

## Characterization of ‘Cabernet Sauvignon’ pomace extracts and evaluation of antifungal potential of *Alternaria* sp. and *Fusarium* sp.

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### Abstract

This study was conducted to capitalize on the waste produced by the vinification process which proved to be important sources of bioactive compounds with significant antifungal properties. ‘Cabernet Sauvignon’ grape pomace extracts were characterized in terms of total polyphenol content, antioxidant potential, but also evaluated in terms of antifungal effect against phytopathogenic fungi (*Fusarium* sp. and *Alternaria* sp.). The isolates used in this study were grown on three potato-dextrose-agar culture media, Czapek Dox and Malt-Agar. The highest amount of polyphenols was determined from the extract obtained by the microwave-assisted method (42.76 mg/g GAE), followed by the extract obtained by maceration (30.37 mg/g GAE). The lowest amount was obtained by the ultrasound-assisted method (15.06 mg/g GAE). However, the highest antioxidant activity was determined in the macerated extract, TEAC = 0.0523. The results of *in vitro* tests clearly indicated a high inhibitory percentage on the mycelium growth rate and, respectively, a significant decrease in spore germination power in *Fusarium* sp. (91.56%). However, for the pathogen *Alternaria* sp. further studies are needed to correctly validate the percentage of inhibition, as alcohol has been shown to have a negative effect on it.

**Keywords:** *Alternaria* sp.; antifungal activity; ‘Cabernet Sauvignon’ pomace; extraction methods; *Fusarium* sp.; polyphenols

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## Introduction

Genus *Alternaria* Nees ex Fr. (syn. *Macrosporium* Fr., *Rhopalidium* Mont.) is rich in species, being one of the most common ubiquitous fungal groups, comprising species of saprophytic, endophytic or parasitic nature in all cultures. The small spores of this pathogen are distributed everywhere, contributing to the deterioration of food quality, which leads to lower nutritional profile by producing toxic metabolites and thus the economic value of food. As phytopathogens, they can cause serious problems in agriculture by reducing crop yields, thus causing considerable economic losses to farmers and food processing industries (Ostry, 2008; Garg and Singh, 2016; Meena *et al.*, 2016).

Research on the pathogen *Alternaria solani* was also performed by Mihaescu *et al.* (2021), which demonstrated the inhibitory effects of *Allium cepa* extract, allicin being the compound responsible for inhibiting spore germination as well as the growth of mycelial hyphae (Ledezma, 2006).

*Fusarium* species are pathogenic, toxin-producing fungi that are worldwide, which can cause health problems associated with cell toxicity, cancer, and adverse effects on the growth and development of animals and humans (Edrington *et al.*, 2001; Hussein and Brasel, 2001). It is well known that various pathogens belonging to the genus *Fusarium* cause diseases in agricultural crops such as corn, wheat, rice, potatoes, tomatoes, beans, sorghum, bananas, sugar cane, mango and other important crops. economic (Summerell *et al.*, 2011).

Grapes are a rich source of many classes of natural compounds, namely carbohydrates, vitamins, minerals, organic acids, but the most important being polyphenols (Vauzour *et al.*, 2010). Polyphenols are secondary metabolites that are found mainly in higher plants, their importance being to defend against the aggression of pathogens of plant or animal origin, or to adapt to various conditions of abiotic stress (temperature, ultraviolet radiation, etc.).

The antimicrobial activity of polyphenols has been extensively studied in the last decade, which makes it possible to replace synthetic products with natural alternatives, which are not toxic to humans and the environment. Among the polyphenols, flavan-3-ols, flavonols and tannins received the most attention due to their broad spectrum and higher antimicrobial activity compared to other polyphenols, as well as the fact that most are able to suppress a number of factors of microbial virulence (such as inhibition of biofilm formation, reduction of host ligand adhesion and neutralization of bacterial toxins) showing synergy with antibiotics (Daglia, 2012). The antibacterial properties of phenolic substances may also be due to iron deficiency or hydrogen binding to vital proteins, such as microbial enzymes (Kabir *et al.*, 2015; Sanhueza *et al.*, 2014). However, there are few studies on the antifungal activity of grape pomace with agricultural applications.

Research by Leonora Mendoza *et al.* (2013) has shown a high efficacy of extracts, high in polyphenols, obtained from grape pomace, on the pathogen *Botrytis cinerea*, which confirms the antifungal activity of polyphenols. There are few studies in the literature on the antifungal effect of grape pomace extract on two pathogens of high economic importance in agriculture, namely *Alternaria* sp. and *Fusarium* sp. Therefore, the purpose of this study was to recover plant waste obtained from the vinification process in order to recover the optimal amount of phenolic compounds needed to suppress the development of mycelial hyphae of selected phytopathogens (*Alternaria* sp. and *Fusarium* sp.).

## Materials and Methods

### *Biological material*

After performing their pathogenicity test their culture was maintained on potato-dextrose-agar medium, Czapek Dox and malt-agar,  $27 \pm 10$  °C (Mardare *et al.*, 2015). The sterilized tissue was dried on sterile filter paper on a clean bench, plated on potato-dextrose-agar (PDA; potatoes 20 g, glucose 20 g, agar 20 g) and

incubated at 25 °C. Mycelium growth was observed and transferred to a new plate containing sterilized potato-dextrose-agar. Pure cultures obtained through hyphal tip method and single spore subculture techniques.

The efficacy of extract was tested against *Alternaria* and *Fusarium* for radial growth inhibition on the potato-dextrose-agar medium using poisoned food technique under *in vitro* condition. Suitable check was maintained without addition of fungicide. Mycelial disc of 5 mm taken from the periphery of 14 days old colony was placed in the center of Petri incubated at  $27 \pm 10$  °C for 14 days and three replications were maintained for each treatment. The diameter of the colony was measured in two directions and an average value was recorded. Per cent inhibition of mycelial growth of the fungus was calculated by using the formula by Vincent (1947).

$$I = (C-T)/C \times 100$$

Where:

I = Per cent inhibition

C = Radial growth in control

T = Radial growth in treatment (fungicide)

One of the current drawbacks of using extract techniques is the type of solvent used, which is most often methanol or other such substances that are toxic to humans and the environment. Although attempts are made to remove solvents from these preparations by various techniques, there is no guarantee that they will be completely eliminated. Under these conditions, it is necessary to use solvents or excipients that can be administered without risk of intoxication or contamination.

Thus, both the classic maceration process and the green extraction techniques were used in the process of obtaining 'Cabernet Sauvignon' plant extract, namely ultrasonic-assisted extraction and microwave-assisted extraction. A binary mixture of ethyl alcohol: distilled water in a ratio of 60: 40 was used as solvent. Before being extracted, the plant material was processed primarily by: drying for 7 days at a temperature of 50 °C and grinding for 4 minutes at 6000 rpm in pulses. The relative humidity determined by the thermobalance was 4.27%.

#### *Maceration (MC)*

The following experimental design was used to obtain the macerate: 20 g of plant material were immersed in 200 ml of solvent (distilled water: pharmaceutical ethyl alcohol) for a total extraction time of 4 days. For a better dispersion of the plant particles in the solvent, the extract was subjected to magnetic stirring at a speed of 500 rpm.

#### *Ultrasound Assisted Extraction (UAE)*

Ultrasound-assisted extraction (UAE) stands out as a sustainable alternative that requires a moderate investment of solvents and energy. In addition, it is easy to handle, safe, economical and reproducible, due to the fact that this technology takes place under conditions of atmospheric pressure and ambient temperature (Soria *et al.*, 2010; Vieira *et al.*, 2013). 20 g of plant material immersed in 200 ml of solvent were sonicated for 10 minutes at an amplitude of 72  $\mu$ m.

#### *Microwave Assisted Extraction (MAE)*

A microwave system, model NEOS-GR (Microwave Extraction System from Milestone Inc) was used for this type of extraction, the extract being obtained at the following experimental parameters: total power delivered to the environment 220 W, for 10 minutes, temperature 80 °C.

The extracts obtained from the three techniques were centrifuged at a speed of 6000 rpm for 5 minutes, then filtered and kept in brown glass vials at + 4 °C until further analysis.

#### *Determination of total polyphenol content (TPC)*

The total polyphenol content (TPC) was determined using the Folin-Ciocalteu spectrophotometric method (ISO 14502-1). The present method involves the reduction of the Folin-Ciocalteu reagent by the phenolic compounds in the tested extract, which leads to the formation of a blue complex. From the experimental variants different volumes were taken which were diluted, following that from each dilution a volume of 200  $\mu$ L was taken over which 1 mL of Folin-Ciocalteu reagent diluted 10 times was added, and after about 5-6 minutes it was added 800  $\mu$ L of 7.5%  $\text{Na}_2\text{CO}_3$  solution. The samples were kept in the dark at room temperature for 60 minutes and then analyzed spectrophotometrically at a wavelength of 765 nm.

The results obtained from the analysis were expressed as mg gallic acid/g plant (mg AG/g) based on the calibration curve constructed for different concentrations of the standard, respectively for 7 points of the concentrations from 10 to 70  $\mu$ g/mL gallic acid.

#### *Determination of antioxidant capacity (DPPH)*

The antioxidant capacity of the extracts was determined by the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging method. The analysis was performed according to Shimamura *et al.* (2014), with some modifications.

The antioxidant reaction of the control (in this case Trolox) and of the experimental variants was highlighted by adding in the test tube the DPPH solutions and the standard/extract in a volumetric ratio of 1:7. The wavelength at which the absorbance of each sample was read was 517 nm, after 5 minutes of stabilization under UV influence, after which the calibration curve for Trolox was plotted. The calibration curve for Trolox was plotted for 6 different reagent concentrations, and the antioxidant capacity of each sample was expressed as Trolox equivalent antioxidant capacity (TEAC), using the formula:

$$\text{TEAC} = \text{IC}_{50} \text{ Trolox} / \text{IC}_{50} \text{ Extract}$$

Where:

IC<sub>50</sub> Trolox = half the maximum concentration of Trolox inhibitors

IC<sub>50</sub> Extract = half of the maximum inhibitory concentration of Extract

#### *Data analysis*

The experimental plan for all variants was performed completely randomized. The data were processed using the one-way ANOVA method, followed by Šidák's multiple comparison test. Processed data were expressed as mean  $\pm$  standard error (SE). This analysis was performed using GraphPad Prism 9.0.0.0 software.

## **Results and Discussion**

Grape pomace is an economically valuable plant material because it is obtained at low cost, and is also a notable source of bioactive compounds, the most important being polyphenols.

#### *Polyphenol analysis*

The total polyphenol content varied depending on the extraction method used. In the case of the extract obtained by the microwave-assisted technique, the highest concentration of polyphenones was recorded, namely  $42.76 \pm 0.76$  mg GAE/g plant, followed by the extract obtained by maceration where a concentration of  $30.37 \pm 0.49$  mg GAE/g plant was obtained. The extract that had a low polyphenol content was the one obtained by the ultrasound-assisted method ( $15.06 \pm 0.18$  mg GAE/g plant).

The total polyphenol concentrations obtained from the analyzed samples are shown in Table 1.

**Table 1.** Variation of the total polyphenol content according to the extraction technique used

Sample code	Ratio EtOH: distilled water (v/v)	TPC (mg/g GAE)
Tesc_C_s_MC	60: 40	30.37 ± 0.49
Tesc_C_s_UAE	60: 40	15.06 ± 0.18
Tesc_C_s_MAE	60: 40	42.76 ± 0.76

Obreque Slier *et al.* (2010) used 'Carménère' and 'Cabernet Sauvignon' grape seeds, the total polyphenolic content of ethanol extracts (1: 9 v/v ethanol/water) obtained from them ranging from 21.8 to 16.6 mg GAE/g and 20.4 to 17.5 mg GAE/g, respectively, depending on the time of harvest. Also, Carmine Negro *et al.* (2019) observed a high value of polyphenols content, namely 38.1 mg GAE/g, using as extraction solvent methanol and water, the extract being obtained from 'Cabernet Sauvignon' variety.

By ultrasound-assisted extraction Goula *et al.* (2016) reported a polyphenol value of 28 mg GAE/g. The extraction time used was 30 minutes, the solvent used to be water: ethanol in a ratio of 60:30 and a temperature of 60 °C.

The polyphenol concentrations identified in this paper were close to the values reported in the specialized studies for 'Cabernet Sauvignon' and 'Merlot' or other red grape varieties (Casazza *et al.*, 2010; Deng *et al.*, 2011; Medouni Adrarb *et al.*, 2015; Yilmaz and Toledo, 2006).

#### *Testing of antioxidant activity by DPPH method*

The stable free radical DPPH was used to evaluate the antioxidant capacity of the extracts. Thus, in Table 2 it can be seen that the best antioxidant capacity was identified in the extract obtained by maceration, the next being in the extract obtained by microwave-assisted technique, and the lowest value was recorded in the extract obtained by ultrasound-assisted technique.

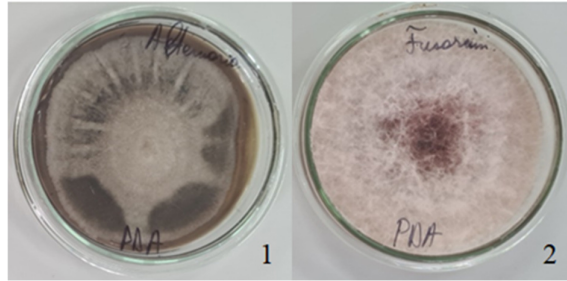
**Table 2.** Variation of the antioxidant potential of the pomace extract according to the extraction technique used

Sample code	IC50 (µg/mL)	TEAC
Tesc_C_S_MC	704.96	0.0523 ± 0.0023
Tesc_C_S_UAE	3122.70	0.0118 ± 0.0018
Tesc_C_S_MAE	743.53	0.0496 ± 0.0066

The results of our study are correlated with those obtained by Rockenbach *et al.* (2011) who showed that the antioxidant activity of extracts from skins and grape seeds ranged from 2.032 µmol to Trolox equivalent/ 100 g for skins and 8.281 Trolox equivalent/ 100 g for seeds. Their experimental study shows a much higher antioxidant activity of the extract obtained from the skin compared to that obtained from the seeds.

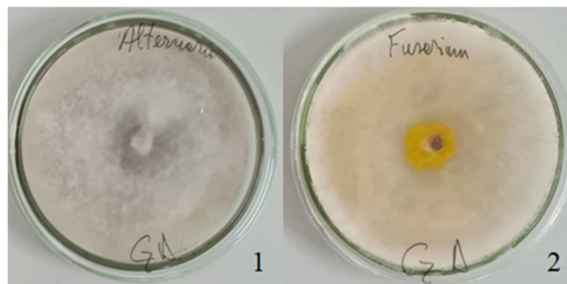
#### *The pathogen Alternaria and Fusarium in vitro on culture medium*

The two phytopathogens used in this study (*Alternaria* sp. and *Fusarium* sp.) were isolated from the leaves of Rosa sp. respectively beet leaves, on PDA medium (potato-dextrose-agar). Mycelial dynamics were rapid, with a radial development from the inoculation point. The diameter of the colonies at *Alternaria* sp. was on average 1.6/ 1.6 cm at 3 days and 7/ 7 cm at 10 days, while phytopathogen *Fusarium* sp. showed a much faster growth, with a diameter of the colonies averaging 3/ 3.1 cm at 3 days and 7/ 7 cm at 5 days after inoculation (Figure 1).



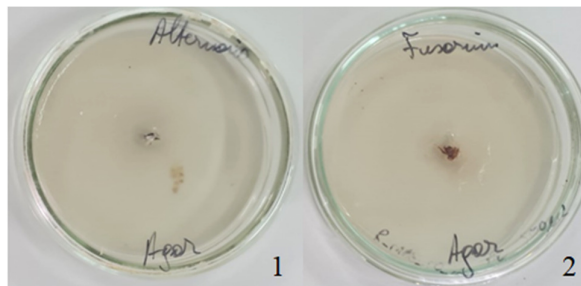
**Figure 1.** *Alternaria* sp. (1) & *Fusarium* sp. (2) on PDA plate

On the Czapek Dox medium (Figure 2) the mycelial hyphae at *Alternaria* sp. they had a slow growth, the diameter of the colonies being 1/ 1 cm at 3 days and 6.5/ 6.8 cm at 14 days. Also, the mycelium in *Fusarium* sp. showed a slow growth on this culture medium, the colonies developing from the 5th day of observation, their diameter being 0.8/1 cm and 6.7/ 6.9 cm at 14 days.



**Figure 2.** *Alternaria* sp. (1) & *Fusarium* sp. (2) on Czapek Dox plate

Phytopathogenic fungi were also grown on malt agar (Figure 3). During the 14 days of observation, the rate of growth of mycelial hyphae on these culture media was zero in the case of both fungi, which indicates that an environment poor in carbohydrates is unsuitable for the development of these pathogens.

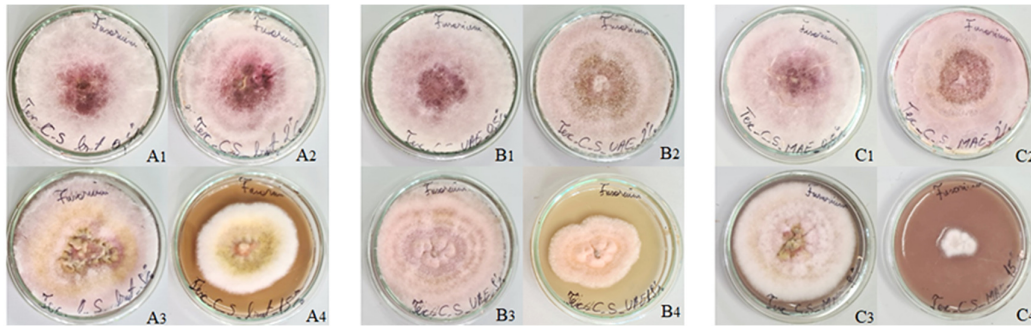


**Figure 3.** *Alternaria* sp. (1) & *Fusarium* sp. (2) on malt-agar plate

#### *In vitro* evaluation of bio-fungicides

In the present study, in order to establish a notable inhibitory percentage on pathogen dynamics, four concentrations of grape pomace were used: 0.5%; 2%; 9% and 15% respectively. Compared to synthetic products, in the case of natural extracts, the fungicidal or fungistatic effect on the phytopathogen is directly proportional to the concentration used.

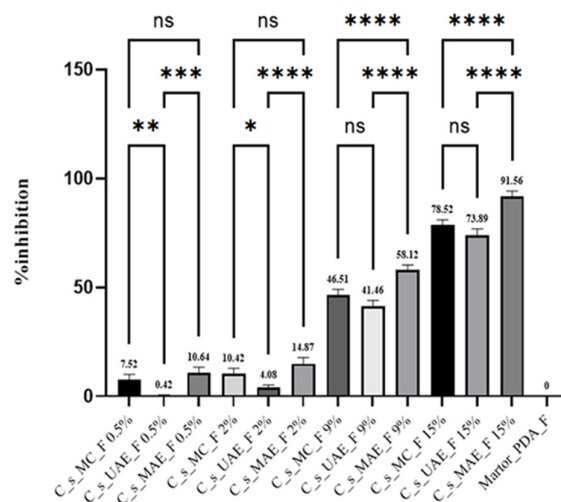
Figure 4 shows the action of the grape pomace extracts obtained by the three extraction techniques on the mycelial hyphae in the fungus *Fusarium* sp.



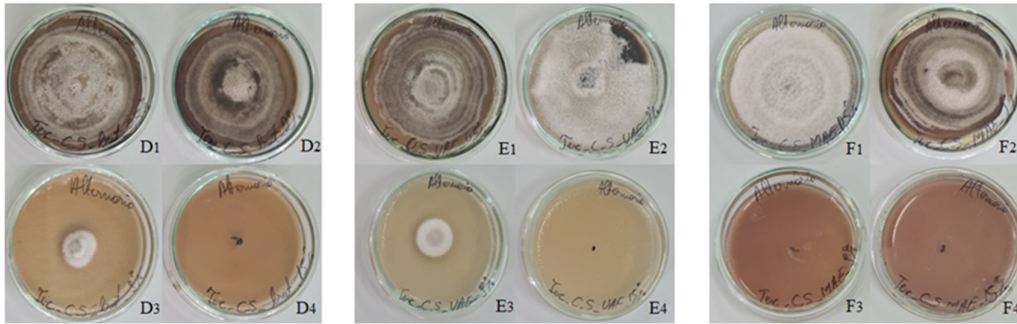
**Figure 4.** Growth of *Fusarium* sp. isolate on PDA amended with ethyl extract of ‘Cabernet Sauvignon’ MC (A1=0.5%; A2=2%; A3=9%; A4=15%), UAE (B1=0.5%; B2=2%; B3=9%; B4=15%) and MAE (C1=0.5%; C2=2%; C3=9%; C4=15%)

Statistical analysis of the results showed a high percentage of inhibition of mycelial growth and spore germination in the extract obtained by microwave-assisted extraction (MAE) on the phytopathogen *Fusarium* sp. compared to the control variants, the differences being strongly significant ( $p < 0.0001$ ) (Figure 5). Consistent with previous research, it has been reported that the presence of phenolic compounds, including simple molecules (phenolic acids) and complex structures (flavanols, flavonols and anthocyanins) have been directly involved in plant defense mechanisms against pathogenic fungi (Ahmed *et al.*, 2017). Similarly, Luo *et al.* (2016) confirmed that the presence of phenolic compounds could increase cell permeability, thus reducing fungal growth. Other research has shown that the mechanisms of inhibition of phytopathogens vary depending on the fungus tested, but also depending on the phenolic compounds present in the tested extracts. Thus, the phenolic extract obtained from a mixture of varieties such as ‘Cabernet Sauvignon’, ‘Carmènere’ and ‘Syrah’, subsequently fractionated with hexane, chloroform or ethyl acetate, tested against the phytopathogenic fungus *Botrytis cinerea*, has a strong fungicidal effect (Joaquín-Ramos *et al.*, 2020).

Figure 6 shows the action of grape pomace extracts on the fungus *Alternaria* sp. In all variants of 0.5%; 2%; 9% and 15%, respectively, the extracts show a strong fungistatic or even fungicidal effect on this pathogen, the inhibition rate being directly proportional to the concentration.



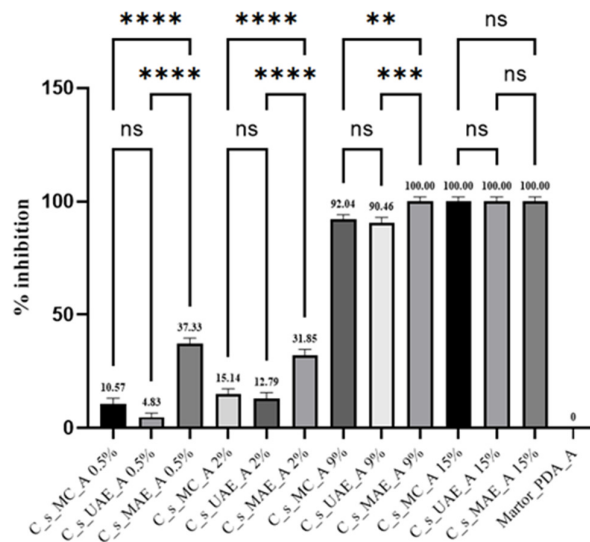
**Figure 5.** Effect of concentrations of the three types of extracts on the pathogenic fungus *Fusarium* sp. The data are expressed as the mean  $\pm$  SD values of three independent experiments performed in triplicate, and the values of  $p$  were calculated by the one-way ANOVA method followed by Šídák's multiple comparison test. \*\*\*\*  $p < 0.0001$ , \*\*\*  $p = 0.0001$ , \*\*  $p = 0.0085$ , \*  $p = 0.0242$ , <sup>ns</sup>  $p = 0.1266$



**Figure 6.** Growth of *Alternaria* sp. isolate on PDA amended with ethyl extract of 'Cabernet Sauvignon' MC (D1=0.5%; D2=2%; D3=9%; D4=15%), UAE (E1=0.5%; E2=2%; E3=9%; E4=15%) and MAE (F1=0.5%; F2=2%; F3=9%; F4=15%)

The statistical analysis presented in Figure 7 shows that the extract obtained from 'Cabernet Sauvignon' extract shows an inhibitory effect on mycelial dynamics in *Alternaria* sp. much stronger than in the case of the pathogen *Fusarium* sp., an aspect that cannot yet be scientifically explained.

Similar studies have been performed by Ranjitha *et al.* (2014), who used grape seed extract to determine the antifungal potential. From the results obtained by them, it was found that the extract obtained from this type of plant material inhibited the mycelial growth of the species *Colletotrichum capsici*, drastically reducing its development, by 68% compared to the control variant.



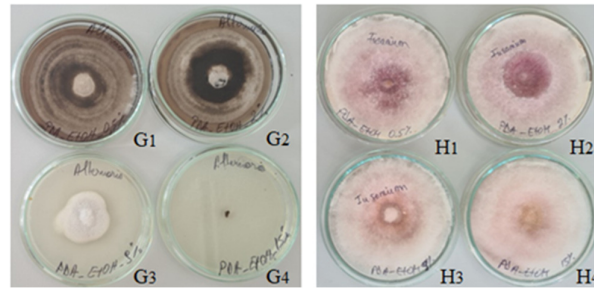
**Figure 7.** Effect of concentrations of the three types of extracts on the pathogenic fungus *Alternaria* sp.

The data are expressed as the mean  $\pm$  SD values of three independent experiments performed in triplicate, and the values of p were calculated by the one-way ANOVA method followed by Šídák's multiple comparison test. \*\*\*\* p < 0.0001, \*\*\* p = 0.0004, \*\* p = 0.0031, ns p = 0.9999

For the correct interpretation of the *in vitro* results of the antifungal potential of the extracts, the effect of ethyl alcohol on the development of mycelial hyphae was also studied, representing one of the control variants (Figure 8).

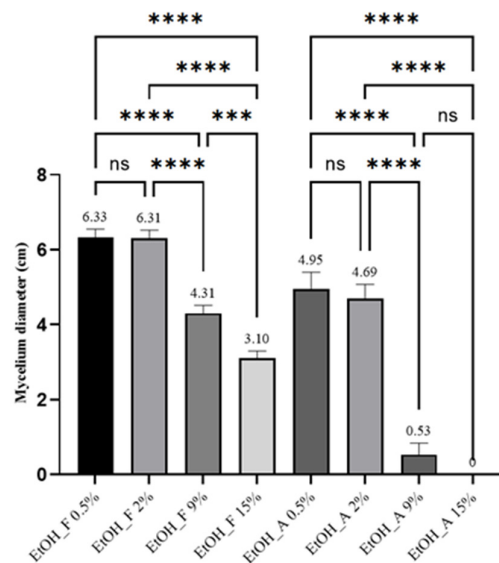
Thus, we found that ethyl alcohol, even in a concentration of 15%, used against the pathogenic fungus *Fusarium* sp. has not been shown to have an inhibitory effect on the development of mycelium, which

demonstrates that the antifungal activity was due to the bioactive compounds present in the grape pomace extract.



**Figure 8.** Growth of *Alternaria* sp. (G1=0.5%; G2=2%; G3=9%; G4=15%) & *Fusarium* sp. (H1=0.5%; H2=2%; H3=9%; H4=15%) isolate on PDA amended with ethyl alcohol

On the pathogenic fungus *Alternaria* sp. ethyl alcohol has been shown to have a strong inhibitory effect at the maximum concentration used in our experiments. At a concentration of 9%, small differences can be observed in its mycelial development (Figure 9), but at a lower concentration no significant differences were noticed compared to the experimental variants in which the extract obtained from grape pomace was used.



**Figure 9.** Dynamics of mycelial growth on PDA medium with ethyl alcohol (F = *Fusarium* sp., A = *Alternaria* sp.)

The data are expressed as the mean  $\pm$  SD values of three independent experiments performed in triplicate, and the values of p were calculated by the one-way ANOVA method followed by Šidák's multiple comparison test. \*\*\*\* p < 0.0001, \*\*\* p = 0.0009, ns p = 0.9999

Thus, at the 15% concentration used in our experiment, ethyl alcohol completely inhibits the growth of the mycelium of the pathogenic fungus *Alternaria* sp. This demonstrates that the inhibitory effect was produced by the presence of ethyl alcohol and less by the bioactive compounds in the tescovine extract.

## Conclusions

For an evaluation of the overall effectiveness of a plant protection product, several aspects are taken into account, such as: the duration of its biological activity (persistence of the action), compatibility with different protection strategies or cultural practices, ease of application, etc. The grape variety analyzed presented an important source of bioactive compounds that could be applicable in various fields, including agriculture. It is important to note that the techniques for obtaining the extracts used in this study showed a significant influence on the total concentration of polyphenols. Thus, for the recovery of a significant amount of phenolic compounds from 'Cabernet Sauvignon' pomace, it is recommended to use the microwave-assisted method (MAE). Grape pomace extract contains ethyl alcohol, which is why *in vitro* research has been directed in two directions: first, the influence of ethyl alcohol on the growth and development of *Fusarium* sp. and *Alternaria* sp. pathogens; secondly, the influence of grape pomace extract in different concentrations (0.5%; 2%; 9% and 15% respectively) on the dynamics of the two pathogens. Ethyl alcohol was an inhibitory factor on the development of *Alternaria* colonies compared to those of *Fusarium*, due to which the influence of grape pomace extract on this fungus cannot be a starting point in the elaboration of a biocontrol protocol. On the other hand, compared to the pathogen *Fusarium*, the fungicidal effect of the grape pomace extract was manifested at a maximum concentration of 15%, the results not being influenced by the presence of alcohol. This positive aspect may be a plausible starting point in the development of biocontrol strategies for this pathogen in agroecosystems.

## Authors' Contributions

Conceptualisation: AD and RM; Writing - original draft and Writing - review and editing: AD, DV, GC, CM and RM; Experimentation: AD, DV, GC, DN and SM; Supervision and validation: AD, CM and RM.

All authors read and approved the final manuscript.

## Ethical approval (for researches involving animals or humans)

Not applicable.

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## Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

## References

- Ahmed E, Arshad M, Khan MZ, Amjad MS, Sadaf HM, Riaz I (2017). Secondary metabolites and their multidimensional prospective in plant life. *Journal of Pharmacognosy and Phytochemistry* 6:205-214.
- Athanasia MG, Konstantinos T, Kyriakos KM (2016). Valorization of grape pomace: Drying behavior and ultrasound extraction of phenolics. *Food and Bioproducts Processing* 100:132-144. <https://doi.org/10.1016/j.fbp.2016.06.016>
- Casazza AA, Aliakbarian B, Mantegna S, Cravotto G, Perego, P (2010). Extraction of phenolics from *Vitis vinifera* wastes using non-conventional techniques. *Journal of Food Engineering* 100(1):50-55. <https://doi.org/10.1016/j.jfoodeng.2010.03.026>
- Daglia M (2012). Polyphenols as antimicrobial agents. *Current Opinion in Biotechnology* 23:174-181. <https://doi.org/10.1016/j.copbio.2011.08.007>
- Deng Q, Penner MH, Zhao Y (2011). Chemical composition of dietary fiber and polyphenols of five different varieties of wine grape pomace skins. *Food Research International* 44(9):2712-2720. <https://doi.org/10.1016/j.foodres.2011.05.026>
- Garg A, Singh S (2016). *Alternaria* species in aerospora of vegetable and fruit market at Agra and their mycotoxigenic potential. *Asian Journal of Life Sciences* 1:4-7.
- Harvey R, Edrington T, Kubena L, Rottinghaus G, Turk J, Genovese K, Nisbet D (2001). Toxicity of moniliformin from *Fusarium fujikuroi* culture material to growing barrows. *Journal of Food Protection* 64:1780-1784.
- Hussein H, Brasel J (2001). Toxicity, metabolism, and impact of mycotoxins on humans and animals. *Toxicology* 167:101-134. [https://doi.org/10.1016/s0300-483x\(01\)00471-1](https://doi.org/10.1016/s0300-483x(01)00471-1)
- Joaquín-Ramos AJ, López-Palestina CU, Pinedo-Espinoza JM, Altamirano-Romo SE, Santiago-Saenz YO, Aguirre-Mancilla CL, Gutiérrez-Tlahque J (2020). Phenolic compounds, antioxidant properties and antifungal activity of jarilla (*Barkleyanthus salicifolius* [Kunth] H Rob & Brettell). *Chilean Journal of Agricultural Research* 80:352-360. <https://doi.org/10.1590/jst.02221>
- Kabir F, Sultana MS, Kurnianta H (2015). Antimicrobial activities of grape (*Vitis vinifera* L.) pomace polyphenols as a source of naturally occurring bioactive components. *African Journal of Biotechnology* 14(26):2157-2161. <https://doi.org/10.5897/AJB2015.14617>
- Sanhueza L, Tello M, Vivanco M, Mendoza L, Wilkens M (2014). Relation between antibacterial activity against food transmitted pathogens and total phenolic compounds in grape pomace extracts from Cabernet Sauvignon and Syrah varieties. *Journal of Advances in Microbiology* 4:225-232. <https://doi.org/10.4236/aim.2014.45029>
- Ledezma E, Apitz-Castro R (2006). Ajoene the main active compound of garlic (*Allium sativum*): A new antifungal agent. *Revista Iberoamericana de Micología* 23(2):75-80.
- Luo JJ, Li ZB, Wang JJ, Weng QF, Chen SH, Hu MY (2016). Antifungal activity of isoliquiritin and its inhibitory effect against *Peronophythora litchi* Chen through a membrane damage mechanism. *Molecules* 21(2):237. <https://doi.org/10.3390/molecules21020237>
- Mardare ES, Cristea S, Gadea M, Tamba-Berehoiu R (2015). The influence of some abiotic factors on the development of *Alternaria* sp. pathogen (“*in vitro*”). *Romanian Biotechnological Letters* 20(5):10880-10884.
- Medouni-Adrar S, Boulekbache-Makhlouf L, Cadot Y, Medouni-Haroune L, Dahmoune F, Makhoukhe A, Madani K (2015). Optimization of the recovery of phenolic compounds from Algerian grape by-products. *Industrial Crops and Products* 77:123-132. <https://doi.org/10.1016/j.indcrop.2015.08.039>
- Meena M, Zehra A, Dubey KM, Aamir M, Gupta KV, Upadhyay SR (2016). Comparative evaluation of biochemical changes in tomato (*Lycopersicon esculentum* Mill.) infected by *Alternaria alternata* and its toxic metabolites (TeA, AOH, and AME). *Frontiers in Plant Science* 7:1408. <https://doi.org/10.3389/fpls.2016.01408>
- Mendoza L, Yanez K, Vivanco M, Melo R, Cotoras M (2013). Characterization of extracts from winery by-products with antifungal activity against *Botrytis cinera*. *Industrial Crops and Products* 43:360-364. <https://doi.org/10.1016/j.indcrop.2012.07.048>
- Mihaescu C, Sturzeanu M, Din A (2021). *In vitro* evaluation of fungicide and bio-fungicides against isolates of *Alternaria solani* Sorauer. *Romanian Biotechnological Letters* 26(1):2295-2301. <https://doi.org/10.25083/rbl/26.1/2295.2301>

- Negro C, Aprile A, De Bellis L, Miceli A (2019). Nutraceutical properties of mulberries grown in Southern Italy (Apulia). *Antioxidants* 8:223. <https://doi.org/10.3390/antiox8070223>
- Obreque Slier E, Peña-Neira Á, López-Solís R, Zamora Marín F, Ricardo-da Silva JM, Laureano O (2010). Comparative study of the phenolic composition of seeds and skins from Carménère and Cabernet Sauvignon grape varieties (*Vitis vinifera* L.) during ripening. *Journal of Agricultural and Food Chemistry* 58:3591-3599. <https://doi.org/10.1021/jf904314u>
- Ostry V (2008). *Alternaria* mycotoxins: An overview of chemical characterization, producers, toxicity, analysis and occurrence in foodstuffs. *World Mycotoxin Journal* 1(2):175-188. <https://doi.org/10.3920/WMJ2008.x013>
- Ranjitha CY, Priyanka S, Deepika R, Smitha Rani GP, Sahana J, Prashith Kekuda TR (2014). Antimicrobial activity of grape seed extract. *World Journal of Pharmacy and Pharmaceutical Sciences* 3(8):1483-1488.
- Rockenbach II, Gonzaga LV, Rizelio VM, Any Elisa de Souza Schmidt Gonçalves, Genovese MI, Fett R (2011). Phenolic compounds and antioxidant activity of seed and skin extracts of red grape (*Vitis vinifera* and *Vitis labrusca*) pomace from Brazilian winemaking. *Food Research International* 44:897-901. <https://doi.org/10.1016/j.foodres.2011.01.049>
- Shimamura T, Sumikura Y, Yamazaki T, Tada A, Kashiwagi T, Ishikawa H, Matsui T, Sugimoto N, Akiyama H, Ukeda H (2014). Applicability of the DPPH assay for evaluating the antioxidant capacity of food additives – inter-laboratory evaluation study. *Analytical Sciences* 30(7):717-721. <https://doi.org/10.2116/analsci.30.717>
- Soria AC, Villamiel M (2010). Effect of ultrasound on the technological properties and bioactivity of food: A review. *Trends in Food Science & Technology* 21:323-331. <https://doi.org/10.1016/j.tifs.2010.04.003>
- Summerell BA, Leslie JF, Liew ECY, Laurence MH, Bullock S, Petrovic T, Bentley AR, Howard CG, Peterson SA, Walsh JL, Burgess WL (2011). *Fusarium* species associated with plants in Australia. *Fungal Divers* 46:1-27. <https://doi.org/10.1007/s13225-010-0075-8>
- Vauzour D, Rodriguez-Mateos A, Corona G, Oruna-Concha MJ, Spencer JPE (2010). Polyphenols and human health: prevention of disease and mechanisms of action. *Nutrients* 2:1106-1131. <https://doi.org/10.3390/nu2111106>
- Vieira GS, Cavalcanti RN, Meireles MAA, Hubinger MD (2013). Chemical and economic evaluation of natural antioxidant extracts obtained by ultrasound-assisted and agitated bed extraction from jussara pulp (*Euterpe edulis*). *Journal of Food Engineering* 119:196-204. <https://doi.org/10.1016/j.jfoodeng.2013.05.030>
- Vincent JM (1947). The esters of 4-hydroxybenzoic acid and related compounds. Part I. Methods for the study of their fungistatic properties. *Journal of the Society of Chemical Industry* 66:149-155. <https://doi.org/10.1002/jctb.5000660504>
- Yilmaz Y, Toledo RT (2006). Oxygen radical absorbance capacities of grape/wine industry byproducts and effect of solvent type on extraction of grape seed polyphenols. *Journal of Food Composition and Analysis* 19(1):41-48. <https://doi.org/10.1016/j.jfca.2004.10.009>
- \*\*\* GraphPad, <https://www.graphpad.com/>.
- \*\*\*ISO 14502-1 (2005). Determination of substances characteristic of green and black tea, Part. 1: Content of total polyphenols in tea - Colorimetric method using Folin-Ciocalteu reagent.



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