SUSTAINABLE AND INTEGRAL EXPLOITATION OF AGAVE

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CONACYT

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EXTRACTION OF SAPONINS FROM LEAVES OF AGAVES

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SUMMARY

The agave or maguey is endemic to America and Mexico is the place where you will find the largest number of species of agave. In Zacatecas, Mexico, in the south and southeast regions of the state grown Agave *tequilana* Weber (blue agave) and *A. salmiana* ssp. *crassispina* (green agave) for the main production of beverages like "aguamiel, "pulque" and "mezcal", but due to the growth of this last activity, are being generated large amounts of waste solid such as bagasse and leaves of agave, which are important to evaluate their possible use. Zacatecas generated annually at least 21000 tons being about 30 percent agave leaves and the rest agave bagasse. Currently they are not given an application to the leaves of agave and has been found to exist in these residues some secondary metabolites of interest as saponins. Agave saponins are amphipathic glycosides with triterpene or steroid skeletons whose structural diversity is related to the wide range of biological effects are source sapogenins basis for synthesis of therapeutic importance sterols. But the extraction, purification and identification of these compounds are methodological challenges. The aim of this work is to extract saponins and characterize the waste generated of the agribusiness mezcal. Characterized by optical and scanning electron microscopic bagasse fiber and infrared spectroscopy the content of organic matter, and calcium carbonate was evaluated. The analysis of the composition of the leaves indicate a high content of crude fiber but low content in fat and protein. Extracts from fresh leaves which were purified by column chromatography and analyzed by thin layer chromatography were obtained to evaluate saponins. All with the intention of seeking the possible uses of these compounds and the reduction of waste generated in the production of mezcal.

Palabras clave: Cromatography, maguey, mezcal, microscopy and sapogenins.

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INTRODUCTION

The state of Zacatecas, accounts for 3.8% of Mexico. The climate in the state varied, semiarid predominating (Medina-Garcia et al. 2003). In Zacatecas, the cultivation of Agave *tequilana* Weber and *A. salmiana* ssp. for mezcal production is concentrated in the southern and southeastern regions of the state. From the granting of the designation of origin of mezcal, the business has grown and thus the generation of waste, so it is important to evaluate their possible use. At present the greatest economic interest of this plant lies in the production of alcoholic beverages such as pulque, tequila and mezcal. From these processes large amount of by-products and wastes that can be usable as the fiber leaves and bagasse of stem agave (Caspeta et al. 2014). In the mezcal industry, the first residue generated are the leaves that correspond to 14% by weight of agave being its extremely slow degradation, but because of its sugar content, fiber, minerals and secondary metabolites could be useful (Narváez-Zapata and Sánchez-Teyer, 2009). Plants synthesize a variety of secondary metabolites, either as part of normal growth and development or in response to pathogen attack. Among the secondary metabolites of agave, of particular importance are saponins, glycosides with skeletons amphipathic triterpenes (C30 aglycone based) or steroids (aglycone C27) whose structural diversity is related to its wide range of biological effects. Furthermore saponins are a ready source of sapogenins basis for synthesis of sterols of therapeutic importance. Applications of saponins varied, as also used in the beverage, food and cosmetics as foaming agents, taste modifiers and the custodian, in the pharmaceutical industry to have anti-inflammatory, anti-fungal and take effect antitumor (Yoong-Cheok et al. 2014).

METHODOLOGY

Leaves of agave species of *A. tequilana* Weber and *A. salmiana* from the municipalities of Pinos, Zacatecas (southeast of the state) and Teúl Gonzalez Ortega, Zacatecas (south region) were used. From the agave bagasse micro-structural characterization was performed by optical microscopy, infrared spectroscopy (FTIR) and scanning electron microscopy (SEM). As the extracts which fresh leaves were cut into small pieces and air dried for one month later dried in an oven at temperatures between 50-60 °C were used. Once the samples were ground (particle size ½ mm) dehydrated. The powders were macerated in absolute methanol while maintaining constant agitation and oxygen-free atmosphere. The supernatant was filtered and concentrated under reduced pressure, after liquid-liquid extraction was performed with dichloromethane and the soluble fraction was lyophilized for storage and calculations yields. A second extract consisting of the juice of fresh stalks that obtained with a commercial juicer was prepared. The juice was repeatedly filtered and centrifuged to remove suspended solids. Finally lyophilized powder you had a liquid-liquid extraction with light petroleum for removing fats present, filtered leaving dust completely dry for storage. The compositional analysis was performed according to the Mexican Official Norms and the determination of reducing sugars was performed by the DNS method. Separation of saponins was performed on gel filtration and the compounds were monitored by thin layer chromatography with the intention of having more pure sub-fractions and in terms of their increased activity or content of saponins, fractions of interest again were separated by silica gel. To ensure the presence of saponins in work samples, an evaluation was performed on the hemolytic activity with fractions, for which bovine blood was collected, with

erythrocytes a solution of 3% v / v was made, in which were immersed loaded with fractions where have suspected presence of saponins (Sharm et al. 2013).

RESULTS AND DISCUSSION

Agaves are different in their morphological characteristics (Fig. 1). Leaves of *A. salmiana* are lanceolate, measured on average 60 cm long, their spines were subulate, color ranged from green to a grayish color. Leaves of *A. tequilana* Weber are unchanged lanceolate way with 80 cm long with apical spine, hooked and numerous, presenting a blue color. The description of these agaves corresponds to that already described by other authors.

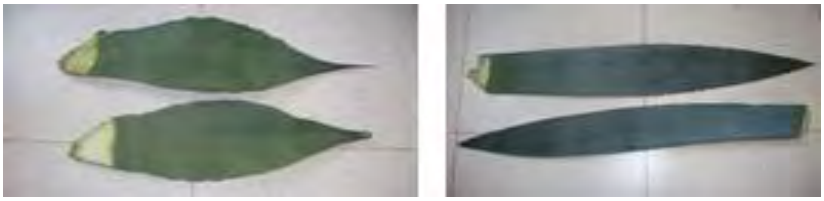

Figura No. 1. Figure No. 1 Leaves of *A. salmiana* (left) and A. *tequilana* (right).

A micro-structural level, the fibers of *A. salmiana* have a diameter ranging from 269 µm to 680 µm, in the case of **A.** *tequilana* the fiber diameters ranging from 164 microns to 363 µm (Fig. 2).

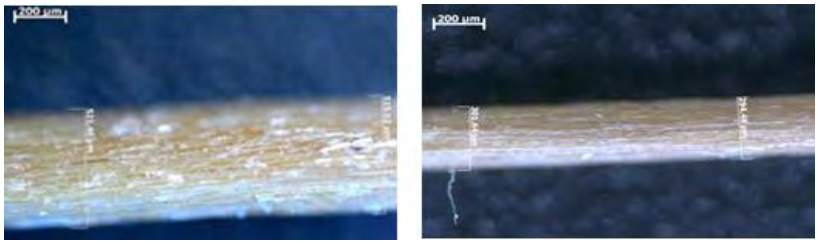

Figure No. 2. Micrographs of the fibers taken with optical microscope of bagasse of **A.** *salmiana* (left) and *A. tequilana* (right). View 5x reference bars indicate 200 microns.

The absorption spectra IR all studied samples revealed the presence of characteristic bands and by scanning electron microscope the type of fiber and the weight percent of elements for the samples were carbon, oxygen and calcium inferring the presence of calcium carbonate in the agaves. (data not shown). In the chemical composition analysis (Table No. 1) shows that the percent of ash, fiber and ether extract varies between the two species of agaves, while the percent protein is greater in the case of *A. salmiana* both fresh leaves as the extract.

Tabla 1. Composition analysis from leaves of *A. salmiana* and *A. tequilana.*

Agave	Humidity %	Ashes %	Ether extract %	Fiber %	Protein %	Reducing sugars mg/mL
A. salmiana[a]	82.40	14	0.58	20	5.1	58.47
A. salmiana[b]		14	0.08	21	7.3	57.08
A. tequilana[a]	85.30	14	0.99	21.5	5.2	64.64
A. tequilana[b]		15	0.16	22	6.4	65.98

[a] to refer to fresh leaves and [b] to the residue after extraction conducting.

Crude extracts were fractionated by column chromatography to give a total of 7 fractions E1 and E2 of 14 *A. salmiana*; and 7 fractions E1 and E2 3 *A. tequilana* Weber. In all cases, the fractions that were obtained with the mixture water: methanol 25:75 to 0: 100 were positive with anisaldehyde reagent and had erythrocyte hemolysis which indicated the presence of saponins (Figure 3). To confirm the presence saponins hemolytic activity of the extracts was assessed in erythrocytes.

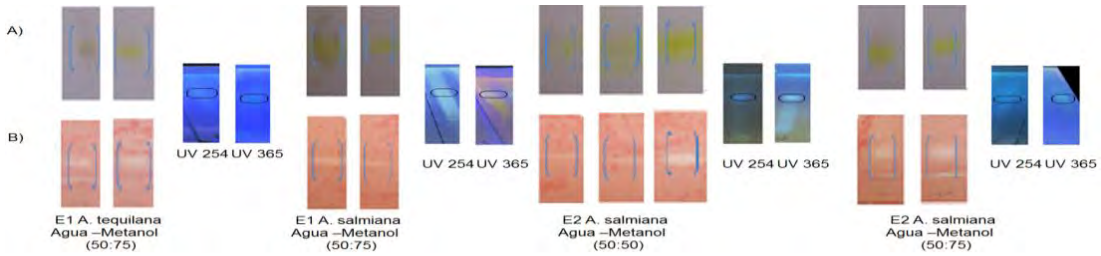

Figure 3. The photographs represent CCD plates. In the image are marked by brackets those fractions that were positive for anisaldehyde reagent (A) and showed hemolysis of erythrocytes (B) as well as the plates 254 views light and E2 have 365.

CONCLUSIONS

The phytochemical characterization showed the presence of including saponins important compounds confirmed by thin layer chromatography secondary metabolites.

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REFERENCES

- Caspeta L., Caro-Bermúdez M.A., Ponce-Noyola T., Martínez A. 2014. Enzymatic hydrolysis at high-solids loadings for the conversión of *Agave bagasse* to fuel etanol: Applied Energy 113: 277-286.
- Narváez-Zapata J.A., Sánchez-Teyer L.F. 2009. Agaves as a Raw Material: Recent Technologies and Applications. Recent Patents on Biotechnologa 3:185-191.
- Medina-García G., Rumayor-Rodríguez A., Cabañas-Cruz B., Luna-Flores M., Ruiz-Corral J.A., Gallegos-Vázquez C., Madero-Tamargo J., Gutiérrez-Sánchez J.R., Rubio-Díaz S., Bravo-Lozano A.G. 2003. Potencial productivo de especies agrícolas en el estado de Zacatecas. Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias. Centro de Investigación Regional Norte Centro, Campo Experimental Zacatecas., México, D.F. ISBN 968-800-557-6, pp. 80-84.
- Sharm O.P., Kumar N., Singh B., Bhat T.K. 2013. An improved method for thin layer chromatographyc analysis of saponins; Food chemistry 132: 671-674.
- Yoong-Cheok CH., Karim-Salman H.A., Sulaiman R. 2014. Extraction and quantification of saponins: A review; Food Research International 59: 16-40.