August 2013. Vol. 4, No. 2 ISSN2305-8269
International Journal of Engineering and Applied Sciences

www.eaas-journal.org

SURFACTANT PROPERTIES OF THE SAPONINS OF *Agave Durangensis*, APPLICATION ON ARSENIC REMOVAL

Laura Silvia Gonzalez-Valdez [1], Norma Almaraz-Abarca[1], José Bernardo Proal-Nájera[1], Fabian Robles-Martinez[2], Gustavo Valencia-Del-Toro[2], Manuel Quintos-Escalante[1].
Instituto Politécnico Nacional CIIDIR Durango[1] Instituto Politécnico Nacional UPIBI[2]
lsgv2001@yahoo.com

ABSTRACT

The saponin composition of Agave durangensis foliar tissue was assessed by HPLC/DAD to determine the diversity and abundance of these secondary metabolites in that taxon, and also to analyze, the surfacting properties of those terpenic derivatives for removing arsenic in water. Four different steroidal saponins were detected at concentrations ranging from 0.380 to 3.472 mg/g dry tissue, in the leaves of this species of Agave, which are removed and left in the field as waste, when the plants are gathered to elaborate mescal. The total concentration of saponins estimated ((6.165 mg/g dry tissue) suggests that A. durangensis is an important source of steroidal saponins, having relevant properties for removing arsenic from water (above 90%). This study is a contribution to the knowledge of that species of plant and reveals a potential additional use to the integral management of this resource.

Key words: saponins, Agave durangensis, surfactant

1. INTRODUCTION

Genus *Agave* belongs to the family Agavaceae, which is endemic of American Continent. In Mexico, *Agave* reaches its highest level of species diversity with at least 150 among the around 200 species recognized [1]. Durango is among the richest States of Mexico in species of *Agave*, with 24 to 29 [2]. Several species have been used by the different native cultures of American Continent since pre-Columbian times. The broad spectrum of uses includes food, beverages, medicine, tools and fabrics [3, 4].

Agave durangensis is distributed in scattered colonies of Southern Durango and Northern Zacatecas [1, 2]. Plants of this species may reach 120 cm tall and 180 cm broad, with broadly leaves of 40-90 x 14-22 cm [1]. The present major use of *A. durangensis* is the elaboration of an alcoholic beverage named mescal. This beverage is elaborated solely from the stem and the leaf bases and the leaves, representing around 50% in weight of the whole plants, are left in the field as waste material [5]. *Agave* and other genera of Agavaceae are rich in secondary metabolites, meanly steroidal saponins [6]. Among the species of *Agave* studied for their saponin composition are *Agave cocui* [7], *Agave americana* [8] and *Agave cantala* [9], and until further research has been conducted, there are at present no data about the evaluation of saponin composition of *A. durangensis.*

Plant saponins are secondary metabolites with high molecular weight. They are meanly found as glycosides, consisting of a sugar moiety linked to a steroidal or triterpenic structure. These metabolites are broadly used in the pharmaceutical industry for their anti-inflammatory, antihypertensive, and anticancerigen properties [10, 11]. Saponin extracts has been used to improve the feed efficiency for cattle and lambs [12]. Steroidal and triterpenic saponins also have relevant surfactant properties, so that they can be seen as important agents of heavy metal removal for remediation of contaminated sites [12], since they have been probed as natural chelating agents to remove Cr, Cd, Cu, Pb and Zn from soil and wastewater [13, 14, 15]. It is important to mention that assays for arsenic removal have not been previously reported.

Several countries around the world have contamination problems by arsenic in groundwater, due principally to natural process, agricultural and industrial activities. Severe health effects have been observed in human populations drinking arsenic-rich water over long periods. Inorganic arsenic has been reported as

carcinogenic for human, and concentrations exceeding national and international regulations (permissible maxim limit of 0.025 and 0.010 mg/l, respectively) have been detected in Mexico [16, 17]. Treatment process of enhanced coagulation and precipitation for arsenic includes complexation with polyvalent heavy metals, such as Fe, and coprecipitation with the metal hydroxide. These treatments were not very effective due to re-dissolution of contaminants; the use of a surfactant increases the efficiency of removal owed change the nature hydrophilic of the particles to hydrophobic, preventing its redissolution [18]. Sodium oleate has been using as surfactant due to its tensoactive properties, with value of dehydrophilic-lipophilic balance of 18 [19].

Saponin extraction has been performed using polar organic solvents like methanol, ethanol and *n*-buthanol by maceration for 18 to 72 h, even for 15 days, and by elimination of lipids by extraction with solvents non-polar like hexane or chloroform [20]. Some authors have hydrolyzed the saponin extracts for characterizing them like sapogenins (the aglycone form of saponins) and thus avoid a probably interference of other compounds. The identification and quantification of saponins can be done by several techniques; one of the most frequently used is HPLC with detectors as light scattering and MS, either from total extracts or fractions [10, 21, 22], while diode array detector (DAD) has been used for the simultaneous quantification of compounds from vegetal extracts [23].

Most of saponins exhibit major absorption peaks in the range of 250 to 350 nm. The spectra of saponins are typical, different from those of other metabolites like flavonoids, which are a kind of phenols frequently extracted along with the saponins during the extract procedures [24].
The aim of this study was to determine the saponin composition, by a gradient method of HPLC/DAD, of foliar tissue of *A. durangensis* and evaluate their surfactant properties for removing arsenic in water.

2. MATERIALS AND METHODS

2.1 Sampling

Foliar tissue of *A. durangensis* was collected in Sierra del Registro, Durango, Mexico (23° 59’ 38’’ N, 104° 22.5’ 13’’ W) in June-July, 2009. A voucher sample was deposited at the Herbarium CIIDIR. The tissues were divided into small pieces and dried at 60°C until constant weight was achieved.

2.2 Preparation of Extracts

Ten extracts were individually prepared. Samples (100 g) of dried and grinded foliar tissue were macerated in 200 ml ethanol-water solution (70%, v/v), at room temperature, in dark, at 30 rpm on a stirrer Barnstead/Thermolyne M49235 for 72 h. The extracts were filtered (Whatman paper No. 2) and the solid materials were reextracted at the same conditions for 12 h. The filtrates were brought together (total extracts) and evaporated to dryness at low-pressure.

2.3 Preliminary Tests of Saponins

Dried extracts were tested for the presence and type of saponins by the method of stable froth and by the Liebermann-Burchard test for steroidal saponins [25, 26].

2.4 HPLC/DAD Analysis

Each of ten extracts was individually analyzed by triplicate. The extracts were resolved in 10 ml ethanol-water solution (70%, v/v) and aliquots (20 µl) were used to determine the saponin composition by HPLC/DAD. The analysis were done according to a method previously described for phenols [27, 28], on a Perkin Elmer HPLC system and Perkin Elmer Brownlee Analytical C18 column (4.6×250 mm, 5 µm), by an acidified acetonitrile-water gradient. Standard chromatograms were plotted at 260 and 340 nm. Spectral data for all peaks were accumulated in the range 220-400 nm using diode-array detection (Perkin Elmer Series 200). The structural information was obtained by direct comparison of retention time and UV spectra of references (agavoside A, agavoside B, agavoside C, JT Baker 3388-045), and according to data reported [29].

2.5 Determination of Saponin Concentrations

The saponin concentration of each of ten extracts was determined by linear regression analysis from a standard curve representing a

linear response (Abs_{260nm} = 4.3224 [Saponin] +1.7653, R^2 = 0.9797), constructed with five concentrations (0.05 to 2.50 mg/l) of a saponin reference vs. the respective absorbance (260 nm). The absorbances were registered by a Spectronic Genesys 2 spectrophotometer. Saponin concentrations were expressed in mg/g of dried tissue.

2.6 Colloidal Particle Sizes

Colloid particle sizes were determined by scanning electron microscopy in a JEOL JSM-5800LV microscope.

2.7 Surfacting Properties of the Saponins

One study of arsenic removal, using a hydrophobization process by the jars method ASTM D2035-80, with ferric hydroxide as adsorbent for forming colloids, was performed to evaluate the surfacting properties of the saponins of *A. durangensis*. Aqueous arsenic solutions (200 ml) at concentration of 1.0 mg/l were prepared and stirred at 200 rpm while saponin extract (0.02-2.0 g) were added. After 15 seconds, the stirrer condition was reduced to 25 rpm for 25 minutes, and then left to settle for 30 min. The assays were performed at room temperature. Sodium oleate (JT Baker) was analyzed in the same manner, as reference.

2.8 Determination of Residual Arsenic.

Residual arsenic concentrations were determinate with an atomic absorption spectrophotometer (Perkin Elmer-Aanalyst 700), with graphite furnace, at 193.7 nm.

2.9 Data Analysis

Data were analyzed by an analysis of variance ($p \leq 0.05$) and means separated by Duncan's range test. The results were processed by COSTAT computer program.

3. RESULTS

3.1 Preliminary Test of Saponin in Extracts

The foliar ethanolic extracts of *A. durangensis* gave a positive response for the foam test, forming an emulsion. The Liebermann-Burchard test developed a green-blue solution, typical of steroidal saponins.

3.2 HPLC/DAD Analysis

Under the experimental chromatographic conditions in which HPLC profiles were obtained, patterns comprising three saponins from total extracts of foliar tissue of *Agave durangensis* were detected between 20 and 36 minutes (Figures 1 and 2).

Retention times and UV spectra of saponin extracts and reference material are shown in Figure 3 ; four saponin structures were detected, all presenting a major absorption band at 275 nm, and retention times of 20.156 (saponin 1), 30.628 (saponin 2) and 35.57 (saponin 3). Retention times for the reference were 20.937, 30.584 and 35.629.

ISSN2305-8269
International Journal of Engineering and Applied Sciences

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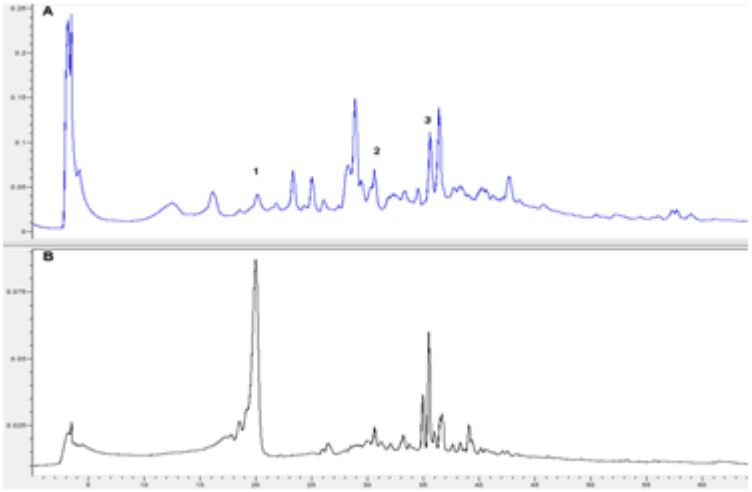

Figure 1. Chromatographic profiles at 260 nm of *A. durangensis* extract (above) and saponin references

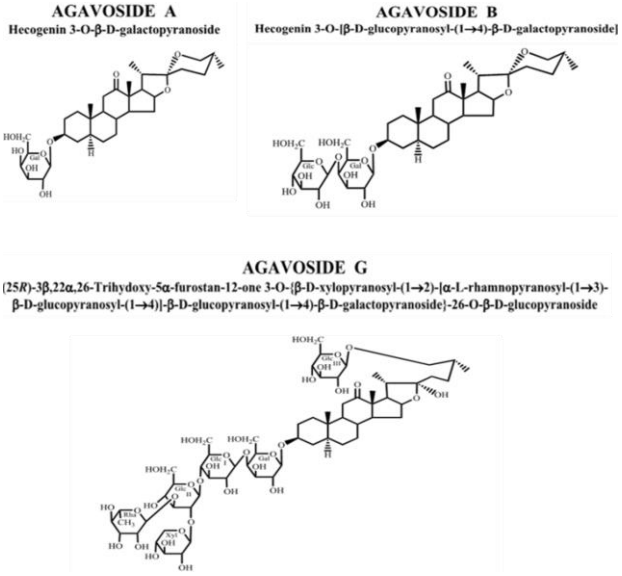

Figure 2. Chemical structures of agavosides (saponin references).

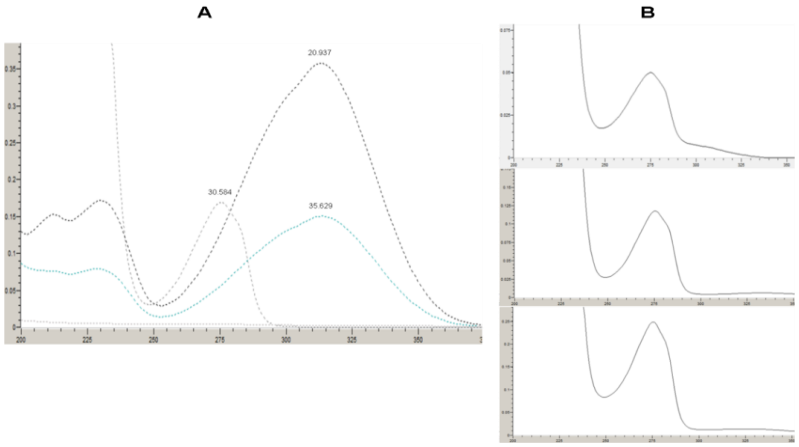

Figure 3. UV spectra of saponins A: Saponin references. B: Saponins present in extract of foliar tissue of *A. durangensis*, saponin 1 (above, RT: 20.156), saponin 2 (middle, RT: 30.628), saponin 3 (below, RT: 35.57)

3.3 Saponin Concentration

Table 1 shows the concentration of every three saponin compounds found from 10 individual samples of foliar tissue of *A. durangensis* (values are the mean of samples analyzed in triplicate and standard deviation), according to the experimental conditions of extraction and to the HPLC/DAD method which with those compounds were detected. The saponin presenting the highest concentration was the number 2, with 3.472±0.035 mg/g dried tissue, while the one found at the lowest concentration was the number 1, with

1.138±0.021 mg/g dried tissue. The total concentration of saponins in the extract was 5.785 mg/g dried tissue.

Table 1. Concentrations of foliar tissue saponins of *A. durangensis*.

Compound	Concentration (mg/g dry weight)
Saponin 1	1.138±0.021
Saponin 2	3.472±0.035
Saponin 3	1.175±0.028

3.4 Surfactant Properties

Colloid particle sizes were confirmed through scanning electron microscopy (Figure 4), and ranged between 0.1 and 10 µ.

Under our experimental conditions, values of 83.9-93.0% and 78.3-90.8% of arsenic removal, for sodium oleate and saponin extract, respectively, were estimated (Table 2).

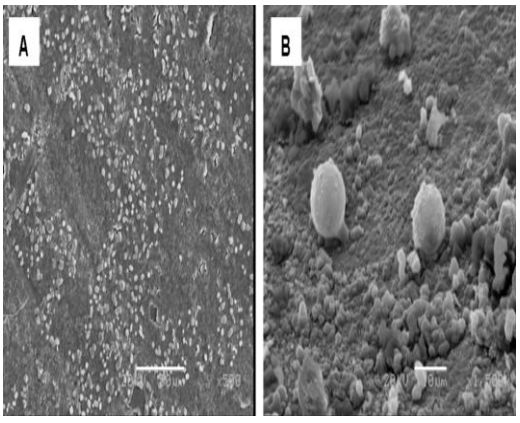

Figure 4. Micrographs of scanning electron microscopic (SEM) of ferric hydroxide colloids. A: scale bar 50 µm, magnification 500. B: scale bar 5 µm, magnification 1.500.

	Sodium oleate		Saponin	
Concentration %	As (mg/l)	As (% Removal)	As (mg/l)	As (% Removal)
0.000	0.993 ± 0.006 a		0.998 ± 0.005 a	
0.005	0.255 ± 0.012 c	74.500	0.380 ± 0.011 b	61.967
0.010	0.163 ± 0.002 g	83.667	0.217 ± 0.003 d	78.333
0.015	0.153 ± 0.007 h	84.733	0.196 ± 0.005 e	80.400
0.020	0.143 ± 0.006 hi	85.700	0.184 ± 0.005 f	81.567
0.025	0.135 ± 0.008 ij	86.467	0.167 ± 0.002 g	83.000
0.030	0.128 ± 0.004 j	87.167	0.147 ± 0.006 h	85.267
0.035	0.127 ± 0.004 jk	87.300	0.146 ± 0.002 h	85.433
0.500	0.095 ± 0.003 l	90.500	0.119 ± 0.004 k	88.133
1.000	0.071 ± 0.004 l	92.900	0.092 ± 0.002 l	90.800

Table 2 Comparison of arsenic removal properties between sodium oleate and saponins of A. durangensis (means and standard deviation, different letters means differences, $p \leq 0.05$)

4. DISCUSSION

4.1 Saponins of Foliar Tissues of *A. durangensis*

According to some authors [26, 27], in the test of Liebermann-Burchard, an oxidation reaction between the acetic anhydride and sulphuric acid occurs, causing the acetylation of OH groups of steroidal saponins and developing a green-blue color; thus the green-blue color developed in the extracts of *A. durangensis* suggested the presence of this kind of saponins in the foliar tissue of that species. The HPLC/DAD method for detecting saponins was originally developed for analyzing phenols, however, this method also allowed detecting saponins.

The detection of saponins with that method was due to the conditions of gradient cover a broad spectrum of polarities, in which several saponins bearing a variable glycosidation patterns can be eluted [27, 28]. Other authors analyzed steroidal saponins by employing gradient methods of acetonitrile-water, with flow rates 1 ml/min, and injection volumes of 20 µL the value of these last two parameters (being the same in the present study), reporting six saponins between 17 and 47 min [21], and 10 saponins between 14 and 63 min [30]. Mi-Jeong (2005), used a different acetronitrile-water gradient (40% to 85%) and flow rate of 0.3 ml/min, reporting two saponins between 3 to 60 minutes of analysis [31]. The comparison of our results with those of the mentioned authors suggests that the retention times of steroidal saponins of *A. durangensis* in which these compounds are detected are relatively affected by changes in the eluent proportions, in the time length in which these proportions are maintained, and in the flow rates. However, the method used in the present study allowed the detection of saponins at a more narrow retention time range (20 to 36 min) than the other methods [21, 30, 31], all presenting a major absorption band at 275 nm (Figure 1).

By comparing the retention times and the UV spectra of the saponins of *A. durangensis* with those of standards (Figure 2) the saponin 1 was identified as agavoside A, the 2 as agavoside B, and the 3 as agavoside C. The number of saponins (four) detected for foliar tissue *A. durangensis* was higher than the number found for *A. cantala* (two saponins), equal to the number reported for *A. macroacantha*, and lower than the detected for *A. sisalana* (five saponins), *A brittoniana* (six saponins), and *A. americana* (ten saponins) [32-36].

4.2 Quantification of Saponins

The concentration of each saponin detected in the foliar tissue of *A. durangensis* ranged from 0.380 a 3.4704 mg/g dried weight (Table 1). Concerning to the total concentration of saponins (6.165 mg/g dried tissue) present in the foliar tissue of *A. durangensis*, similar studies reported 0.832 mg/g dried tissue for *Agave attenuate* [37], 8.2 to 10.1 mg/g dried tissue for *Agave salmiana* [38], and 4.39 to 8.94 mg/g dried tissue for *Agave lechuguilla* [39]. The concentration assessed for the extracts of *A. durangensis* lies between the ranges reported for

those species of *Agave*. The total amount of saponins in *A. durangensis* was around 6% of ethanolic extract. This amount is in the interval of the values (1.17 to 28.30%) reported for plant species considered as valuable sources of saponins [39].

4.3 Surfacting Properties of *A. durangensis* Saponins

The statistical analysis showed significant differences between the sodium oleate and saponin as surfactants for arsenic removal ($p \leq 0.05$) and among the several concentrations evaluated; the surfacting activity increasing with the increment of surfactant concentrations (Table 2). Practically, twice as much quantity of saponin was required to reach the arsenic removal level showed by sodium oleate.

One study of Kilic et al. (2010) about surfacting properties of saponins from quillaja bark to dewatered tannery sludge for recovering Cr, reported 70% of efficiency using one 3% saponin solution [14]; and Hong et al. (2000) reported, using one 3.75% saponin solutions, removals between 20 to 45% of Cr, 50 to 60% of Cu, and 100% of Pb, from the fly ashes [15]. The arsenic removal here reported was more efficient than those reported for Cr, Cu, and Pb, since to remove 90.8% of As, one 1% saponin solution of *A. durangensis* was required (Table 2). Comparing our results with those of those autors [14, 15], and considering that the saponins of *A. durangensis* comes from foliar material regarded as waste, that the extraction method is simple, and that the use of sodium oleate as surfactant is expensive, it can be assumed that the saponins of *A. durangensis* are an important arsenic removal material that can be used to treat contaminated water.

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