Finanziato dall'Unione europea NextGenerationEU
Ministero dell'Università e della Ricerca
Italiadomani PIANO NAZIONALE DI RIPRESA E RESILIENZA
agritech National Research Center for Technology in Agriculture

STILBENOID PROFILING OF GRAPEVINE CANE EXTRACTS AND EVALUATION OF THEIR BIOACTIVITY ON PLANTS

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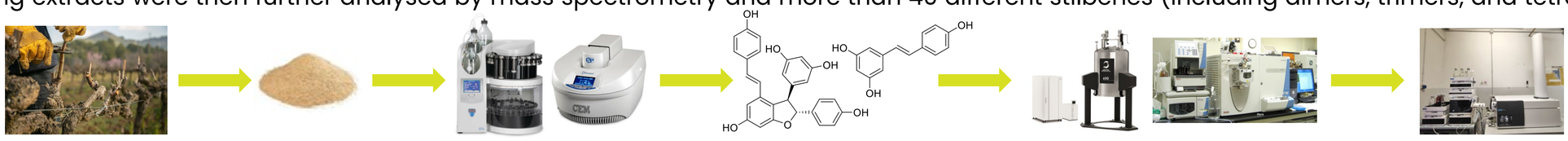
SPOKE, WP E TASK DI APPARTENENZA

8.1.1 & 8.1.2

INTRODUCTION

Every year, more than a billion of tons of agri-food wastes is produced; these residues could be a priceless source of income since they still contain several high value compounds, such as polyphenols, well known for their important biological activities (e.g., antioxidant, antimicrobial).
Grapevine canes are the major by-product of grapevine production, estimated to be around 2-5 tons/hectare each year. The extracts derived from these residues have shown to contain several bioactives: stilbenoids, phenolic acids, flavonoids.[1] The aim of our research is the optimization of convenient extractive methodologies for the production of polyphenol enriched extracts from grape pruning canes to be used as crop protective agents and biostimulants. For this purpose, different techniques (e.g., microwave-assisted extraction (MAE), accelerated solvent extraction (ASE) and enzyme-assisted extraction (EAE)) and experimental parameters have been evaluated and combined.[2] The extracts were analysed by NMR spectroscopy to obtain a fingerprint of the crude, and the mass recovery was calculated. The most promising extracts were then further analysed by mass spectrometry and more than 40 different stilbenes (including dimers, trimers, and tetramers) were identified.



MATERIAL AND METHODS

Grape pruning canes were collected from a winery in Cuneo during 2018 spring. They have been dried and stored at 4 °C. Before extraction the canes were firstly chopped and then minced to a coarse powder, that was stored in closed vials at -20 °C. Reagents and solvents were obtained from commercial suppliers and used without further purification. NMR spectra were recorded on an Avance NEO 400 (400 MHz) spectrometer using the residual signal of the deuterated solvent as internal standard. 1H chemical shifts (δ) are expressed in ppm . For the MAE, CEM discover was utilized. For the ASE, Dionex ASE 350 was utilized.

The stilbenoid composition in cane extracts was analyzed by LC-ESI-MS/MS. Extracts were resuspended in 75% methanol with 0.1% formic acid (FA, v:v), diluted, and analyzed using an Agilent 1290 Series pump coupled with a Jet Stream ESI source on a 6546 LC/Q-TOF mass spectrometer. LC runs were performed on an InfinityLab Poroshell 120 SB-C18 column (2.1 x 100 mm, 1.9 μm) under acidic conditions, applying a 65-minute non-linear gradient of acidified acetonitrile (0.1% FA, v:v). Mass spectra were searched against a custom database containing the main stilbenoids, and relative quantification was done on MS spectra by extracting the EIC for $[M-H]^-$ ions. Targeted MS/MS analyses with 20 V and 25 V collision energies refined compound identification.

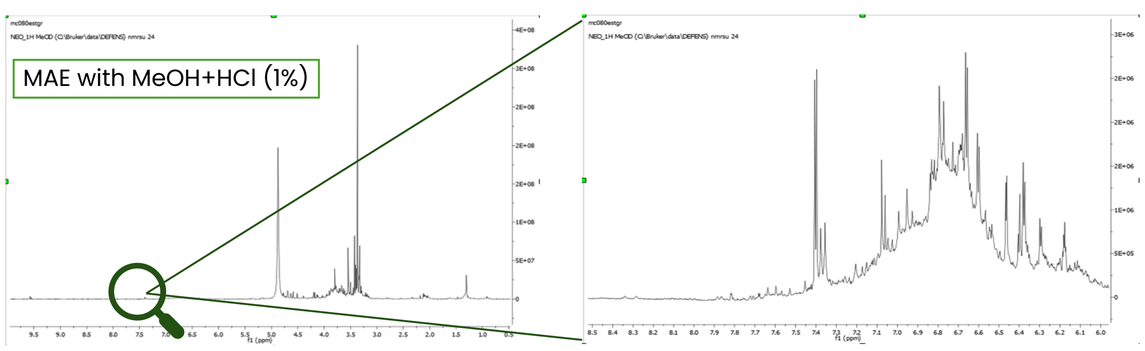
RESULTS

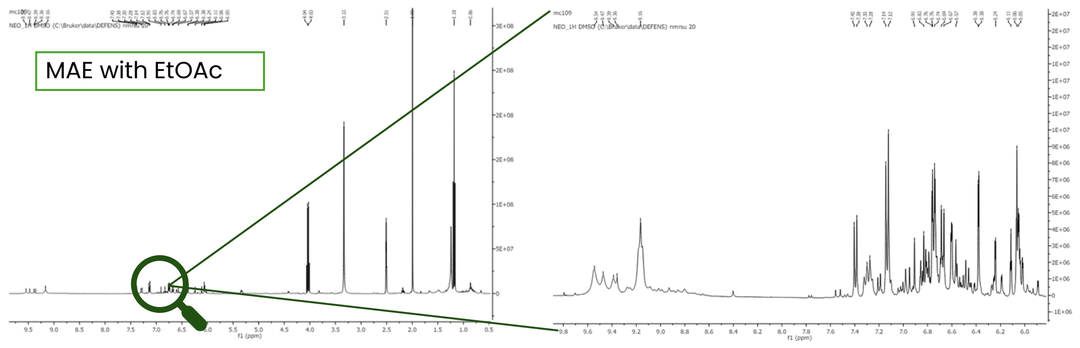
A) Extraction optimization

With the aim of obtaining extracts enriched in stilbenoids from grape pruning canes, different extraction procedures (EAE, MAE, ASE) and experimental parameters (time, temperature, solvents) were evaluated. Moreover, an enzymatic mixture of cellulase, cellobiosidase and β-glucosidase was utilized to pretreat the biomass to increase the extractive yields. NMR spectroscopy was employed to produce a fingerprint of the crudes and together with the recovered mass yield allowed to compared the best extractive methods. Here reported in Table 1 the comparison between the four best extractive protocols.

Entry	Extraction technique	Matrix weight	Solvent	Solv. : Matrix ratio	Temperature	Time	Extractive yields[b]
1	MAE	1 g	MeOH+1%HCl	10 : 1	100 °C	10 min	23,4 %
2	MAE	1 g	EtOAc	10 : 1	100 °C	20 min	2,7 %
3	Enzyme + MAE	1 g	Phosphate buffer (pH=5.5) + EtOAc	2,5 % [a] + 10 : 1	40 °C + 100 °C	24 h + 10 min	0,7 %
4	ASE	18,5 g	EtOAc	2,8 : 1	100 °C	2 x 20 min	2,2 %

Table 1. a) volume of enzyme stock solution / weight of dried matrix; b) calculated as (recovered mass weight / dried matrix weight) * 100





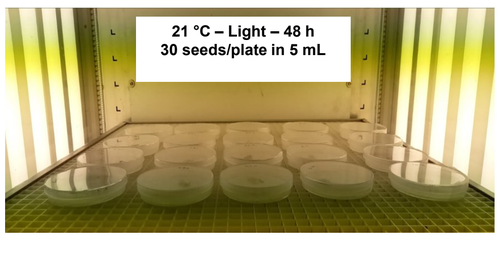
B)Preliminary mass spectrometry analysis

For the spectrometric analysis we selected entries **1**, **2** and **4** from Table 1. Entry **1** was sequentially portioned in three fractions: a) the first one soluble in EtOAc, b) the second one soluble in acetone, c) and the last one soluble in MeOH; the first two were analysed. Entries **2** and **4** were analysed as whole.
The analysis showed the presence of more than 40 stilbenoids in the four extracts. The composition varied based on the original extraction methodologies and the single fraction analysed (ANOVA test, $p \leq 0.05$, n=3). It is noteworthy, that the mass analysis highlighted the presence of different oligomers, ranging from dimers (*i.e.* ε-viniferin) up to tetrameric structures.

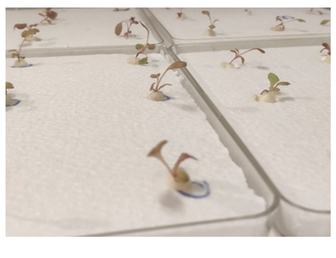
C) Plant growth trials

The potential impact of the aforementioned extracts on plant growth is currently being assessed through the implementation of germination and growth trials using lettuce (*Lactuca sativa* L.), a model species widely used to test phytotoxicity and biostimulant activities. The evaluation of the effects of increasing concentrations of the extracts at different growth stages, such as seed germination, seedling establishment and vegetative development, is ongoing.

Evaluation of the effects on germination



Evaluation of the effects on seedling's growth



Evaluation of the effects on plant's development

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This study was carried out within the Agritech National Research Center and received funding from the European Union Next-GenerationEU (PIANO NAZIONALE DI RIPRESA E RESILIENZA (PNRR) – MISSIONE 4 COMPONENTE 2, INVESTIMENTO 1.4 – D.D. 1032 17/06/2022, CN00000022). This manuscript reflects only the authors' views and opinions, neither the European Union nor the European Commission can be considered responsible for them.