstrated for 2 to 10 g/L, as well as for 0.2 to 1 g/L as shown in Fig. 4. Similar to the HPLC results, lower saponin contents for Quillaja saponin (Sigma) were reflected in lower optical densities for antimony pentachloride and vanillin–sulfuric acid colorimetric assays. All three assays are shown to successfully quantify saponin solution in pure water.

Table 2. ¹³C NMR data for reference (Lavaud *et al.*, 2001) and the 14th, 15th, 16th fractions

Position	Reference	No. 14	No. 15	No. 16
Ara-1	104.6	98.6	98.6	98.6
Ara-2	76.0	76.0	76.0	76.0
Ara-3	74.1	74.1	74.1	74.1
Ara-4	69.6	69.8	69.8	69.8
Ara-5	65.4	66.9	66.8	65.3
C-1	39.6	39.3	39.3	39.4
C-2	26.5	27.2	27.2	27.2
C-3	82.3	83.5	82.5	82.7
C-4	43.9	43.2	43.2	43.2
C-6	18.9	19.0	19.0	19.0
C-7	34.3	33.7	33.7	33.7
C-8	40.4	40.4	40.4	40.4
C-9	48.1	47.5	47.6	47.6
C-10	37.6	37.8	37.8	37.8
C-11	24.5	24.4	24.4	24.4
C-12	122.3	123.5	123.5	123.5
C-13	146.7	145.1	145.1	145.1
C-14	43.1	42.6	42.6	42.6
C-15	29.2	28.7	28.7	28.7
C-16	24.5	24.8	24.5	24.6
C-17	48.1	47.1	47.1	47.1
C-18	43.4	42.8	42.8	42.8
C-20	31.7	31.5	31.5	31.5
C-21	35.4	34.7	34.7	34.7
C-22	33.5	33.3	33.3	33.3
C-23	64.5	63.7	63.7	63.7
C-24	13.7	12.7	12.7	12.7
C-25	16.3	16.2	16.2	16.2
C-26	17.9	17.7	17.7	17.7
C-27	26.4	26.4	26.4	26.4
C-29	33.5	33.5	33.5	33.5
C-30	24.3	23.9	23.9	23.9
Rha-1	101.2	95.7	95.7	95.3
Rha-2	71.6	72.0	72.0	72.0
Rha-3	82.1	82.6	82.0	82.3
Rha-4	72.7	72.9	72.	72.9
Rha-5	69.9	70.2	70.2	70.2
Rha-6	18.1	18.0	18.0	18.0
Xyl-1	106.5	98.7	98.7	98.7
Xyl-2	75.2	74.9	74.9	74.9
Xyl-3	77.5	77.8	77.8	77.8
Xyl-4	71.0	70.6	70.6	70.6
Xyl-5	66.9	67.0	67.0	66.9

Comparison of quantification methods on a commercial soap base solution

The response for the 5–50 g/L organic extracted *S. mukurossi* saponin spiked in commercial clear glycerin soap base analyzed by HPLC, antimony pentachloride and vanillin sulfuric acid colorimetric assays are summarized in Table 3. In two colorimetric assays the optical density readings were multiplied by the dilution factors to obtain the total absorption. The contents of the commercial clear glycerin soap base interfered with the responses of the antimony pentachloride and vanillin sulfuric acid colorimetric assays, thereby giving some positive readings for the pure glycerin soap base. To construct the calibration curves, total absorptions of antimony pentachloride and vanillin sulfuric acid colorimetric assays were regressed compared to the spiked saponin contents. In addition, the area of the major peak at 12.5 minutes and the summation of the major peaks from 10 to 20 minutes were also regressed. The objective was to include the peaks between 10 and 20 minutes in order to account for all the compounds with similar saponin related structures.

Linearity was evident for the regression with respect to the spiked saponin contents for the two colorimetric assays in Fig. 5A. For the antimony pentachloride assay,

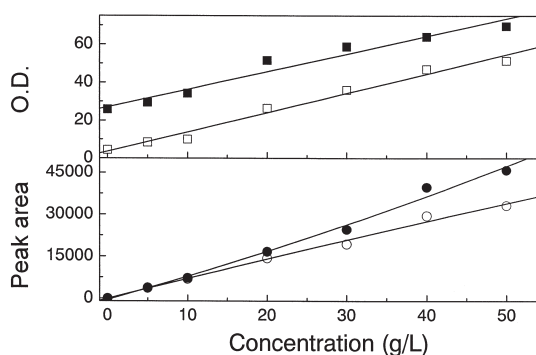


Fig. 5. Correlations between responses and the contents of organic extracted *S. mukurossi* saponin spiked in the commercial clear glycerin soap base areas analyzed by antimony pentachloride and vanillin sulfuric acid colorimetric assays (A); the major peak at 12.5 minutes and total area of all major peaks between 10 to 20 minutes in HPLC assay (B).

there was total absorption at 535 nm = $0.9282 \times$ saponin content (g/L) + 27.076, $R^2=0.9589$. For the vanillin–sulfuric acid assay, total absorption was at 544 nm = $1.0212 \times$ saponin content (g/L) + 3.6457, $R^2=0.9816$. It was shown that vanillin–sulfuric acid assay gave a slight better estimation. Linearity was shown for regression with respect to the spiked saponin contents for HPLC analysis in Fig. 5B, for both the major peak area and the summation of all peak areas from 10 to 20 minutes. Points of zero were included in the regression, since there were no peaks in the chromatograms without spiked saponin. For the major peak area, area = $684.69 \times$ saponin content (g/L), $R^2=0.9926$. For the summation of all peak areas from 10 to 20 minutes, area = $912.17 \times$ saponin content (g/L), $R^2=0.9866$. The areas of the peaks were not affected by the components in the commercial clear glycerin soap base. The correlations were employed to further analyze other commercial products on the market.

Comparison of quantification methods on commercial sanitary products

Saponin contents of 12 commercial cleaning products were analyzed by HPLC, antimony pentachloride and vanillin sulfuric acid colorimetric assays. The summation of the major peaks from 10 to 20 minutes was

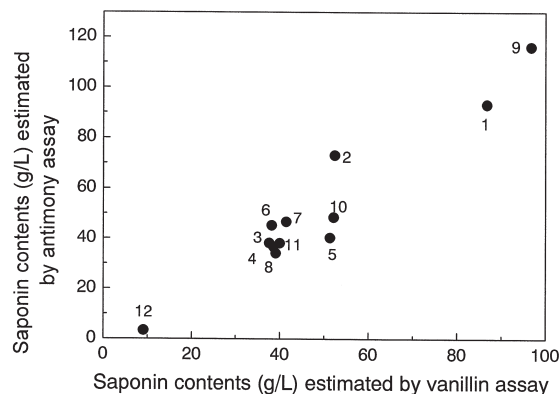


Fig. 6. Correlations between saponin contents of 12 commercial sanitary products estimated by antimony pentachloride and vanillin sulfuric acid colorimetric assays.

Table 3. Response for 5–50 g/L organic extracted *S. mukurossi* saponin spiked in the commercial clear glycerin soap base analyzed by HPLC, antimony pentachloride and vanillin sulfuric acid colorimetric assays

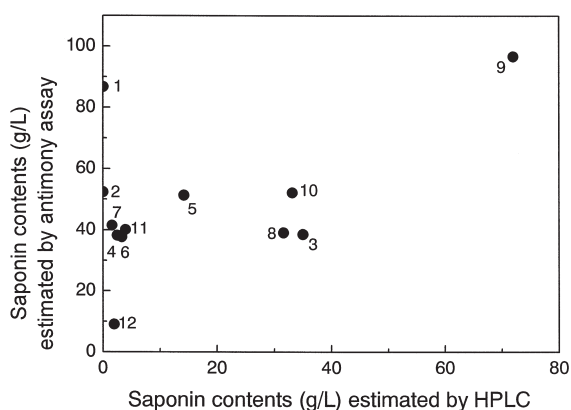
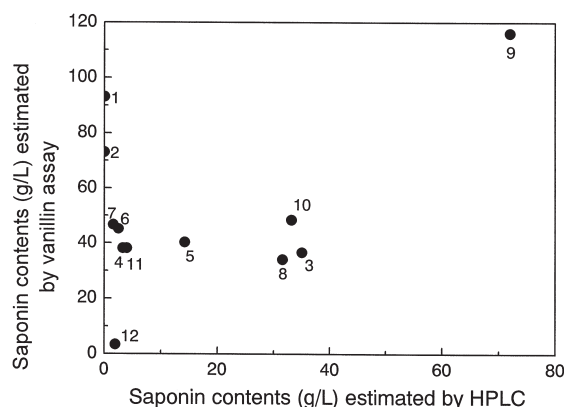
Spiked Saponin contents	Antimony pentachloride assay			Vanillin–sulfuric acid assay			HPLC assay	
	D.F. ¹	O.D. at 535nm ²	Total abs.	D.F. ¹	O.D. at 535nm ²	Total abs.	Area of the major peak at 12.5 min	Area of all major peaks btwn. 10 and 20 min
0	30	0.86	25.8	40	0.11	4.4	–	–
5 g/L	30	0.98	29.4	40	0.21	8.4	3500.3	3783.3
10 g/L	30	1.14	34.2	40	0.25	10.0	6864.9	7191.3
20 g/L	40	1.29	51.6	50	0.53	26.5	14234.9	16588.6
30 g/L	40	1.47	58.8	50	0.72	36.0	19294.5	24546.4
40 g/L	40	1.60	64.0	50	0.94	47.0	29431.3	39740.8
50 g/L	40	1.74	69.6	50	1.03	51.5	33119.1	45822.7

D.F.¹: dilution factors.

O.D.²: optical densities prior to dilution.

Table 4. Saponin contents of 12 commercial sanitary products estimated by antimony pentachloride, vanillin sulfuric acid colorimetric assays and HPLC

Products	Estimated by antimony assay (g/L)	Estimated by vanillin acid assay (g/L)	HPLC	
			Area of all major peaks btwn. 10 and 20 min	valued (g/L)
1 Soapnut multi-purposes cleaner (Hu-Wei TU)	86.74	93.11	65.28	0.07
2 Soapnut saponin cleaner with green tea (Hu-Wei TU)	52.39	73.06	0	0
3 Soapnut saponin purifying toner (Hu-Wei TU)	38.58	36.64	31932.05	35.01
4 Lavender soapnut shampoo (Chin-Wang)	37.64	38.15	2997.04	3.29
5 Multiflower Knotweed soapnut shampoo (Chin-Wang)	51.35	40.28	12951.08	14.20
6 Ginger soapnut shampoo (Chin-Wang)	38.18	45.17	2309.63	2.53
7 Soapnut cleanser (Chin-Wang)	41.48	46.62	1445.57	1.58
8 Soapnut emulsifiers A (5soap)	39.05	34.1	28816.42	31.59
9 Soapnut emulsifiers B (5soap)	96.77	116.1	65686.42	72.01
10 Soapnut cleanser (5soap)	52.17	48.48	30251.77	33.16
11 Soapnut soap bar (Taitung Ma's)	40.01	38.11	3612.51	3.96
12 Soapnut hyaluronic acid (Taitung Ma's)	9.09	3.36	1774.44	1.95

**Fig. 7.** Correlations between saponin contents of 12 commercial sanitary products estimated by antimony pentachloride colorimetric assay and HPLC.**Fig. 8.** Correlations between saponin contents of 12 commercial sanitary products estimated by vanillin sulfuric acid colorimetric assay and HPLC.

selected to account for all compounds with similar saponin related structures. The results, estimated by the calibration curves in Fig. 5, are summarized in Table 4. Estimations by two colorimetric assays were generally relatively consistent. Responses of saponin were detected for all 12 commercial cleaning products. But, the estimated values by antimony pentachloride colorimetric assays were not necessarily higher or lower than the values estimated by the vanillin sulfuric acid colorimetric assay, as shown in Fig. 6. Varieties of compounds present in the ingredients of the 12 commercial cleaning products might interfere with the analysis of the two colorimetric assays.

There was a larger discrepancy between the values estimated by HPLC and the values estimated by antimony pentachloride and vanillin sulfuric acid colorimetric assays. Significant saponin content was detected for products 3, 5, 8, 9 and 10 by HPLC. The Saponin contents estimated by HPLC for products 2, 4, 6, 7 and 11 were quite low, despite the significant responses in the antimony pentachloride and vanillin sulfuric acid colorimetric assays, as shown in Figs 7 and 8. There might be some other compounds that could induce a coloring reac-

tion in both colorimetric assays. However, they couldn't be detected by any published HPLC assays (Hamburger *et al.*, 1993) for detecting saponin. The trends were consistent, as shown in Figs 6 and 7. It is worth noting that the plots for saponin values estimated by antimony pentachloride and vanillin sulfuric acid colorimetric assays still demonstrated a linear trend for products 3, 5, 8, 9, 10 and 12 (see Figs 6 and 7) compared with the values estimated by HPLC. This suggests that the above products might use similar natural soapnut sources and similar commercial soap ingredients. Other products might utilize a different group of raw materials, which might happen to induce color in antimony pentachloride and vanillin sulfuric acid colorimetric assays but fail to give any peaks in the HPLC routine. Different producers may use different materials to produce their respective products, but it is essential that saponin any product claiming to contain saponin addition can be detected and verified by analytical measures.

CONCLUSION

Capabilities to analyze saponin from the soapberry

(*Sapindus mukorossi* Gaertn.) added into cleaning products were compared by high performance liquid chromatography (HPLC), vanillin-sulfuric acid and antimony pentachloride colorimetric assays. The chemical structural features of *S. mukorossi* saponin for the major peak in the HPLC chromatograms were identified by LC/MS/MS, ^1H and ^{13}C NMR spectroscopy. Linearity for the calibration graphs were found for the range down to 0.2 g/L aqueous solutions of the organic extract of crude saponin, for all three assays studied. Linearity was also found for the HPLC analysis and two colorimetric assays for commercial glycerin clear soap base with predetermined soapberry extracts. However, there was a discrepancy between the saponin contents analyzed by HPLC for 12 commercial cleaning products sold in Taiwan claiming to have saponin added and the values estimated by the antimony pentachloride and vanillin-sulfuric colorimetric assays. These results could separate products into two groups: one group that uses similar natural soapberry sources and similar commercial soap ingredients; and the other group that utilizes different raw materials. Since globally there are an increasing number of household products claiming to contain natural ingredients, more rigorous analytical routines are needed to ensure that the products contain what they claim, and that the consumer gets what s/he paid for.

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