MOL2NET, International Conference Series on Multidisciplinary Sciences
I International Biotechnology Congress, UDLA, Quito, Ecuador, 2019

Steroidal saponins isolation of *Agave americana* subsp. *andina* by enzymatic hydrolysis: a new approach.

Cristhian P. Murillo Lozano

Instituto Superior Tecnológico "17 de Julio"
e-mail: cristhianpaulmurillolozano@gmail.com

Abstract.
Nowadays, researching of steroidal saponins (SE) is not present in Ecuador, a country where there is a less the science investment; in spite of owning endemic flora unexplored like *Agave americana* subps. *andina* (or the famous "penco azul"); it is a great specie for phytochemical and biotechnician studies. On the other hand, China has been the major SE productor since the end of twentieth century, and actuality, it progresses in prodrugs enhance. For this reason, the author of this short-paper explains how Ecuadorian biotechnicians can do an experimental design with a novel method of enzymatic hydrolysis. This innovative procedure uses a potato-dextrose subtract, which is a culture medium for yeasts of *chaguarmishki*. This method is useful for extracting *O-glucuronidase*, an enzyme that catalyze *O-glycosides*. These molecules are the active agent on *Agave* genus. Finally, the importance of this research is not for the advantages of SE on pharmaceutics or its human body effect; really, the principal benefit is the enhance of Ecuadorian pharmaceutical industry through a mass production with an investment on science investigation.

Introduction

Penco azul (*Agave americana* subsp. *andina*) is part of the landscape of the Ecuadorian Andean region, in the same way, of its culture and tradition [1]. Moreover, it is interesting from the agricultural perspective, due to its type of asexual reproduction (of which the genus *Agave* or *Agavaceae* is characterized), since it does so through its rhizomes (underground stems that form the rhizomes or genetically identical replicas) [2]. Thus, it is common in Latin American countries, such as Mexico and Ecuador, that there are crops taking advantage of this property [3]. However, the advantages are also observed from a biotechnological perspective, due to the possible separation of steroidal saponins (SE) such as *hecogenin* and *tigogenin*, from the rhizomes by enzymatic hydrolysis. Among the properties of these molecules, the one described by Santos, Fajardo, Romo & Uribe, who concluded in their work with *Agave salmiana*, that the anticancer effect of SE is verified by their first evaluation of the apoptotic potential of *kammogenins* and *mangenins* found in mead. Therefore, thanks to these SE, "programmed cell death" can be caused against colon cancer [4]–[7].

With respect to the SE of this short-paper, as *O-glycosides* from which sucrose and aglucones can be synthesized (such as alcohols, phenols or aldehydes) by acidic, basic or enzymatic hydrolysis [8], it should be considered that they are also produced by the Koenigs-Knorr reactions, where acetobromocellobiose and (3β, 5α, 25R) -3-hydroxyspirostan-11-one (with anhydrous zinc fluoride) are involved as catalysts in acetonitrile, for industrial production, obtaining SE with 93% purity [9]. In addition, the main raw material is herbal extracts, because steroidal saponins are its main active agent [6]. Thanks to this, even in populations of *A. americana* has been found that the content of *hecogenin* and *tigogenin* increases by a ratio of 12: 1 (respectively) during the creation of the plant [10]. For this reason, a new method to separate SE is proposed in this research, using the most adult examples, whose age is approximately twelve years old (determined empirically) and with a high hecogenin content, which is the most active SE [11].

Materials and Methods

At the University of Manchester, Cambridge and Bahuguna Garhwal, special glycosides (steroidal saponins) were found in the structure of rhizomes [12]. However, the traditional method for extraction consists in the following treatment of the sample: it starts with drying at 40°C; macerate with ethanol and water (at room temperature and in the absence of light); the extract obtained is evaporated in vacuo; 10 g of this content are dissolved in water, and extracted again in separatory funnels with hexane and butanol; for hydrolysis, 5 g of the saponin extract is dissolved in 75 mL of ethanol and 75 mL of hydrochloric acid is added at a concentration of 2 M; finally, it is extracted with ethyl acetate [13].

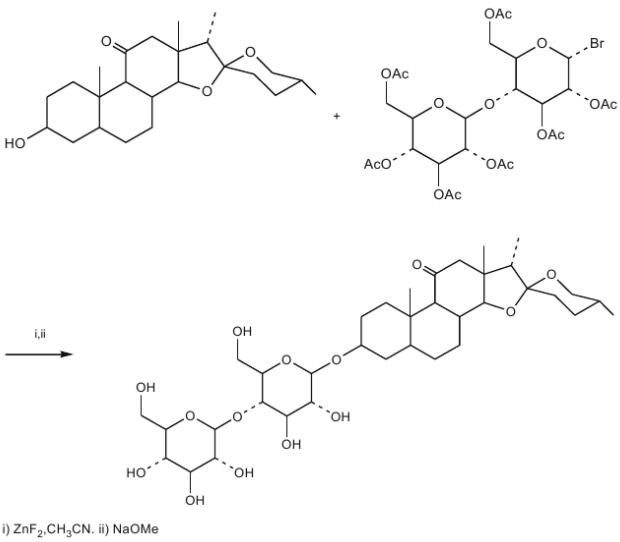

Fig. 1. Synthesis of steroidal saponins [9].

On the other hand, hydrolysis (or a water solvolysis), allows a solvent (nucleophile) to break one or more bonds in the solute [8]. The method proposed with the present study is through enzymatic hydrolysis. In this way, we seek to adapt a technique performed by García et al. [14], who experimented with potato dextrose agar. From this culture medium, the extract of the hydrolyzing enzyme was obtained, inoculating yeasts that ferment the *Agave tequilana* mead. In Ecuador, *A. americana* mead is known as *mishki* or *chaguarmishki*, so it is expected to replicate the experiment on a laboratory scale with this

native drink. As for the treatment of the sample, it is preferable to use the traditional method of extraction [13].

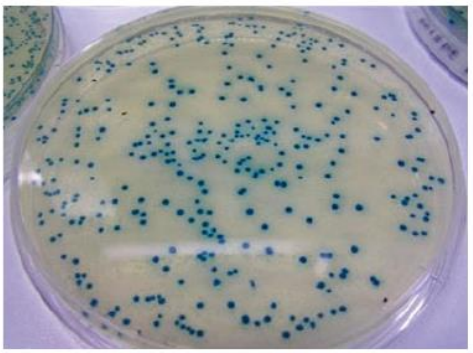

Fig. 2. Precipitated indigo blue is a genetic marker that shows hydrolase activity [5], [9].

Brito [5] also agreed on the use of plant tissues as a source of substrate for culture mediums, so that hydrolases with a high level of purity are obtained [6].

Results and Discussion

The hydrolysis separation methods used by Guerra et al. [13], Chen & Wu [10] and Hernández et al. [3] do not require digestion with organic compounds, moreover, part of their procedure manipulates HCl to separate the saponins. This is convenient at the industrial level because the reagent can be purchased in high quantities. However, Ecuador does not have such a manufacturing skill for saponins as China, compared to the standards set by Chen & Wu [10].

García et al. [14] experimented with enzymatic hydrolysis using potato dextrose agar. The bacteria are inoculated in Petri dishes with *A. americana* mead extract at 1.5% (%w/v). 1 mL aliquot is taken, incubated at 28, 32 and 37 ° C for 24, 48 and 72 hours. Colonies developed with different morphologies are transferred to agar media (nutrients and selective potato dextrose) and incubated again. The procedure is repeated until pure groups are obtained. Once separated, they are stored at 28°C for 24 hours. 100 mL of the biomass samples are transferred to a flask, and mixed at 100 rpm for 48 hours. At the end the phases are separated by centrifugation.

García et al. [14] obtained the extracellular enzyme extract in this way, it may be an alternative to use potato dextrose in an enzymatic hydrolysis that separates saponins from rhizomes of *A. americana* subsp. *andina.*

To add, one aspect of the highly investigated *O-glycoside* hydrolysis in relation to prodrug design is the search for a selective tissue catalysis. Since there are relatively high levels of glucuronidase activity in bowel tumors, their development is a rational strategy. In sum, an aniline mustard alkylating agent that is coupled to glucuronic acid has also been studied, obtaining a correlation between glucuronidase activity and tumoral regression [6]. However, the clinical results were disappointing, apparently due to the loss of glucuronidase as a mechanism of resistance.

Conclusions

Saponins, as reviewed in this short-paper, are found in constant proportions in samples of *A. americana* of different ages. Therefore, they are not only in their rhizomes, but also in their nectar. In summary, hydrolysis method of García et al. [14], and procedure for the sample treatment of Chen & Wu [10], with rhizomes of *A. americana* subsp. *andina* and chaguarmishki, in a 2^2 factorial experimental design, altering the conditions of the substrate and the solvent for digestion.

A brief recommendation is that under the ideas of García et al. [14] and Chen & Wu [10], a sustainable system for the separation of saponins in the country that serves as raw material in the pharmaceutical industry should be developed. This would investigate treatments for diseases such as colon cancer.

References

[1] L. de la Torre, "El penco azul: espinas corazón y miel," *Ecuador: terra incognita*, no. 109, Ecuador, pp. 30–41, 2018.

[2] E. Badano and F. Pugnaire, "Invasion of Agave species (Agavaceae) in south-east Spain: invader demographic parameters on native species," *Divers. Distrib.*, pp. 493–500, 2004.

[3] L. Hernández, *Descripción de Ecosistemas y caracterización de poblaciones naturales de Agave atrovirens*. Buenavista, 2017.

[4] L. Santos, O. Fajardo, I. Romo, and J. G. Uribe, "Fast Centrifugal Partition Chromatography Fractionation of Concetrated Agave (Agave salmiana) Sap to Obtanin Saponin with Apoptic Effect on Colon Cancer Cells," *Plant Foods Hum Nutr.*, 2015.

[5] G. Sonfack *et al.*, "Saponin with antibacterial activity from the roots of Albizia adianthifolia," *Nat. Prod. Res.*, pp. 1–9, 2019.

[6] B. Testa and J. M. Mayer, *Hydrolysis in drug and prodrug metabolism: chemistry, biochemistry, and enzymology*. Alemania, 2003.

[7] T. H. Quang *et al.*, "Phytochemistry Letters Oleanane-type triterpenoid saponins from the roots of Polygala aureocauda," *Phytochem. Lett.*, vol. 34, no. September, pp. 59–64, 2019.

[8] J. Vidal, *Curso de química orgánica y nociones de química biológica*, 18va ed. Buenos Aires: Editorial Stella, 1984.

[9] M. Brito, *Synthesis and Characterization of Glycosides*, 2da ed. Suiza: Springer, 2016.

[10] Y. Chen and Y. Wu, "Progress in Research Manufacturing of Steroidal Sapogenins in China," vol. 3, no. 2, pp. 59–70, 1994.

[11] D. Puentes and L. Nelly, "Interacciones moleculares entre plantas y microorganismos: saponinas como defensas químicas de las plantas y su tolerancia a los microorganismos," *Rev. Estud. Transdiciplinarios*, no. 1, pp. 32–55, 2009.

[12] G. Pant, M. Purohit, G. Morris, A. Halstead, and R. Thompson, "Structure Elucidation and Proton an Carbon-13 Assigment of a Pentasaccharde Glycoside form Agave Americana," *Magn. Reson. Chem.*, no. 32, pp. 213–219, 1994.

[13] J. Guerra *et al.*, "Saponinas esteroidales de la planta Agave brittoniana (Agavaceae) con actividad contra parásito Trichomona vaginalis," 2008.

[14] M. García *et al.*, "Strategy for Biotechnological Process Design Applied to the Enzymatic Hydrolysis of Agave Fructo-oligosaccharides to Obtain Fructose-Rich Syrups," *J. Agric. Food Chem. Artic.*, vol. 57, pp. 10205–10210, 2009.