Analysis on *in vitro* antioxidant capacities and phenolic composition of lichens extracts of Cetrarioid clade based on multivariate statistical analysis

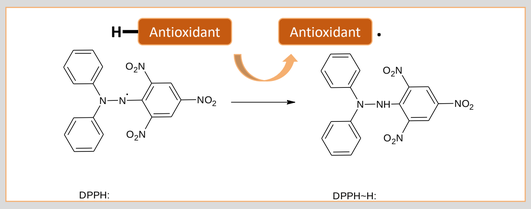
Ureña-Vacas, I.M. [a], **González-Burgos, E.** [a], **Gómez-Serranillos, M.P.** [a]

[a]*Department of Pharmacology, Pharmacognosy and Botanical, Faculty of Pharmacy, Universidad Complutense de Madrid, Plaza Ramon y Cajal s/n, Ciudad Universitaria, 28040, Madrid, Spain*

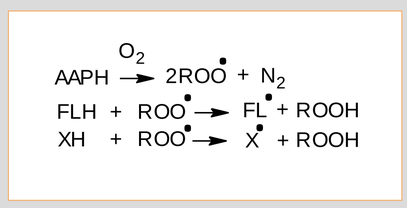
Introduction

Lichens (fungus and unicellular algae/cyanobacteria symbiosis) are one of the least studied organisms from a pharmacological point of view. They contain own secondary metabolites with phenol groups to which an important antioxidant potential has been attributed. The **Cetrarioid clade** is one of the most numerous within the **Parmeliaceae family**.

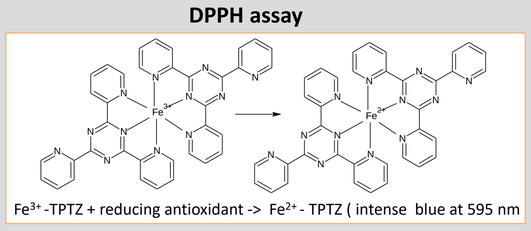
There are several techniques that allow evaluating ***in vitro*** **antioxidant activity**.



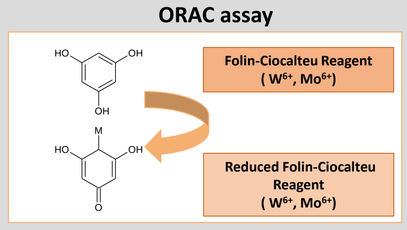
DPPH assay



ORAC assay



FRAP assay



Total phenol content assay

Figure 1. Thallus of the studied lichens of Cetrariod clade.

Objectives

- ❖ Evaluation of the antioxidant properties of 14 samples of **lichen extracts** from **Cetrarioid clade.**
- ❖ Classification of lichen species by **multivariate analysis**.

Material and methods

- Principal component analysis (PCA) and Hierarchical Cluster Analysis (HCA) using IBM SPSS statistics version 25.
- Variables were autoscaled (transformation into z-scores).

Results

A)

B)

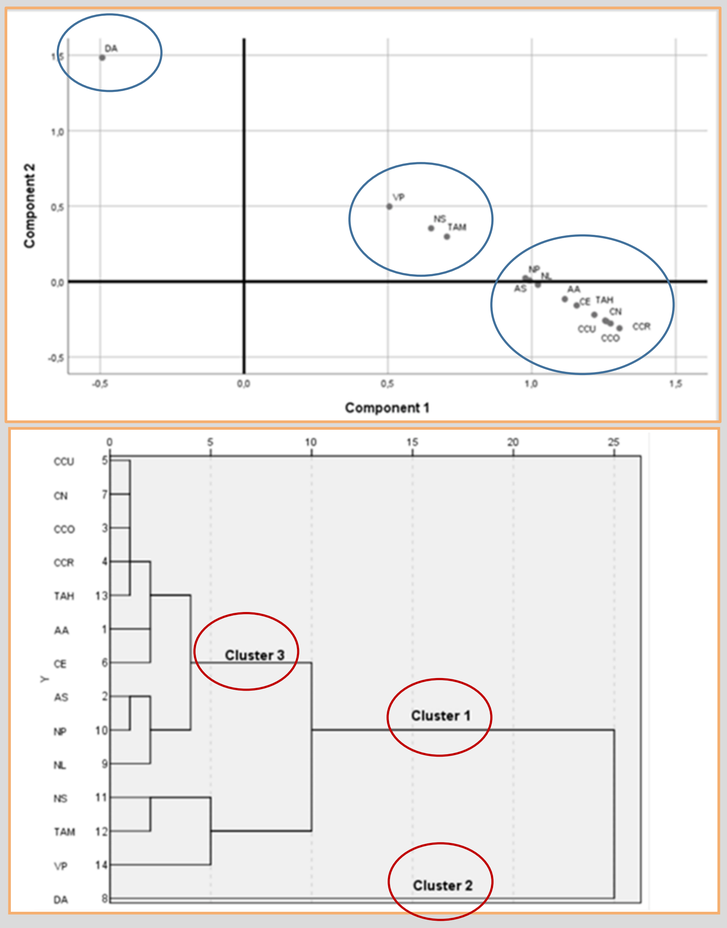


Figure 2. A. Principal component analysis (PCA). B. Dendogram for lichen extracts obtained from HCA. Abbreviations: AA (*Allocetraria ambigua),* AS (*Asahinea scholanderi*), CCO (*Cetraria commixta*), CCR (*Cetraria crespoae*), CCU (*Cetraria cucullata*), CE (*Cetraria ericetorum*), CN (*Cetraria nivalis*), DA (*Dactylina arctica*), NL (*Nephromopsis laureri*), NP (*Nephromopsis pallescens*), NS (*Nephromopsis stracheyi*), TAH (*Tuckneraria ahtii*), TAM (*Tuckermanopsis americana*), VP (*Vulpicida pinastri*).

PCA

Differences and similarities in antioxidant activity and phenolic content.

Dactylina arctica was placed on left half up of the plot (first quadrant). *Nephromopsis stracheyi, Tuckermannopsis americana* and *Vulpicida pinastri* were in the second quadrant (right half up of the plot) and finally, the other lichen species from Cetrarioid clade were placed on right half down of the plot (third quadrant).

Cluster 1: lichen species with moderate phenolic content and antioxidant capacity.
Cluster 2 is composed of a single specie *Dactylina arctica* (DA). (strongest antioxidant activity and the highest total phenolic content).
Cluster 3 characterized by the lowest antioxidant properties and the lowest phenolic content values. It can be divided in two subclusters 3A and 3B.

	Cluster 1	Cluster 2	Cluster 3A	Cluster 3B	p-value*	p-value**
DPPH (IC_{50} µg/mL)	441.7±127.2	346.3±0.2	1445.6^{ab}± 506.83	853.2^{ab}± 196.64	0.001	<0.01
FRAP (µmol of Fe2+ eq/g sample)	25.9^{a}±0.9	29.6±0.2	$12.1^{a,b}$±4.2	$15^{a,b}$±2.4	0.007	<0.01
ORAC (µmol TE/mg dry extract)	2.5^{a}±0.9	8.2±0.1	1.4^{a}±1.1	1.6^{a}±0.9	0.142	<0.01
Total Phenolic content (µg GA/mg)	64.3^{a}±14.8	113.5±0.2	$47,9^{a,b}$±7.9	$66.1^{a,c}$± 15.7	0.008	<0.01

Table 1. *In vitro* antioxidant activity and total phenolic content of clusters of lichen species obtained by Hierarchical cluster analysis (HCA). Results were expressed as mean ± SD. *The Levene's F Test for Equality of Variances. **One-way ANOVA or the Krustal-Wallis test. Statistical significance ($p<0.05$) is presented in letters superscripts a: *versus* cluster 1; b: *versus* cluster 2; c: *versus* cluster 3A, d: *versus* cluster 3B.

Conclusions

- The **antioxidant profiles** were different between ***Dactylina arctica*** (Richardson) Nyl species and the rest of the studied species of the **Cetrarioid clade**. *Dactylina arctica* showed the highest total phenolic content and the highest antioxidant capacity.
- HCA grouped lichen species into three clusters. Cluster 1 and 2 stand out due to their antioxidant activity and total phenolic content higher than the other species from Cluster 3.

Bibliography

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