POSTER COMMUNICATIONS

OLEANOLIC ACID: a Potential Antidiabetic Compound from Aglianico Grape Pomace

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This PhD research project focused on the wine production chain of the Campania region aims at valorizing the production waste by recovering bioactive natural metabolites through sustainable extraction techniques. The Green Chemistry principles will guide the extraction of metabolites, in order to create value-added products in the frame of the circular economy. Red and white grape varieties from the Campania region (Aglianico, Fiano, Greco, and Falanghina) will be studied to identify healthy metabolites and develop optimized extraction protocols. The bioactivity of the extracts will finally be evaluated. The obtained results will suggest the re-use of the identified metabolites in several sectors including the wine, food, pharmaceutical, and nutraceutical industries.

ACIDO OLEANOLICO: un Potenziale Composto Antidiabetico nelle Vinacce di Aglianico

Il progetto di ricerca intende sostenere la filiera vitivinicola della regione Campania attraverso la valorizzazione degli scarti di produzione mediante tecniche di estrazione sostenibili di metaboliti naturali dotati di interessanti proprietà benefiche per la salute dell’uomo. I principi della *Green Chemistry* guideranno l'estrazione dei metaboliti bioattivi e il loro riutilizzo, in modo da creare prodotti dal valore aggiunto nell’ottica dell’economia circolare. Le varietà di uva a bacca rossa e bianca più diffuse in Campania (Aglianico, Fiano, Greco e Falanghina) saranno studiate per identificare i metaboliti di interesse e sviluppare efficienti protocolli di estrazione. La bioattività degli estratti sarà poi valutata. I risultati di tale ricerca suggeriranno un possibile riutilizzo dei metaboliti identificati in campo enologico, alimentare, farmaceutico e nutraceutico.

**Key words**: Grape pomace valorization, Natural triterpenoids, Red grapes, glucose uptake and mitochondrial activity, C2C12 cells

1. Introduction

Polyphenols have been associated with various bioactivities such as antioxidant, vasodilatory, antithrombotic, cardioprotective, anti-inflammatory, anticancer and antimicrobial ones (Kato-Schwartz et al., 2020; Zhu et al., 2019; Peixoto et al., 2018). Recent studies have shown that grape pomace polyphenols and their polysaccharide conjugates also exhibit antidiabetic effects (Campos et al., 2021). However, it is now evident that the remarkable potential of health-related benefits demonstrated by grape pomace extracts, cannot be attributed solely to the presence of polyphenols. Thus, besides polyphenols, additional metabolites, so far undetected, may be likely occurring. In order to verify such hypothesis, an in-depth chemical analysis of Aglianico (*Vitis vinifera*) red grape pomace was conducted by chromatographic, 1H-NMR and LC-MS/MS techniques.

2. Materials and Methods

In November of 2021, Aglianico grape pomace was collected from the Taurasi area in Italy. A 2-Kg sample was lyophilized to obtain 418 gr of dry material. The dry pomace was extracted twice overnight and under stirring with H2O:EtOH 2:8 (v/v) at room temperature. The resulting hydroethanolic extract was partitioned against (A) *n*-hexane, (B) Ethyl Acetate (EtOAc), and (C) *n*-butanol. The dry weights of the *n*-hexane, EtOAc, and *n*-butanol extracts were 0.296 gr, 4.95 gr, and 2.496 gr, respectively. The EtOAc extract (B) was further separated through a 80-gr silica Combiflash column (3.0 mL/min flow) connected to a Teledyne Isco CombiFlash Rf flash chromatography system, eluted with the following gradient elution: dichloromethane:EtOAc 1:1 (200 mL; fraction 1), 100% EtOAc (200 mL; fraction 2), EtOAc:MeOH 9:1 (200 mL; fraction 3), EtOAc:MeOH 7:3 (400 mL; fraction 4). Finally, the extracted compounds in each fraction were identified by 1H-NMR, LC-MS/MS, and HPLC. In order to evaluate their biological activities, measurement of mitochondrial activity and glucose uptake were conducted. The mitochondrial activity was measured by using the mitochondrial selective probe MitoTracker CMXRos. The ability of crude extract and fractions to modulate glucose uptake was measured by monitoring the uptake of NBDG, a fluorescent derivative of deoxyglucose covalently bound to the fluorescent chemical nitro blue tetrazolium.

3. Results and Discussion

## 3.1 Chemical analysis of grape pomace

The most interesting fraction obtained from the Aglianico grape pomace was fraction B. An NMR-based investigation allowed a preliminary identification of triterpenoids, flavan-3-ols, anthocyanins, and polymeric pigments. The occurrence of relatively high quantity of a triterpenoid compound turned out to be the most remarkable datum. Thus, it was purified through chromatographic separation and identified as oleanolic acid by means of NMR spectroscopy and High Resolution ESIMS analysis (Fig.1). The concentration of oleanolic acid in fresh Aglianico pomaces was estimated to hover around 0.45 mg/g.



**Figure 1** 1H NMR spectrum (CD3OD) of fraction 1 containing Oleanolic Acid as major component

3.2 Effect of Oleanoic Acid Enriched Fractions on Mitochondrial and Glucose Uptake Activity of In-Vitro Cultured C2C12 Myoblast

The relatively high amount of oleanolic acid recovered from grape pomace provided the opportunity to further investigate its anti-diabetic activity. To investigate the pro-metabolic effect of oleanolic acid, in collaboration with the Department of Pharmacy, its ability to promote mitochondrial activity in C2C12 myoblasts was tested. Oleanolic acid was able to induce mitochondrial activity at the dosage of 1.8 μg/mL, with a dose-dependent potency. Also, its ability to promote glucose uptake via membrane glucose transporters (GLUT) was investigated. Compared to vehicle (DMSO 0.1%), cells treated with oleanolic acid showed an increase in glucose uptake, thus indicating a modulation of glucose uptake through stimulation.

For the first time, an in-depth chemical analysis of Aglianico grape pomace was conducted by means of different chromatographic (column chromatography, UV–Vis HPLC) spectrometric (HRMS, LC-MS/MS) and spectroscopic (NMR) techniques. This investigation disclosed the presence of a number of bioactive compounds, mainly polyphenols. The most relevant outcome of the reported analysis was the isolation of a remarkable amount of oleanolic acid, in the order of 0.45 mg/g of pomace (fresh weight). A promising antidiabetic activity of oleanolic acid was demonstrated. This allowed us to verify our initial hypothesis that additional major bioactive metabolites along with typical grape polyphenols are responsible for the multiple reported bioactivities shown by grape pomace extracts.

4. References

Campos, F., Peixoto, A. F., Fernandes, P. A., Coimbra, M. A., Mateus, N., de Freitas, V., ... Fernandes, A. (2021). The antidiabetic effect of grape pomace polysaccharide-polyphenol complexes. Nutrients, **13**(12), 4495.

Kato-Schwartz, C. G., Corrˆea, R. C. G., de Souza Lima, D., de S ́a-Nakanishi, A. B., de Almeida Gonçalves, G., Seixas, F. A. V., ... Bracht, A. (2020). Potential anti-diabetic properties of Merlot grape pomace extract: An in vitro, in silico and in vivo study of α-amylase and α-glucosidase inhibition. Food Research International, **137**, Article 109462.

Peixoto, C. M., Dias, M. I., Alves, M. J., Calhelha, R. C., Barros, L., Pinho, S. P., & Ferreira, I. C. F. R. (2018). Grape pomace as a source of phenolic compounds and diverse bioactive properties. Food Chemistry, **253**, 132–138.

Zhu, M., Huang, Y., Wang, Y., Shi, T., Zhang, L., Chen, Y., & Xie, M. (2019). Comparison of (poly) phenolic compounds and antioxidant properties of pomace extracts from kiwi and grape juice. Food Chemistry, **271**, 425–432.