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A rapid preparative-TLC/GC-MS methodology for discriminating between two filmy ferns (*Hymenophyllaceae*) native from the temperate rain forest of Southern Chile based on their soluble carbohydrates

[Una metodología de CCF-preparativa/CG-EM rápida para discriminar entre dos helechos película (Hymenophyllaceae) nativos del bosque templado del sur de Chile basada en sus hidratos de carbono]

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Abstract: Filmy ferns are a conspicuous epiphytic component of the temperate rain forest of southern Chile and the more abundant genus is *Hymenophyllum* represented by 19 species, they are a primitive fern family sharing their limited water lost control (poikilohydricity) with lower non-vascular plants, such as bryophytes and lichens. Because, carbohydrate accumulation is a desiccation tolerance mechanism proposed for desiccation-tolerant vascularized plants, the aim of this investigation was to propose a methodology for soluble carbohydrate analysis in native ferns. Preparative- TLC showed to be a simple and rapid technique for separating *Hymenophyllum* sugars. GC-MS analysis allowed identifying glucitol (2.0%), sucrose (2.0%), α or β-melibiose (5.0%) and salidroside (4.4%) in *H. cruentum* carbohydrate profile from *H. dentatum* showed significant differences presenting only two sugars sucrose (1.4%) and α or β-melibiose (3.2%). Furthermore, salidroside, an antioxidant compound, was identified only in *H. cruentum*. Our results suggest that *H. cruentum*, has higher preventive mechanisms than *H. dentatum*; that would allow delay the effects of desiccation (prevent a rapid dehydration) and thus survive to short periods of drought present in the study area. Due to the presence of salidroside in *H. cruentum* this species should be more resistant to oxidative stress that *H. dentatum*.

Keywords: *Hymenophyllum*, Chilean native filmy ferns, carbohydrates, desiccation, GC-MS

Resumen: Los helechos película se pueden encontrar en el bosque templado lluvioso del sur de Chile, siendo el género más abundante *Hymenophyllum* representado con 19 especies. Son una familia de helechos primitivos que comparten la limitación de control de pérdida de agua (poiquilohidrícos) con plantas no vasculares, tales como briófitas y líquenes. Como el contenido de hidratos de carbono ha sido asociado a la protección de la planta durante la desecación, su análisis es una herramienta importante para dilucidar el mecanismo asociado a este fenómeno. El objetivo de esta investigación fue proponer una metodología fácil y rápida para el análisis de hidratos de carbono en helechos nativos. La CCF-preparativa permitió la separación de hidratos de carbono presentes en plantas del género *Hymenophyllum*. El análisis por GC-MS logró identificar glucitol (2,0%), sacarosa (2,0%), α o β-melibiosa (5.0%) y salidrosido (4,4%) en *H. cruentum*. El perfil de hidratos de carbono de *H. dentatum* mostró diferencias significativas, mostrando sólo dos compuestos sacarosa (1,4%) y α o β-melibiosa (3.2%). Además, salidrosido, un compuesto antioxidante, sólo fue identificado en *H. cruentum*. Nuestros resultados sugieren que ante la menor tolerancia a la desecación observada previamente en *H. cruentum*, esta especie presenta mecanismos que le permitirían retardar los efectos de la desecación (prevención de una rápida pérdida de agua) y así sobrevivir a los periodos cortos de sequía presentes en la zona de estudio. Debido a La presencia de salidrosido en *H. cruentum*, esta especie debería ser más resistente al estrés oxidativo que *H. dentatum*.

Palabras clave: *Hymenophyllum*, helechos chilenos nativos, hidratos de carbono, desecación, CG-EM.

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INTRODUCTION

The literature define 'resurrection plants' as those able to tolerate desiccation to an extent where almost all protoplasmic water is lost, and then they are able to re-wetting, and restored their physiological functions in a short period of time (Oliver *et al.*, 1998; Oliver *et al.*, 2004; Illing *et al.*, 2005; Farrant 2007). This phenomenon is quite common in bryophytes and lichens, but is unusual in pteridophytes and angiosperms, occurring in only 0.02% of vascular plants (Alpert & Oliver, 2002). It has been reported that desiccation process in bryophytes and lichens is a fast event, allowing little time for protection to occur. Protection is based mainly preexisting protection or on rehydration-induced repair processes (Oliver *et al.*, 2004). Drying process can produce serious damage to the different plant tissues. To prevent this injury or to minimize secondary cost associated to repairing damaged structures during rehydration, several mechanisms have been proposed. For instance, controlling the excessive formation of reactive oxygen species (ROS) produced by photosynthetic tissues during desiccation and rehydration (Tuba *et al.*, 1994; Sherwin & Farrant, 1998; Farrant *et al.*, 2003;) by increasing the concentration of antioxidants is a protective biochemical mechanisms reported in desiccation tolerant Angiosperms (Sgherri *et al.*, 1994; Kranner *et al.*, 2002; Illing *et al.*, 2005; Kranner & Birtic, 2005; Farrant *et al.*, 2009). Accumulation of disaccharides and trisaccharides, such as sucrose, trehalose and raffinose have also been related to plant protection during desiccation Saccharide accumulation, such as sucrose, trehalose and raffinose have also been related to plant protection during desiccation (Norwood *et al.*, 2000; Peters *et al.*, 2007). Sugars have been proposed to contribute to the maintenance of turgor during stress, and the prevention of protein denaturation and membrane fusions in the cell (Crowe *et al.*, 1998). Sucrose for instance, is capable of forming biological glasses (a process called vitrification) within the dried cell. Vitrification of the cytoplasm may not be due to the effects of sugars only, but probably results from the interaction of sugars with other molecules, most likely proteins (Hoekstra, 2005). The formation of an intracellular glass phase is believed to be indispensable to survival during desiccation. Some species of pteridophytes are desiccation tolerant (Alpert & Oliver, 2002; Helseth & Fischer, 2005), but the information of the desiccation mechanisms in pteridophytes is scarce. However, Oliver *et al.* (1998) suggested that it could have some similarities with angiosperms because its drying rate is slow, would provoke an *in situ* accumulation of protective compounds. Moreover, frond curling and wall folding occur during drying in *Polypodium polypodioides* (Muslin & Homann, 1992; Helseth & Fischer, 2005), suggesting some mechanisms in common with angiosperms.

The *Hymenophyllaceae* is a primitive family from the class Filicopsida, Pteridophyta (Pryer *et al.*, 1996)). They constitute a "rare example of an evolutionary shift back from a typical vascular plant to poikilohydry, typical from briophites" (Proctor, 2012; Garcés *et al.*, 2014). Filmy ferns possess transparent and delicate fronds, most of photosynthetic lamina is formed by a single layer of cells, lacking stomata (Tryon & Tryon, 1982), and lacking effective water lost control, depending their growth strongly on very humid and shady environments (Kappeen & Valladares, 2007; Farrant *et al.*, 2009;). These filmy ferns can be found at moderate elevations in the tropics, in Atlantic Western Europe, south east Australia, New Zealand and they are an important epiphyte component in the temperate rain forest of southern Chile. The most abundant genus in Chile is *Hymenophyllum* represented with 19 species (Parra *et al.*, 2012). As explained above, sugar accumulation is a desiccation tolerance mechanism proposed for vascular and cryptogams plants (Martinelli, 2009; Farrant *et al.*, 2009), qualitative and quantitative analysis of sugars in native filmy ferns is an important tool for elucidate the mechanism associated to the desiccation tolerance phenomenon.

The aim of this investigation is to propose a methodology for the identification and characterization of sugar in native filmy ferns.

MATERIALS AND METHODS

Plant material

The vegetable materials were collected from a second-growth forest stand located in Katalapi Park in the Cordillera of Quillaipe, Region de los Lagos, Chile (41° 31'07 .5 "S, 72° 45'2 .2" W). Saldaña *et al.* (2014) have described extensively this Park previously. Small pieces of fallen tree trunks containing epiphytes *Hymenoglossum cruentum* (Cav.) Presl and *Hymenophyllum dentatum* (Cav.) were translocate to a nursery garden at Universidad de La Frontera (Laboratory of Plant Physiology and

Molecular Biology). Trunks with the epiphytes were installed in a vertical wall consisting of Acma stainless steel mesh panels which were vertically hanged inside the nursery garden. Panels were coated with two or three layers of burlap to keep a moist surface upon watering.

Reagents and standards

All organic solvents, i.e., dichloromethane, methanol and hexane (Burdick & Jackson, MI, USA) were GC grade. Sugar standards were purchased from Fluka/Aldrich/Sigma (St. Louis, MO, USA). Derivatizations of sugars were performed using N, O-bis-(trimethylsilyl) trifluoroacetamide (BSTFA) containing 1% trimethylchlorosilane (TMCS) and pyridine (silylation grade) both from Pierce (Rockford, IL, USA). Individual standard stock solutions were prepared in methanol at concentrations varying from 120 to 200 µg/mL. Individual solutions of glucose, sorbitol, levoglucosan and sucrose were diluted serially using methanol to prepare calibration curves ranging from 200 to 1.2 µg/mL, and a composite standard solution was also prepared, varying from 100 to 2.4 µg/mL, to assess sugar recoveries. The BSTFA and pyridine reagents, as well as the individual and composite standard solutions were stored at 4º C

Extraction of sugars

For the extraction of sugars 500 mg of fully hydrated filmy fern fronds of both species were processed. Fronds were powdered in mortar using liquid nitrogen, then the plant material was transferred to a falcon tube and 2 mL of ethanol 86% was added and incubated for 30 min with constant stirring at room temperature, then tubes were centrifuged at 8.500g for 15 min at 4° C and the supernatant was stored at -20° C until analysis.

Thin layer Chromatography

Previously to spotted, the sugar extract were centrifuged at 12.000 g for 10 min at 4ºC for eliminated vegetative tissue remainder. Sugar extract (20 µL) were analyzed by thin layer chromatography using silica gel coated aluminum chromatofoils (Merck, GF_{254}) and developed using ethyl acetate: glacial acetic acid: methanol: water (12:3:3:2.5 v/v/v/v), a saturation time of 30 min was used in the chromatography chamber. The plate migration distance allowed was 180 mm for approximately 120 min then the plates were air-dried. To detect sugars and glycosides, dry chromatofoils were sprayed with a solution of ethanol + phosphoric acid + α-naphthol (50 mL + 6 mL+ 0.5 g). The plates were then heated at l00º C until colored spots appeared on a light brown background. For separation of different sugars compounds, several plates of each species were run until a total amount of 10 mg of each compound was collected. Individual plates were developed separately for each filmy fern sugars extract and one small section of each chromatography plate was revealed for determining the Rf values of each compound, the area of individual spots were localized and scraping off the individual zones onto small glass beaker with ethanol 86% and extracted by sonication, then the extracts were concentrated under reduced pressure. These samples were used to prepare the silylated derivatives.

Sample derivatization

Sample derivatization was based on the methodology reported by Medeiros & Simoneit (2007). Ten milligram of each compound was dissolved in 1.5 mL of a mixture dichloromethane/methanol (2:1 v/v), and then the solution was concentrated to 500 µL using a stream of high-purity nitrogen (Linde, Temuco, Chile). Aliquots of the total extracts (20–25 µL) were evaporated completely using a stream of filtered nitrogen gas, and then converted to their trimethylsilyl derivatives using BSTFA containing 1% TMCS (20–25 µL) and pyridine (10 µL) for 3 h at 70° C. Immediately before GC–MS analysis, derivatives were evaporated to dryness using nitrogen gas and dissolved in 20–25 µL of hexane for injection.

GC-MS Analysis

The identification of the sugars was carried out applying the methodology reported by Medeiros & Simoneit (2007) with some modifications. Aliquots of 1 µL of silylated samples were analyzed using GC-MS (Focus DSQ, Thermo Electron Corporation). The separation of compounds was performed using a capillary column BP-1 (30 m × 0.22 mm I.D. and film thickness of 0.25 µm). Helium was used as the carrier gas (1.3 mL/min). The temperature of the oven was programmed for starting at 65° C and hold by 2 min, increasing at a rate of 6° C/min to 300° C and hold by 15 min. The MS was operated in the electron impact mode with ionization energy of 70 eV. Mass spectrum acquisition was performed in the mass range of 50 to 650 m/z at 1.27 scan/s. The

injection mode was splitless mode (splitless time: 30 s). The injector and MS source temperatures were maintained at 280 and 230° C, respectively. Data were acquired and processed with the Xcalibur software. The identification of compounds was performed by searching a library of mass spectra NIST (Mass Spectral Library Version 2.0) using a matching algorithm with a reverse search technique to verify that the highest peaks of the reference compounds are present in the mass spectra problem. The mass spectra were interpreted based on their fragmentation patterns. In addition, a comparison of their Kovats indices (KI) was performed by injecting an alkenes series (C9-C26). The experimental KIs were compared with theoretical KI compounds reported in the database "NIST" (NIST ver. 2.0, Thermo) (Babushok *et al.*, 2007).

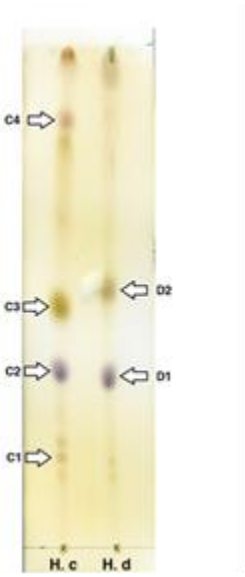


Figure 1
Thin layer chromatogram of sugar extracts from *H.cruentum* (C) and *H. dentatum* (D). Mobile phase: ethyl acetate: glacial acetic acid: methanol: water (12:3:3:2.5 v/v/v).

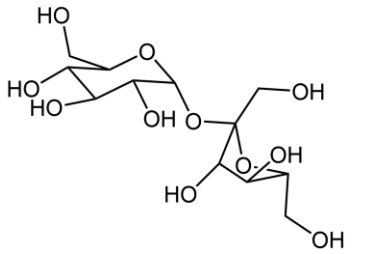
RESULTS AND DISCUSSION

TLC Analysis

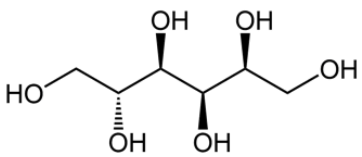
Thin layer chromatography allowed identifying 4 main spots in *H. cruentum* sugar extract (Figure 1) with RF of 0.2, 0.35, 0.49 and 0.86 named C1, C2, C3 and C4 respectively, and 2 spots from *H. dentatum* (Figure 1) with RF of 0.39 and 0.52 named D1and D2 respectively. C4 is an interesting spot, because in relatively intense (abundant in the extract) and is present only in the extract of *H. cruentum.* There were other less intense spots that were not identified because its low amount in the extract. The total amount of sugars isolated by TLC was 67 mg for *H. cruentum* corresponding to 13.4% of yield and 23 mg for *H dentatum*, corresponding to 4.6% of yield.

GC-MS Analysis

The identification of sugars in samples of the filmy ferns *H. cruentum* and *H. dentatum* was carried out by GC-MS. Total ion chromatograms of all samples showed one major chromatographic signal (> 90%), indicating the compound extracted from each spot was highly pure. Figure 2 shows that C1-spot is formed mainly by glucitol (retention time (tr) = 25.79 min). This compound also known as sorbitol, come from the photosynthesis in adults leaves of certain plants from Rosaceae and Plantaginaceae families. This poly alcohol was identified only in *H. cruentum* with a yield of 2%. The respective TMS derivate is characterized by 319, 73, 147, 205 and 217 m/z fragments. Glucitol, also called sorbitol, is a sugar alcohol that can be obtained by reduction of glucose. It can be converted to fructose by sorbitol-6-phosphate-2-dehydrogenase. Levels of the polyol sorbitol were also found to be elevated in the leaves of higher plants during drought stress (Mundree *et al.*, 2000)



Sucrose



Glucitol

and the accumulation of sorbitol has been demonstrated in some plants subjected to chronic hyperosmosis (Ahmad *et al.*, 1979) confirmed the osmoprotectant role of this molecule in plant cell in

the process of desiccation. C2 and D1 were the same compounds corresponding to sucrose (t.r. 35.82 min) (Figure 3) yielding 2.0 and 1.4% respectively. Sucrose as TMS derivate is characterized by the ion fragments 361, 217 and 437 m/z and Kovats index (KI) of 2710. This assignation was corroborated by Medeiros & Simineit (2007), who reported a KI of 2712.

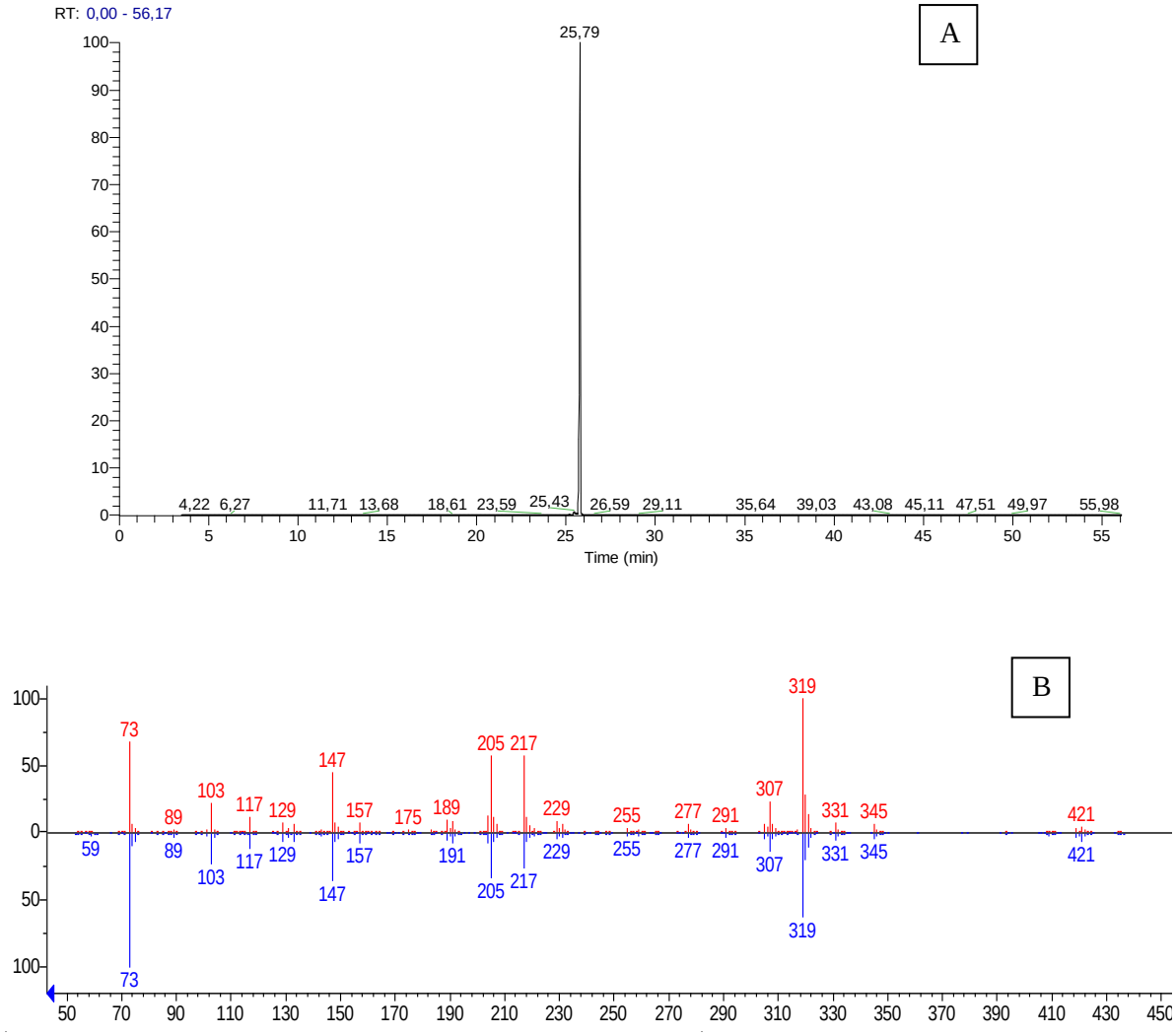


Figure 2
A: Total ion chromatogram (TIC) corresponding to spot C1 (Glucitol-TMS). B: Experimental mass spectrum (red line) and mass spectrum from NIST library (blue line).

One of the functions described for sucrose is osmoprotectant, stabilizing cellular membranes and maintaining turgor (Mundree *et al.*, 2000). According with this result, studied realized in the resurrection plants *Sporobolus staphianus* and *Craterostigma wilmsii* also detected sucrose accumulation in response to drought stress (Whittaker *et al.*, 2001; Cooper & Farrant, 2002). C3 (tr 32.40 min; KI = 2930) and D2 (tr = 33.44 min; KI = 2955) corresponded to either α or β anomers of the melibiose yielding 5.0 and 3.2% respectively, this sugar also had previously been associated to heat and drought stress in *A. thaliana* (Rizhsky *et al.*, 2004). By means of this analysis was not possible to difference both isomers related to the 1α or 1β-configuration of the OH group on the pyranose ring

of melibiose (Figure 4). The most characteristic mass fragments corresponded to 204, 73, 191, 217 and 361 m/z and KI of 29. Medeiros & Simoneit (2007) reported similar KI values for both isomers.

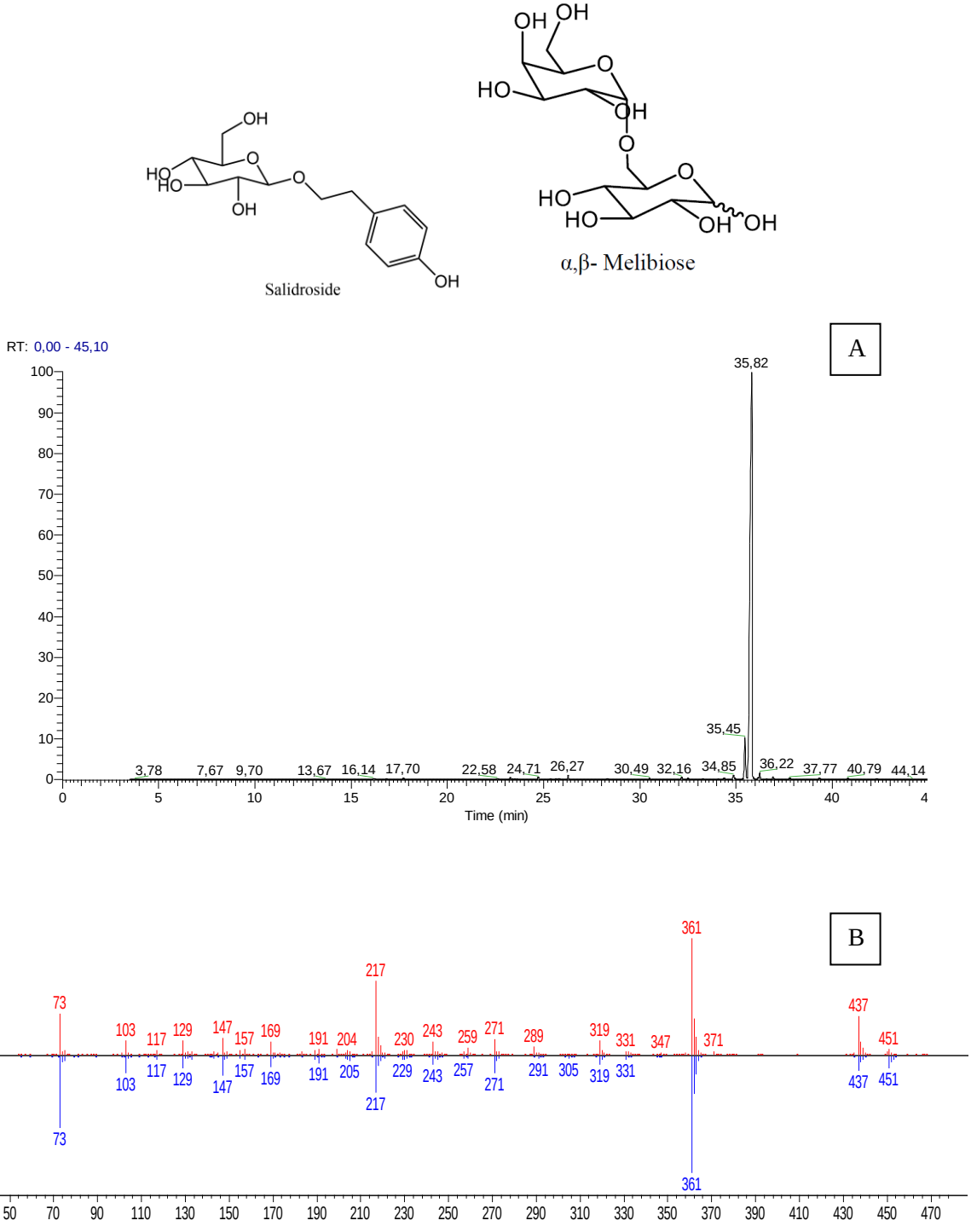


Figure 3
A: Total ion chromatogram (TIC) corresponding to spots C2 and D1 (Sucrose-TMS). B: Experimental mass spectrum (red line) and mass spectrum from NIST library (blue line).

The higher amount of sugars in *H. cruentum* than *H. dentatun* is consistent with a previous work exhibiting higher soluble sugars in the former species (Fallard *et al.*, 2015, submitted). The higher sugar content seems not related with desiccation tolerance because it has been shown that *H. dentatum* exhibited a better desiccation recovery than *H. cruentum* (Parra, 2005). Nonetheless, as sugars are implicated in osmotic cell adjustments it is likely that the higher sugar content in *H. cruentum* could restrict or delay dehydration. This would be consistent with the faster initial dehydration exhibited by *H. dentatum* (Parra *et al.*, 2015). Additionally, some sugars, such as sucrose increased later during the desiccation process in *H. dentatum* but not in *H. cruentum* (Garcés *et al.*, 2014) showing the relevance of sucrose in the desiccation phenomenon in *Hymenophyllum*.

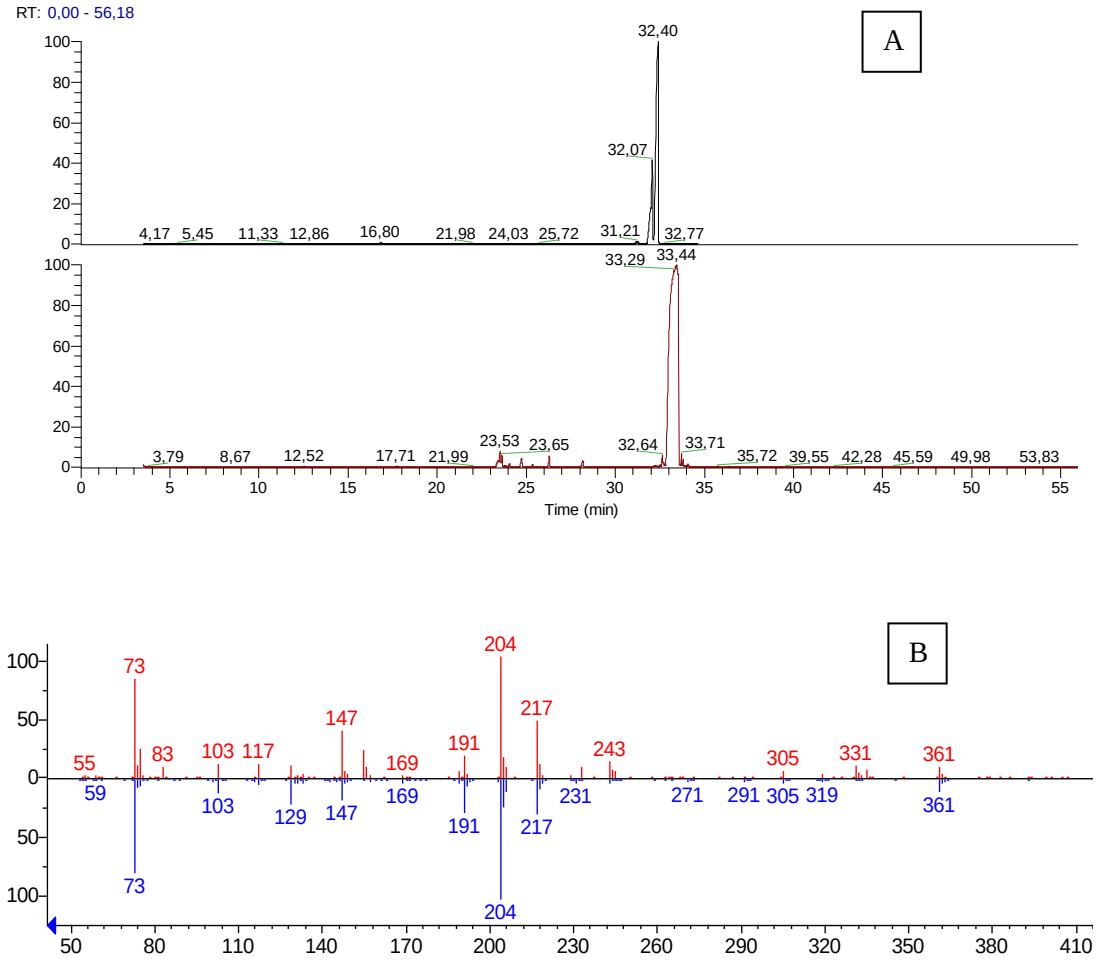


Figure 4

A: Total ion chromatograms (TIC) corresponding to spots C3 (t.r. 32.40 min) and D2 (t.r. 33.44 min) (α o β-Melibiose-TMS). B: Experimental mass spectrum (red line) and mass spectrum from NIST library (blue line).

Finally, C4 was only present in *H. cruentum*, and identified as the TMS derivative of salidroside (2-[4-hidroxy-phenylethyl]-β-D-glucopyranoside) (Figure 5) corresponding to the 4.4% of yield. This compound showed peaks in the mass spectrum at m/z: 204,193,192 and 73, consistent with previously published data for the TMS derivative of saliroside (Vainiotalo *et al.*, 1991; Wu *et al.*, 2003). Salidroside

is a phenolic antioxidant compound present in several natural sources, such as olive oil, and *Rhodiola rosea* (Vlachogianni *et al.*, 2015). Although it is not clear the physiological role of salidroside in *H. cruentum*, it is possible that is a defense compound against fungal infections as previously reported in olive trees (Aabidine *et al.*, 2010). Free tyrosol may be also a substrate for polyphenol oxidase which has been related to mechanical wounding and very rapid frond brownish produced in *H. cruentum* in desiccated state (Acuña K. unpublished data).

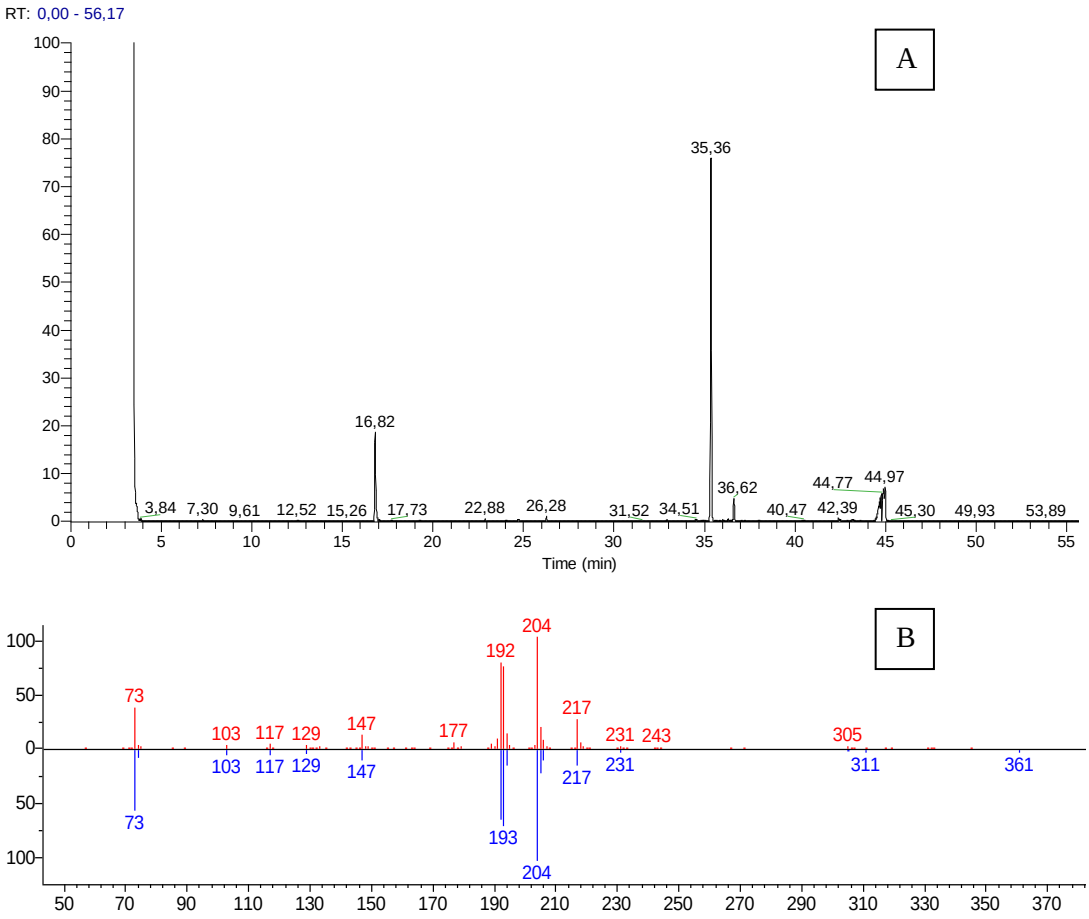


Figure 5
A: Total ion chromatogram (TIC) corresponding to spot C4 (Salidroside-TMS). B: Experimental mass spectrum (red line) and mass spectrum from NIST library (blue line)

CONCLUSIONS

In summary, TLC analysis showed to be a very reliable technique for separating sugars present in filmy ferns hydro alcoholic extracts. By means of GC-MS analysis was possible to identify glucitol (2.0%), sucrose (2.0%), α or β-melibiose (5.0%) and salidroside (4.4%) in *H. cruentum*. Qualitative and quantitative differences were found in *H. dentatum*, identifying lower amount of sucrose (1.4%) than *H. cruentum* and lower amount of α or β-melibiose (3.2%) than *H. cruentum*, corroborating the TLC separation and consistent with the content of total soluble sugars measured previously. Interestingly, salidroside, an antioxidant phenolic compound, only present in *H. cruentum* would confer protection against herbivores together with a set of other phenolic compounds found in this species.

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REFERENCE

Aabidine AZE, Baissac Y, Moukhili A. Jay-allemand C, Khadari B, Modafar CE. 2010. Resistance of olive tree to *Spilocaea oleagina* is mediated by the synthesis of phenolic compounds. **Int J Agr Biol** 12: 61 - 67.

Ahmad I, Larher F, Steward GR. 1979. Sorbitol, a compatible osmotic solute in *Plantago maritime*. **New Phytol** 82: 671 - 678.

Alpert P, Oliver MJ. 2002. **Drying without dying.** In: Desiccation and Survival in Plants – Drying without Drying (Black M, Pritchard HW, eds). CABI Publishing, Wallingford and New York, USA.

Babushok VI, Linstrom PJ, Reed JJ, Zenkevich IG, Brown RL, Mallard WG, Stein SE. 2007. Development of a database of gas chromatographic retention properties of organic compounds. **J Chromatogr** 1157: 414 – 421.

Cooper K, Farrant JM. 2002. Recovery of the resurrection plant *Craterostigma plantagineum* from desiccation: protection versus repair. **J Exp Bot** 53: 1805 - 1813.

Crowe JH, Carpenter JF, Crowe LM. 1998. The role of vitrification in anhydrobiosis. **Annu Rev Physiol** 60: 73 - 103.

Fallard A, Rabert C, Reyes-Díaz M, Alberdi M, Bravo LA. 2015. Compatible solutes and metabolites during a desiccation-rehydration cycle in *Hymenoglossum cruentum* and *Hymenophyllum dentatum* (Hymenophy-llales: Hymenophyllaceae) two filmy ferns with contrasting vertical distribution and desiccation resistance. **Rev Chil Hist Nat** submitted.

Farrant JM, Bartsch S, Loffell D, Vander Willigen C, Whittaker A. 2003. An investigation into the effects of light on the desiccation of three resurrection plants species. **Plant Cell Env** 26: 1275 - 1286.

Farrant JM. 2007. **Mechanisms of desiccation tolerance in angiosperm resurrection plants**. In plant desiccation tolerance (Jenks MA, Wood AJ, eds). CABI Press, Wallingford, USA.

Farrant JM, Lehner A, Cooper K, Wiswedel S. 2009. Desiccation tolerance in the vegetative tissues of the fern *Mohria caffrorum* is seasonally regulated. **Plant J** 57: 65 - 79.

Garcés M, Claverol S, Alvear C, Rabert C, Bravo L. 2014. Desiccation tolerance of Hymenophyllacea filmy ferns is mediated by constitutive and-inducible cellular mechanisms. **CR Biologies** 337: 235 - 243.

Helseth LE, Fischer TM. 2005. Physical mechanisms of rehydration in *Polypoidium polypodioides*, a resurrection plant. **Phys Rev** 71: 061903–1.

Hoekstra FA. 2005. Differential longevities in desiccated anhydrobiotic plant systems. **Integr Comp Biol** 45: 725 - 733.

Illing N, Denby K, Collett H, Shen A, Farrant JM. 2005. The signature of seeds in resurrection plants: a molecular and physiological comparison of desiccation tolerance in seeds and vegetative tissues. **Integ Comp Biol** 45: 771 - 787.

Kappen L, Valladares F. 2007. **Opportunistic grow and desiccation tolerance: the ecological success of poikilohydrous autotrophs.** In: FI Pugnaire, F Valladares (Eds.), Functional Plant Ecology, CRC Press/Taylor & Francis Group, Boca Raton, USA.

Kranner I, Birtic S. 2005. A modulating role for antioxidants in desiccation tolerance. **Integ Comp Biol** 45: 734 - 740.

Kranner I, Beckett RP, Wornik S, Zorn M, Pfeifhofer HW. 2002. Revival of a resurrection plant correlates with its antioxidant status. **Plant J** 31: 13 - 24.

Martinelli T. 2009. In situ localization of glucose and sucrose in dehydrating leaves or *Sporobolus stapfianus*. **J Plant Physiol** 165: 580 - 587.

Medeiros PM, Simoneit BRT. 2007. Analysis of sugars in environmental samples by gas chromatography–mass spectrometry. **J Chromatogr A** 1141: 271 - 278.

Mundree SG, Whittaker A, Thomson JA, Farrant JM. 2000. An aldose reductase homolog from the resurrection plant *Xerophyta viscosa* Baker. **Planta** 211: 693 - 700.

Muslin E, Homann PH. 1992. Light as a hazard for the desiccation-resistant ‘‘resurrection’’ fern *Polypodium polypodiodes* **L Plant Cell Env** 15: 81 - 89.

Norwood M, Truesdale MR, Richter A, Scott P. 2000. Photosynthetic carbohydrate metabolism in the resurrection plant

Craterostigma plantagineum. **J Exp Bot** 51: 159 - 165.

Oliver MJ, Wood AJ, O'Mahony P. 1998. 'To dryness and beyond'' – preparation for the dried state and rehydration in vegetative desiccation-tolerant plants**. Plant Growth Reg** 24: 193 - 201.

Oliver MJ, Dowd SE, Zaragoza J, Mauget SA, Payton PR. 2004. The rehydration transcriptome of the desiccation-tolerant bryophyte *Tortula ruralis*; transcript classification and analysis. **BMC Genomics** 5: 89.

Parra MJ. 2005. **Patrones de distribución geográfica de la flora Pteridofítica de Chile continental, con especial énfasis en la transición climática mediterráneo templada de Chile central.** Tesis de Magíster en Ciencias mención Botánica. Universidad de Concepción. Concepción, Chile.

Parra MJ, Acuña K, Corcuera LJ, Rodríguez R. 2012. Presencia de la familia Hymenophyllacea (Pterophyta) en el Parque Katalapi, Cordillera de Quillaipe, Provincia de Llanquihue, Chile. **Gayana Bot** 69: 384 - 387.

Parra MJ, Acuña KI, Sierra-Almeida A, Sanfuentes C, Saldaña A, Corcuera LJ, Bravo LA. 2015. Photosynthetic light responses may explain vertical distribution of Hymenophyllaceae species in a temperate rainforest of Southern Chile. **PLoS One** submitted

Peters S, Mundree SG, Thomson JA, Farrant JM, Keller F. 2007. Protection mechanisms in the resurrection plant *Xerophyta viscosa* (baker): both sucrose and raffinose family oligosaccharides (RFOs) accumulate in leaves in response to water deficit. **J Exp Bot** 58: 1947 - 1956.

Proctor MC. 2012. Light and desiccation responses of some Hymenophyllaceae (filmy ferns) from Trinidad, Venezuela and New Zealand: poikilohydry in a light-limited but low evaporation ecological niche. **Ann Bot Lond** 109: 1019 - 1026.

Pryer K, Smith A¸Skog J. 1996. Phylogenetic relationships of extant ferns based evidence from morphology and rcbI sequences. **Am Fern J** 85: 205 - 282.

Rizhsky L, Liang H, Shuman J, Shulaev V, Davletova S, Mittler R. 2004. When defense pathways collide. The response of *Arabidopsis* to a combination of drought and heat stress. **Plant Physiol** 134: 1683 - 1696.

Saldaña A, Parra MJ, Flores-Bavestrello A, Corcuera LJ, Bravo LA. 2014. Effects of forest successional status on microenvironmental conditions, diversity, and distribution of filmy fern species in a temperate rainforest. **Plant Spec Biol** 29: 253 - 262.

Sgherri CLM, Logginin B, Bochicchio A, Navari-Izzo F. 1994. Antioxidant system in *Boea hygroscropica*: changes in response to desiccation and rehydration. **Phytochem** 73: 277 - 381.

Sherwin HW, Farrant JM. 1998. Protection mechanism against excess light in the resurrection plants *Craterostigma wilmsii* and *Xerophyta viscosa*. **Plant Growth Reg** 24: 203 - 210.

Tryon RM, Tryon AF. 1982**. Fern and allied plants, whit special reference to tropical America**, Spinger-Verlag, New York, USA.

Tuba Z, Lichtenthaler HK, Csintalan Zs, Nagy Z, Szente K. 1994. Reconstitution of chlorophylls and photosynthetic CO_2 assimilation, upon rehydration in the desiccated poikilochlorophyllous plant *Xerophyta scabrida*. **Planta** 192: 414 - 420.

Vainiotalo P, Julkunen-Tiitto R, Juntheikki M-R, Reichardt P, Auriola S. 1991. Chemical characteristics of herbivore defenses in *Betula pendula* winter-dormant young stems. **J Chromatogr A** 547: 367 - 316.

Vlachogianni IC, Fragopoulou E, Kostakis IK, Antonopoulou S. 2015. *In vitro* assessment of antioxidant activity of tyrosol, resveratrol and their acetylated derivatives. **Food Chem** 177: 165 - 173.

Whittaker A, Bochicchio A, Vazzana C, Lindsey G, Farrant J. 2001. Changes in leaf hexokinase activity and metabolite levels in response to drying in the desiccation-tolerant species *Sporobolus staphianus* and *Xerophyta viscosa.* **J Exp Bot** 52: 961 - 969.

Wu S, Zub Y, Wu M. 2003. High yield production of salidroside in the suspension culture of *Rhodiola sachalinensis.* **J Biotech** 106: 33 - 43.