ANALYSIS OF AN IMMUNOADJUVANT SAPONIN FRACTION FROM QUILLAJA BRASILIENSIS LEAVES BY ELECTROSPRAY IONIZATION ION TRAP MULTIPLE-STAGE MASS SPECTROMETRY.

Authors: Federico Wallace[a], Zhora Bennadji[b], Fernando Ferreira[a,c], Cristina Olivaro[a].
Affiliation: [a] Espacio de Ciencia y Tecnología Química, Centro Universitario de Tacuarembó, UdelaR; Ruta 5 Km 386, Tacuarembó 45000, Uruguay., [b] Programa Nacional de Investigación en Producción Forestal, Instituto Nacional de Investigación Agropecuaria, Ruta 5 Km 386; Tacuarembó 45000, Uruguay, [c] Laboratorio de Carbohidratos y Glicoconjugados, Departamento de Química Orgánica, Facultad de Química, Udelar. Instituto de Higiene, Av. Alfredo Navarro 3051, Montevideo 11600, Uruguay.
E-mail: cristina.olivaro@cut.edu.uy
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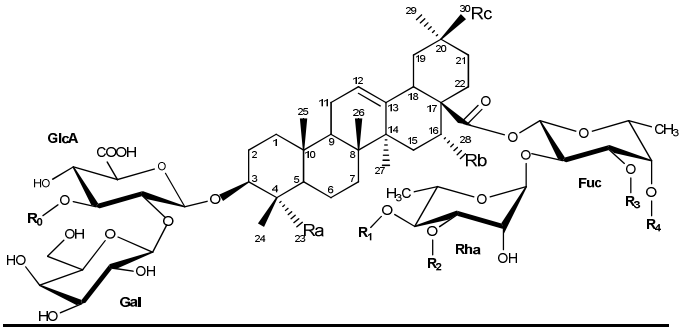
Introduction:
Saponins are natural surfactant compounds whose aqueous solutions can form stable foam and micellar solutions. They are glycosides formed by one, two or three oligosaccharide chains linked to a steroid or triterpene aglycone through acetal and/or ester-acetal bonds.
Quillaja brasiliensis is an endemic tree species in southern Brazil and northern Uruguay. It has been shown in experimental vaccines in animal models that the aqueous extract and some of the purified saponin fractions obtained from leaves of *Q. brasiliensis* have immunoadjuvant activity comparable with Quil-A®, the main commercial adjuvant product based on *Q. saponaria* saponins [1, 2]. These saponins are also able to form ISCOM- type nanometric micellar structures which are even more effective vaccine adjuvants, generating both humoral and cellular immune responses [2].
The aim of this study was to perform a preliminary structural characterization of the immunoadjuvant fraction of saponins designated as Fraction B.

Materials and methods:
The aqueous extract of *Q. brasiliensis* was subjected to a separation on an SPE column to remove non-saponin components and the fractions containing saponins were reunited (Fraction B). This fraction was investigated by direct infusion and liquid chromatography/electrospray ionization ion trap multiple-stage mass spectrometry in negative ion mode (DI-ESI-IT-MS^n and LC-ESI-IT-MS^n). The aglycone and the sequence of the oligosaccharide residues at C-3 and C-28 were characterized based on MS^2 and MS^3 experiments of the $[M-H]^-$ ions.

Results and discussion:
According to their $[M-H]^-$ ions, characteristic product ions and retention times, twenty seven bidesmosidic saponins, bearing four types of triterpenic aglycones (Fig.1) were identified [3].



Aglycones	Ra	Rb	Rc
23-O-acetylphytolaccinic acid	CH_2OCOCH_3	H	CH_3
Gypsogenin	CHO	H	CH_3
Phytolaccinic acid	CH_2OH	H	$COOCH_3$
Quillaic acid	CHO	OH	CH_3

Figure 1: Bidesmosidic saponins feature four different triterpenic aglycones. R_0-R_4: different mono- or oligosaccharide substituents.

Conclusion:
This is the first detailed report about the structure of *Q. brasiliensis* saponins. Further work will be necessary to fully characterize all the immune stimulating saponins present in the *Q. brasiliensis* species.

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References:
[1] Yendo, A.C.A., de Costa, F., Cibulski, S.P., Teixeira, T.F., Colling, L.C., Mastrogiovanni, M., Soulé, S., Roehe, P.M., Gosmann, G., Ferreira, F.A., Fett-Neto, A.G. (2016). *Vaccine*, 34: 2305–2311.
[2] Cibulski, S.P., Silveira, F., Mourglia-Ettlin, G., Teixeira, T.F., dos Santos, H.F., Yendo, A.C., de Costa, F., Fett-Neto, A.G., Gosmann, G., Roehe, P.M. (2016). *Comp. Immunol. Microbiol. Infect. Dis.*, 45: 1–8.
[3] Wallace F., Bennadji Z., Ferreira F., Olivaro C. (2017) *Phytochem. Lett.*,20: 228-233.