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Variability of Saponins Concentration in Guishe Collected in Different Geographical Areas and Weather Conditions

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Abstract

Lechuguilla (*Agave lechuguilla* Torrey) is a plant traditionally used to obtain a natural fiber known as ixtle (or sisal). This activity represents the main source of income of the people which has living in some semi-arid regions of north of Mexico. The yield of ixtle in one lechuguilla plant is only 15 %, while 85% is a succulent by-product (named guishe) that has no use whatsoever. The guishe is mainly composed of saponins, which are very important chemical compounds in several applications; it is possible to take advantage of their properties as precursors of steroidal compounds in the production of plant hormones. This paper presents a study of the variability of saponins concentration in guishe extracts, which have been collected in different geographical areas and at different times of the year. It was confirmed that the concentration of this metabolite depends on the harvest area and on the weather conditions.

Keywords. Agave lechuguilla, ixtle by-products, saponins, guishe

1. Introduction

Agave lechuguilla Torrey is a succulent rosette shrub, which can measure between 30 and 40 cm wide and 20 to 70 cm high (Figure 1). Its roots are long, thin and fibrous; they are distributed to a depth between 8 and 12 cm from the ground. Besides, the leaves are formed from a developed apical bud.

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Figure 1: *Agave lechuguilla* Torrey

The lechuguilla is one of the two species of agave with the natural distribution broader and higher density in North America, where their productivity is higher than the average for the species in its environment (Kings et al., 2000). According to Nobel (1998), it is a species that occurs in 100.000 km^2, from Texas and New Mexico (in USA), to Queretaro, Hidalgo and Guanajuato (in Mexico). Reyes & Aguirre (1999) reported that the distribution of lechuguilla corresponds to the harvest pattern of Mexican plateau and adjacent areas (Figure 2), while Rzedowski (1978) says that this species is an element of the high plateau and plain provinces of the northeast Mexican xerophylic region. De la Garza (1985) and Gentry (1982) claim that lechuguilla is distributed from 200 to 2400 meters above sea level.

Figure 2: Geographical Distribution of Lechuguilla in Mexico

As a part of the genus *Agave*, the lechuguilla is mainly composed of mineral salts, saponins and calcium oxalate (Orozco et al., 1977; Nobel, 1988; Diaz-Jimenez et al., 2008; Wall et al., 1962). It has been estimated that the average weight of a fresh sprout is of 356 g and contains 14.6 % of fiber and 85.4 % of guishe (succulent tissue and water from the leaves). The guishe is obtained as a by-product of an artisan process which main objective is to obtain the fiber known as ixtle (Figure 3). The concentration of saponins in *A. lechuguilla* has been reported about from 1 to up 18 % by weight, and has been found both in the leaves and guishe (Hernandez-Soto, 2005; Flores, 1991; Wall et al, 1962).

Figure 3: Ixtle and its by-Product (Guishe)

It has been determined that the guishe is a rich source of saponins that has been remained untapped (García-Contreras, 2010). Until now there is some evidence that the guishe has been used to produce handmade soaps and shampoos, however the study of this by-product is very limited (Flores and Perales, 1989; Zapien, 1981).

Saponins are glycosides common in a large number of plants as secondary metabolites with diverse biological activities, such as anticarcinogenic, antioxidant, antifungal (Francis et al., 2002). These compounds consist of a lipophilic core with an adjacent steroid or triterpenoid structure, with one or more rings of carbohydrates. The lipophilic core is called aglycone, due to the group that is bound to an anomeric carbon atom, which is the carbon atom linked to one or two oxygen atom, or any other hetero-atom, such as nitrogen (Wade, 2004).

When the carbohydrates are separated from the aglycone, either enzymatically or by acid hydrolysis, the lipophilic portion generated is named "sapogenin"; when a saponin is hydrolyzed, is changed the ending -in by -genin, for example, dioscin becomes diosgenin. When a saponin is hydrolyzed into sapogenin, it loses the most distinctive features that had initially (García-Contreras, 2010).

On the other side, the saponins in the lechuguilla could also be used as: i) a source of steroids since besides smilagenin (steroidal sapogenin), it has been reported the presence of other 8 saponins, ii) raw material for the development of cortisone (anti-inflammatory), and iii) raw material to obtain estrogen and progesterone, because their leaves contain between 1 and 2 % of dry weight of steroidal saponins (Wall et al., 1962).

Furthermore, it is known that plants produce in their metabolism a series of chemical compounds that are used to cover different roles in their life. The primary metabolites are those essential for the survival of the plant, while the secondary metabolites are chemical compounds used to maintain the basic metabolism in circumstances in which the common substrates may not be used for the normal development (Dominguez, 1973). Among the most popular secondary metabolites can be mentioned pigments, flavonoids, alkaloids or saponins in the case of agaves. The production of secondary metabolites in plants is sometimes determined by the geographical area and the conditions of stress to which the plant is subjected.

The main contribution of this paper is the generation of knowledge concerning the composition of this by-product. From the obtained results it is possible to determine the potential of guishe to be used as raw material for the synthesis of added value products. Also it will be possible to develop new methodologies which can be transferred to industrial applications and the environmental risks associated to this by-product can be eliminated or at least minimized.

Thus, this work analyzed the variability in the concentration of saponins in extracts of guishe collected in three different geographical areas of the State of Coahuila, Mexico, as well as the influence of collecting season in two times of the year.

2. Materials and Methods

2.1. Raw Material

The samples collection was done from these places since the main ixtle producers are located there. The dates for the collection were selected in March and June in order to study the effect of different weather conditions, it is important to remark that some other conditions were evaluated in a previous work. So, the collection of fresh guishe was conducted in three locations in the southeast of Coahuila, Mexico and two times of the year. The localities of collection were: Ejido Tortuga in the county of Ramos Arizpe (Latitude: 25.833, Longitude: -101.267), Ejido Buñuelos of Saltillo (Latitude: 25.05, Longitude: -101.183) and Ejido El Porvenir of the county of Parras de la Fuente (Latitude 25.550, Longitude 101,720). The times of collection were on March and June. The material was transported to the laboratory in sealed polyethylene bags.

2.2. Pretreatment of the Raw Material

The collected guishe was mechanically pressed using a press steel. From this action, it was obtained a thick juice which is identified as a "liquid extract". Also, a solid material is produced; it is a fiber which was scrapped and sent to a final disposal site. The "liquid extract" was heated to 94 °C for 15 min in order to achieve enzymatic inactivation.

On the other hand, the saponins were separated from the "liquid extract" by using an extraction with solvents with the methodology described by García-Contreras (2010) with some modifications. Liquid extract (100 mL) was concentrated in a rotary evaporator at 100 °C up to 30 mL approximately. The concentrate was placed in a 500 mL volumetric flask and was made a first removal using ethyl acetate in a proportion solid:liquid from 1:10 (w/v). The mixture was maintained reflux magnetic stirrer, during 7 h at 65 °C. At the end of this time, the mixture was left in standby for 24 hours at 4 °C; after that, it was filtered using a vacuum filter with a Whatman No. 41. The material present in the bottom of the vessel was transferred to a watch glass and dried in an oven at 60 °C. Finally, the particle size of the solid was reduced to obtain a powder referred as solid extract.

2.3. Physicochemical Characterization of the Liquid Extract

Protein, ash, and moisture were determined by AOAC methods (AOAC, 1996). The ether extract was determined by Soxhlet method (AOAC, 1996). The quantification of total sugars was performed by the method of Fenol-Sulfuric acid (Dubois et al., 1956). All determinations were done in triplicate.

2.3. Quantification of Saponins in the Guishe Extracts

An indirect determination of saponins by quantifying the content of reducing sugars by the method of Miller (Hernandez-Soto, 2005) was performed. In the experiment, a curve of dextrose was generated and interpolated with a curve of *Quillaja saponaria* obtaining a new curve (saponins g L^{-1}, sugars g L^{-1}); after that, an equation to determine the reducing sugars corresponding to saponins was deduced.

3. Results and Discussion

3.1. Physiochemical Characterization of Guishe

Table 1 shows the results of the physicochemical analysis carried out on samples of guishe collected in three localities in the state of Coahuila (Parras de la Fuente, Ramos Arizpe and Saltillo), in two times of the year (March and June).

Table 1: Physicochemical Analysis of Guishe Agave Lechuguilla at three Locations in Coahuila harvested in March and June

Parameter	P-PF[a] March	P-PF[a] June	T-RA[b] March	T-RA[d] June	B-S[c] March	B-S[c] June
Total dry mater (%)	21.58	29.78	24.18	34.12	26.23	37.43
Moisture (%)	78.42	70.22	75.82	65.88	73.77	62.57
Ash (%)	3.93	4.68	3.05	4.24	6.25	7.22
Crude fiber (%)	1.64	1.47	2.01	1.92	1.94	1.64
Ether extract (%)	0.5	0.51	0.15	0.48	0.29	0.37
Protein (%)	0.57	0.49	0.32	0.54	0.44	0.52
Carbohydrates (%)	24.88	15.83	15.83	7.34	9.52	7.52

[a]EP-PF: Ejido El Porvenir, Parras de la Fuente.
[b]ET-RA: Ejido Tortuga, Ramos Arizpe.
[c]EB-S: Ejido Buñuelos, Saltillo.

Firstly, with regard to moisture (H), it is noted that the samples collected in June are close to a 10 % less than the samples collected in March. The reason for this decline is primarily related to the weather in June who is much drier, and then the plant has lost a part of its reserves of water. The obtained results for this parameter are in the range previously reported in other research, where moisture values were found between 74 and 69 % (Hernandez-Soto, 2005). Similarly, the values obtained in this work are consistent with those reported for *Agave tequilana* Weber (71% H) (Gonzalez et al., 2005).

Concerning the concentration of total sugars, it was observed a greater concentration in the sample from the Ejido El Porvenir, in Parras de la Fuente. This is related to the percentage of moisture, because this same sample presented the higher water content, and the sugars are highly soluble. This statement is consistent with the results generated for the samples from Ramos Arizpe and Saltillo, which presented sugar content of 15.83 and 9.52 %, and content of H of 75.82 and 73.77 %, respectively.

In general terms, the high content of sugars in the “liquid extract” is the first sign of the potential of this material for the obtaining of polysaccharides or oligosaccharides with possible application in the agrifood chains. Also, this situation implies that it can be applied in bioethanol production, as well as has been conducted with other agro-industrial wastes such as bagasse from sugar cane (Jackson de Morales Rocha et al., 2011; Dias et al., 2013), waste of carrot (Aimaretti et al., 2012), or tangerine peel (Choi et al., 2012).

On the other side, the determinations of ether extract reveal a low concentration of volatile compounds in the samples besides the absence of fats.

In regard to the determination of ashes, it can be observed that on June there was a relatively large increase with respect to March for the three locations. In addition, the variability was presented in the ash content according to the sampling area, being greater for both samples from the county of Saltillo (6.25 and 7.22 on March and June, respectively). This is related to the high temperatures that were presented in June, the temperature recorded at that time was up to 35 °C and the absence of rainfall was also reported to this area (Accuweather, 2012).

Hernandez-Soto (2005) reported that the ash content for leaves of *A. lechuguilla* is between 2.34 and 3.25 %, which is consistent with the results obtained in this work.

It should be noted that this is the first characterization study of guishe with these conditions: comparing three of the locations with the most activity carver activity of *A. lechuguilla* in the region, collecting samples in two seasons of the year (March and June). The obtained results from the characterization show significant differences in the samples collected in March with respect to the ones collected in June; those differences are directly related to the moisture in the atmosphere as well as the quality of the soil in the different regions.

3.2. Indirect Quantification of Saponins in the Extracts of Guishe

Figure 4 shows a comparison of the saponins concentration in the six guishe juices corresponding to the three harvesting areas in two times of the year. In all cases, the content of saponins (g L^{-1}) was higher in the second sampling (June), with values of 98.43, 100.33 and 23.76 (g L^{-1}) for the ejidos Tortuga (Ramos Arizpe), El Porvenir (Parras de la Fuente) and Buñuelos (Saltillo), respectively. The content of saponins in the extract of the Ejido El Porvenir in Parras de la Fuente stands out with a value of 100.33 g L^{-1}. It is clear that the treatment for the removal of saponins with solvents was efficient; it allows the samples obtained in Parras de la Fuente and Ramos Arizpe to be purified. In general, it was confirmed that the concentration of saponins in the different samples is clearly influenced both by the place of origin and by the date of collection.

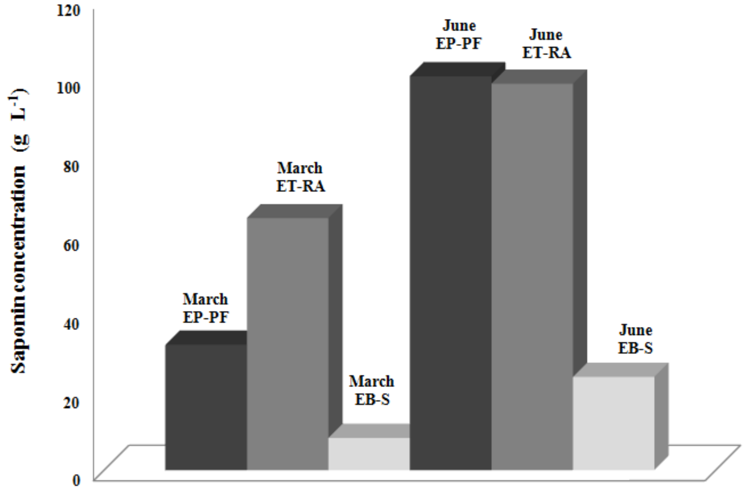

Figure 4: Saponins Concentration (g L^{-1}) in the Guishe Extracts

The high saponin values found in the samples collected in June confirmed that this is the time with lower precipitation of the year in the area, a fact that coincided with a stage of dry season during several months. For that reason, the concentration of water in the plant is smaller and therefore the relative concentration of phytochemicals is greater. On the other hand, the scarcity of water in the area of plant growth, inhibits the presence of another type of vegetation, increasing the risk of predation. The lechuguilla plant tends to increase the production of some secondary metabolites for protection purposes, among them the saponins (Abdel-Khalik et al., 2002).

In regard to the location, it was found that the samples showing a higher concentration of saponins were harvested in the ejidos Tortuga (Ramos Arizpe) and El Porvenir (Parras de la Fuente). From this result, it is deduced that depending on the geographical location of the plant, it is subject to different conditions of environmental stress (Dinan et al., 2001) which implies a different saponins production.

3. Conclusions

It was found that the concentration of saponins in the samples analyzed is different depending on the region collected.

The samples with the highest concentration of saponins were obtained from the ejidos located in Parras de la Fuente and in Ramos Arizpe.

For practical purposes of use of the guishe, specially in a process to obtain compounds of greater value, it is recommended to collect the raw material in the period of greatest dry season, since the concentration of saponins is greater.

It is concluded that due to the high concentration of the saponins contained in guishe, this by-product can be used in order to obtain compounds of high added value, such as plant hormones, bioethanol, and some others.

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5. References

Abdel-Khalik, S. M., Miyase, T., Melek, F. R., El-Shabraway, O. A., Mahmoud I. I. & Mina, S. A. (2002). New steroidal saponins from Agave lophantha Schiede and their pharmacological evaluation. Pharmazie, 57(8), 562-566.

www.accuweather.com/es/mx (November 28, 2012)

Aimaretti, N., Ybalo, C. V., Rojas, M. L., Plou F. J. & Yori. J. C. (2012). Production of bioethanol from carrot discards. Bioresource Technology, 123, 727-732.

Official Methods of Analysis of AOAC International. (1996). Association of Official Agricultural Chemists. 16th edition. Washington, D.C.

Choi, I. S., Kim, J. H., Wi, S. G., Kim K. H. & Bae, H. J. (2013). Bioethanol production from mandarin (Citrus unshiu) peel waste using popping pretreatment. Applied Energy,102, 204-210.

De la Garza, P.F. (1985). Comercialización y costos de producción del ixtle de lechuguilla. Boletín divulgativo Núm. 73. Instituto Nacional de Investigaciones Forestales, SARH. México, D.F.

Dinan, L., Harmatha, J. & Lafont, R. (2001). Chormatographic procedures for the isolation of plant steroids. Journal of Chromatography A, 935,105-123.

Dias, M.O.S., Junqueira, T. L., Rossell, C. E. V., Filho, R. M. & Bonomi, A. (2013). Evaluation of process configurations for second generation integrated with first generation bioethanol production from sugarcane. Fuel Processing Technology. 109, 84-89.

Díaz Jiménez, L., Hernández Soto, R. & Ibarra Arellano, E. (2008). Métodos de cuantificación de saponinas en extractos vegetales. p. 161-177, in C. N. Aguilar, R. Rodríguez, S. Saucedo-Pompa and D. Jasso-Cantú, eds., Fitoquímicos sobresalientes del semidesierto mexicano: de la planta a los químicos naturales y a la biotecnología. Universidad Autónoma de Coahuila. Saltillo, México.

Domínguez, X. (1973). Métodos de investigación fitoquímica. Limusa, México. 93-161.

Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A. & Smith, F. (1956). Colorimetric method for determination of sugars and related substances. Analytical Chemistry. 28(3), 350-356.

Francis, G., Kerem, Z., Makkar, H. P. S. & Becker, K. (2002). The biological action of saponins in animal systems: a review. British Journal of Nutrition, 88, 587-605.

Flores, M. N. (1991). Aislamiento e identificación de saponinas presentes en el Agave lecheguilla. Tesis, Universidad de Guadalajara, Guadalajara, México. p. 7-20.

Flores, F. J. & Perales, G. J. (1989). Primer reporte nacional de las plagas asociadas a la lechuguilla Agave lechuguilla Torrey. Agraria, 5, 57-73.

García-Contreras, A.R. (2010). Extracción, cuantificación y aislamiento de saponinas a partir de Agave lechuguilla y Yucca filifera. Tesis. Facultad de Ciencias Químicas, Universidad Autónoma de Coahuila, Coahuila, México.

Gentry, H. S. (1982). Agaves of continental North America. The University of Arizona Press. Tucson, Arizona. USA, 670 p.

González, Y., González, O. & Nungaray, J. (2005). Potencial del bagazo de agave tequilero para la producción de biopolímeros y carbohidrasas por bacterias celulolíticas y para la obtención de compuestos fenólicos. e-Gnosis 3 (14), 1-9.

Hernández-Soto, R., (2005). Extracción y cuantificación de compuestos bioactivos de Agave lechuguilla Torrey para su aplicación en la industria cosmetológica. Tesis. Centro de Investigación y Asistencia en Tecnología y Diseño del Estado de Jalisco, A.C. Jalisco, México.

Jackson de Morales Rocha, G., Martin, C., Barbosa Soares, I., Souto Maior, A. M., Macedo Baudel, H. & Moraes de Abreu, C. A. (2011). Dilute mixed-acid pretreatment of sugarcane bagasse for ethanol production. Biomass and Bioenergy, 35(1), 663-670.

Nobel, P.S. (1988). Environmental biology of agaves and cacti. Cambridge University Press. New York. USA, 270 p.

Orozco, L.M., Nava, C. R., Medina, T. J. & de la Cruz, J. A. (1977). Investigación silvoagropecuaria de la zonas áridas de México, campo experimental Noria de Guadalupe. Monografía Técnico-Científica, UAAAN, 3, 182-265.

Reyes, A.J. & Aguirre, R. R. (1999). Fitogeografía de la sierra Monte Grande, Charcas, San Luis Potosí, México. Caldasia. 21, 50-69.

Reyes-Agüero, J. A, Aguirre-Rivera, J. R. & Peña-Valdivia, C. B. (2000). Biología y aprovechamiento de Agave lechuguilla Torrey. Boletín de la Sociedad Botánica de México. 67, 75-88.

Rzedowski, J. (1978). La vegetación de México. First edition. Limusa, México, p.135-152.

Wade L.G., Jr. (2004). Química orgánica. 5 edition. Pearson Education, S.A. Madrid, p. 1075-1077.

Wall, M.E., Warnock, B. H. & Willaman J. J. (1962). Steroidal sapogenins. LXVIII. Their occurrence in Agave lechuguilla. Economic Botany, 16, 226-269.

Zapién, B. M., (1981). Evaluación de la producción de ixtle de lechuguilla en cuatro sitios diferentes. Primera reunión nacional sobre ecología manejo y domesticación de las plantas útiles del desierto. Publicación especial Núm. 31. Instituto Nacional de Investigaciones Forestales, SARH. México D.F.