

Characterization of grape marc hydrolysates and their antifungal effect against phytopathogenic fungi of agricultural importance

Olivia Tzintzun-Camacho^{1*}, Valeria Hernández-Jiménez¹, Daniel González-Mendoza¹,
Juan Pedro Pérez-Pérez¹, Rosalba Troncoso-Rojas², Dagoberto Durán-Hernández¹,
Carlos Ceceña-Durán¹, and Carlos Francisco Moreno-Cruz³

¹Universidad Autónoma de Baja California, Instituto de Ciencias Agrícolas, Carretera a Delta s/n, C.P. 21705, Ejido Nuevo León, Mexicali, Baja California, México. *Corresponding author (otzintzun@uabc.edu.mx).

²Centro de Investigación en Alimentación y Desarrollo, A.C. Coordinación de Tecnología de Alimentos de Origen Vegetal, Carretera Gustavo Enrique Astiazarán Rosas, No. 46, Col. La Victoria, Hermosillo, Sonora, C.P. 83304, México.

³Universidad Autónoma Metropolitana-Iztapalapa, Av. San Rafael Atlixco No. 186, Col. Vicentina, Iztapalapa, Ciudad de México, C.P. 09340, México.

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ABSTRACT

Winemaking waste contain a high number of bioactive compounds with antimicrobial properties that can be exploited in agriculture. In the present study, hydrolysates from three wine grape (*Vitis vinifera* L.) marcs were characterized and their antifungal activities against phytopathogenic fungi (*Fusarium oxysporum* and *Alternaria* spp.) were evaluated. Wine grape marcs (red, pink and white wine) collected from Ensenada, Baja California, Mexico, were subjected to an acid hydrolysis treatment. Skin hydrolysates of pink and white marcs obtained high concentrations of reducing sugars (5.8 ± 0.1 and 5.5 ± 0.2 g L⁻¹, respectively). Meanwhile, the highest concentration of total sugars was obtained for skin hydrolysates of white marc (9.7 ± 0.08 g L⁻¹). The seed hydrolysates of white marc obtained high concentrations of phenolic compounds (0.52 ± 0.1 mg mL⁻¹). In addition, the highest antioxidant activity was found for skin hydrolysates of red marc ($96 \pm 0.61\%$). Results of in vitro antifungal assays clearly indicated a marked inhibition of the mycelial growth and spore viability of *F. oxysporum* (100% inhibition using red and white hydrolysates) rather than *Alternaria* spp. (58% inhibition exposed to pink hydrolysates), due to high concentration of phenols. According to HPLC analysis, phenolic acids such as gallic acid, hydroxybenzoic acid, vanillic acid and *p*-coumaric acid were predominant in the hydrolysates. This study demonstrated that the grape marc hydrolysates exhibit a potential antifungal activity, and highlights that the hydrolysates can be exploited in agriculture as a safe alternative of antifungal agents.

Key words: Antifungal activity, grape marcs, hydrolysates, phenolic acids, phytopathogenic fungi, *Vitis vinifera*.

INTRODUCTION

The winery industry produces a huge quantity of byproducts which represent a serious problem of disposal. Winery byproducts, including grape pomace, seeds, skins, stems and leaves, are rich sources of phenolic compounds with antioxidant and antimicrobial properties that have been previously reported (Friedman, 2014). Grape seeds are rich in flavonoids, such as resveratrol, oligomeric procyanidins and linoleic acid, and the skins are abundant in anthocyanins (Friedman, 2014; García-Lomillo and González-SanJosé, 2017). It is important to mention that winemaking byproducts represent a source of phenolic compounds, polysaccharides or proteins that may be valorized (Rockenbach et al., 2011;

Gómez-Brandón, et al., 2019). The antioxidant activities of phenolic compounds have been widely described (Rockenbach et al., 2011; Gülcü et al., 2019), but there are few reports on the antifungal activity of grape marcs with agricultural applications. Intensive application of chemical fungicide causes fungicide-resistant populations and negative effects in the environment. Therefore, eco-safe antifungal agents are necessary to control plant disease development. Particularly, cotton cultivation in the Mexicali valley, Baja California, Mexico, represents an important economic activity. However, fungal diseases are the major causes of reduced yields for this crop. Specifically, Fusarium wilt disease of cotton is widely spread in all cotton regions, and it was reported to be the fifth most important fungal pathogen in the world (Dean et al., 2012). Moreover, one of the most common foliar diseases of cotton is Alternaria leaf spot caused by *Alternaria* spp. (Zhu et al., 2019). The management of Fusarium wilt has been focused on trash management, crop rotation and the use of fungicides (Gaspar et al., 2014). Meanwhile, Alternaria leaf spot has been controlled with chemical fungicides, bioagents and natural extracts. The use of plant extracts has been reported to control of phytopathogens in agriculture. Plants produce a huge variety of secondary metabolites that serve to protect them against plant pathogens. In this sense, a high inhibitory effect on *F. oxysporum* has been reported using plant extracts or formulations based on those extracts (Abd-Elsalam and Khokhlov, 2015; Joaquín-Ramos et al., 2020). Moreover, the potential antifungal activity of different natural formulations to control *Alternaria* spp. has been reported (Wianowska et al., 2016). However, winery wastes contain bioactive compounds with antimicrobial activities that can be used in agriculture. Particularly, previous studies have reported antifungal activities of grape marc fractions against pathogenic bacteria, viruses and fungi (Friedman, 2014). However, the utilization of the grape residues for the development of antifungal agents to control phytopathogens in agriculture is scarce evaluated.

Therefore, the aim of this work was to give a pretreatment and valorization of three wine grape marcs to obtain seed and skin hydrolysates rich in bioactive compounds. Each hydrolysate was characterized and their antioxidant and antifungal properties were also evaluated under in vitro conditions against fungal phytopathogens of cotton.

MATERIALS AND METHODS

Collection of wine grape residues

Samples of each grape marc (10 kg) were obtained from two wine industries located in the Guadalupe Valley in Ensenada, Baja California, Mexico. Red grape marc (RGM) was obtained directly from fermentation tanks, while pink and white grape marcs (PGM and WGM, respectively) were obtained after juice extraction. Winemaking grape (*Vitis vinifera* L.) varieties of byproducts were 'Merlot' (red grape marc), 'Grenache' (pink grape marc), and 'Sauvignon blanc' (white grape marc). All samples were stored into sealed bags at -20 °C until use.

Powder preparation and hydrolysis treatment

Samples of each grape marc (200 g) were weighted using an electronic balance (Mod. HT224RCE, VIBRA, Tokyo, Japan) and skins and seeds were manually separated. All samples were placed in an oven (Mod. LO-201C, The Grieve Corporation, Round Lake, Illinois, USA) at 80 °C for 48 h. Dried samples were finely ground and stored at room temperature in the dark. Grape marc powders were subjected to an acid hydrolysis treatment followed by a steam explosion as previously described Tzintzun-Camacho et al. (2016) with some modifications. All the powders (10 g L⁻¹) were acidified with 1.0% v/v HCl and shaken during 30 min in darkness. Then, two steam explosion treatments were conducted (121 °C, 0.1 MPa, 20 min). After that, pH of mixture was adjusted to 7.0 with a 10 N NaOH solution and filtered through filter paper (Whatman Nr 1). Finally, seed and skin hydrolysates of each grape marc were obtained. All hydrolysates were stored at 4 °C in darkness.

Total and reducing sugar determination

Reducing sugars were quantified spectrophotometrically using the method described by Tzintzun-Camacho et al. (2016) with some modifications: 1 L 3,5-dinitrosalicylic acid (DNS) reagent was prepared with 10 g DNS, 1 g sodium sulfite, 2 g phenol and 10 g sodium hydroxide. The reaction was prepared by mixing 1 mL hydrolysate and 1 mL DNS reagent. Samples were boiled for 15 min; after that, 8 mL distilled water was added. The absorbance of samples was measured at 575 nm using a UV-visible spectrophotometer (Spectronic BioMate 3, Thermo Fisher Scientific, Waltham, Massachusetts,

USA). The reducing sugars were determined using a dextrose calibration curve ranging from 0 to 1.0 g L⁻¹. Analyses were conducted in triplicate. The total sugar concentration of hydrolysates was determined according to method described by Dubois et al. (1956) and modified by 1 mL hydrolysate being mixed with 1 mL 5% w/v phenol solution; then, 2.5 mL sulfuric acid (98% v/v) was added. Samples were maintained in an ice bath for 15 min in darkness. The absorbance was measured at 490 nm. The total sugars were determined using a sucrose calibration curve ranging from 0.1 to 0.5 g L⁻¹. Analyses were conducted in triplicate.

Determination of total phenols and flavonoids

Phenolic compounds were quantified using the spectrophotometric method of Gülcü et al. (2019) with some modifications: 0.1 mL hydrolysate was mixed with 0.5 mL 1 N Folin-Ciocalteu reagent, and samples were incubated at 25 °C for 5 min in darkness. Then, 0.4 mL 1 N sodium carbonate solution was added, and maintained at 25 °C for 30 min in darkness. Samples were measured at 765 nm, and the quantification of total phenolic compounds was determined using a gallic acid calibration curve of (0.1 to 0.5 g L⁻¹) and expressed as gallic acid equivalents (GAE). Analyses were conducted in triplicate.

Total flavonoids were analyzed by the modified aluminum chloride technique described by Gülcü et al. (2019): 1 mL hydrolysate was mixed with 4 mL distilled water; after that, 0.3 mL of a 0.5 g L⁻¹ sodium nitrate solution was added. Samples were incubated at 25 °C for 5 min in darkness. Then, 0.3 mL of 1 g L⁻¹ aluminum chloride solution was added. After 6 min at room temperature in darkness, 2 mL 1 M sodium hydroxide and 2.4 mL distilled water were added. Samples were measured at 510 nm, and total flavonoids were determined using a calibration curve of quercetin ranging from 0.2 to 1.0 mg mL⁻¹ and expressed as quercetin equivalents (QE). Analyses were conducted in triplicate.

Determination of DPPH scavenging activity

A solution of 0.1 mM 1,1-diphenyl-2-picrylhydrazyl (DPPH) was prepared using methanol (96% v/v) as a solvent. The reaction was prepared by mixing 0.1 mL hydrolysate with 2.9 mL DPPH solution. Samples were incubated at room temperature for 30 min. Samples were measured at 517 nm using methanol as a blank. The control consisted of a 0.1 mM DPPH solution. DPPH scavenging activity was determined using the following equation described by González-Mendoza et al. (2011):

$$\% \text{ DPPH scavenging activity} = [(A_c - A_s)/(A_c)] \times 100$$

where A_c is control absorbance and A_s is sample absorbance.

HPLC analysis

The identification and quantification of phenolic compounds were determined according to modified methods described by Mradu et al. (2012) and Troncoso-Rojas et al. (2013). A 1290 Infinity HPLC system (Agilent Technologies, Santa Clara, California, USA) was used equipped with a quaternary pump and a diode array detector. Separation was conducted with a Luna C18 column (200 mm length × 0.5 μm particle size; Phenomenex Ltd., Torrance, California, USA) using a flow rate of 1 mL min⁻¹. An elution gradient was developed using a 0.1% phosphoric acid solution in water (A) and acetonitrile (B). The gradient program started with 8% of solution B for 35 min, then solution B was increased to 78% for 10 min, and finally it was reduced to 8%. Eluates were monitored at 260, 270, 280, 295, 310, 325, 360 and 370 nm, and samples were injected in duplicate. The identification and quantification of phenolic compounds were determined by comparison of retention times with standards of gallic acid, hydroxybenzoic acid, vanillic acid, rutin, *p*-coumaric acid, resveratrol, naringenin, kaempferol and quercetin.

Antifungal activity assays of hydrolysates

The antifungal activity of hydrolysates was evaluated against two phytopathogenic fungi: *Fusarium oxysporum* and *Alternaria* spp. Hydrolysates of seeds and skins used in the assays were RGM, PGM, and WGM. Culture media of each grape marc were prepared containing hydrolysate and agar (15 g L⁻¹). Media were sterilized (121 °C, 0.1 MPa, 15 min) and poured into Petri dishes. After solidification, plates were inoculated with a fragment of mycelia (3 mm in diameter), positioned at the center of the plate and incubated at 30 °C for 12 d. Petri dishes containing only PDA medium and inoculated with the fungal culture were used as controls. The inhibition of mycelial growth was recorded during

the assays. Additionally, the viability of fungal spores was evaluated using an automated cell counter (TC20, Bio-Rad Laboratories, Hercules, California, USA), and 0.4% trypan blue solution as a dye.

Statistical analysis

The experimental design for all experiments was completely randomized and determinations were conducted in triplicate. The statistical analyses of sugars, phenolic compounds, antioxidant activity and spore viability were performed using one way-ANOVA with $P \leq 0.05$. Differences between means were determined by the Tukey-Kramer test, $P \leq 0.05$ (NCSS, Kaysville, Utah, USA). Results were expressed as means obtained from three replicates with their corresponding standard deviations.

RESULTS AND DISCUSSION

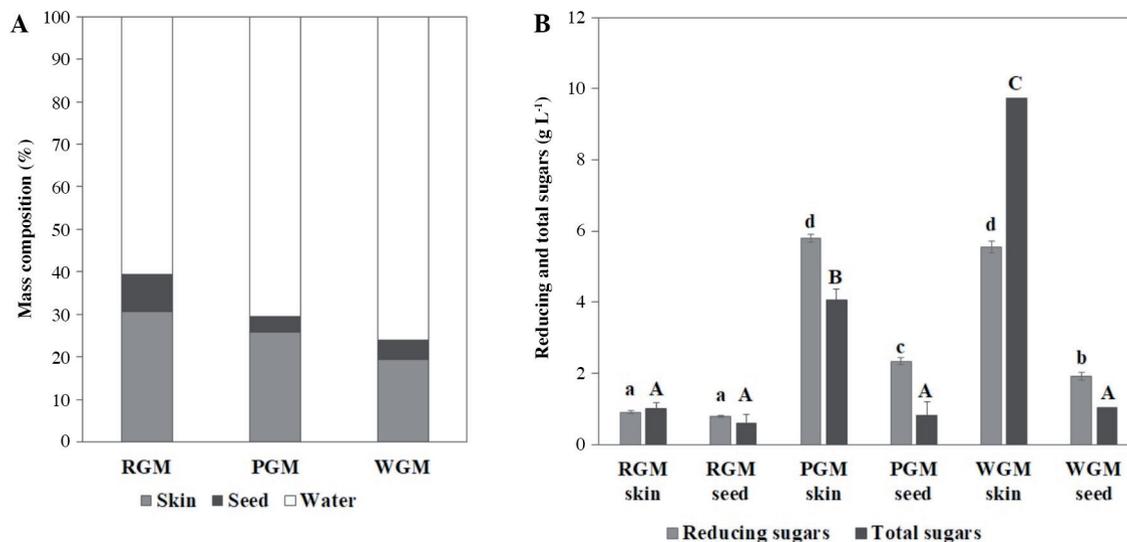
Mass composition of wine grape marcs

The mass compositions of three grape marcs (RGM, PGM and WGM) were determined in this study. A high mass percentage of water was observed for the three grape marcs (Figure 1A). In particular, the skin proportion for RGM was significantly higher than for PGM and WGM ($31 \pm 1\%$, $26 \pm 3\%$ and $19 \pm 1\%$, respectively in dry base). Similarly, seed proportion for RGM was higher than for PGM and WGM (9%, 4%, and $4 \pm 1\%$, respectively in dry base). The differences in the mass composition of grape marcs could be explained by the grape cultivar and the procedure used for wine production. In this study, samples of RGM were obtained directly from fermentation tanks, while PGM and WGM were obtained after juice extraction. According to previous studies, grape marcs are mainly constituted by seeds (52%) followed by skins (46%) and stalks (2%) (Fiori and Florio, 2010). Other studies have reported a different mass composition (51% skins, 47% seeds and 2% stalks; Basso et al., 2018), indicating that constituents can vary considering the type of cultivar.

Effect of acid hydrolysis treatment on wine grape marcs

In this work, the breakdown of polysaccharides contained in the structure of the cell walls from the skins and seeds of grape marcs was shown by the increased concentration of reducing and total sugars from hydrolysates after acid hydrolysis treatment (Figure 1B). Skin hydrolysates of PGM and WGM obtained high concentrations of reducing sugars

Figure 1. Mass composition (A) and reducing and total sugars in hydrolysates of seeds and skins (B) of red (RGM), pink (PGM) and white grape marcs (WGM).



Values are expressed as means \pm standard deviation ($n = 3$). Means with different letters are significantly different according to Tukey's test ($P \leq 0.05$).

(5.8 ± 0.1 and 5.5 ± 0.2 g L⁻¹, respectively). As expected, RGM hydrolysates showed the lowest values of reducing sugars (0.9 g L⁻¹) due to the sugars being consumed during the fermentation process of red wine. Meanwhile, the hydrolysates of skin WGM obtained the highest concentration of total sugars (9.7 ± 0.08 g L⁻¹) followed by skin PGM (4.1 ± 0.3 g L⁻¹), while the hydrolysates of seeds showed concentrations below to 1.0 g L⁻¹ (Figure 1B).

According to previous studies, grape marc is a good source of carbohydrates (31%-54% w/w), containing soluble monomers (dextrose and fructose) and complex polysaccharides (polyphenols, pectins and cellulose) (Corbin et al., 2015). In this sense, several pre-treatments such as physical, chemical and enzymatic methods have been reportedly used to break down polysaccharides of plant materials. However, hydrolysis methodologies (alkaline concentrated and diluted acid) and steam explosion have been extensively used to release monosaccharides from cell walls (Apolinar-Valiente et al., 2015). In our study, the grape marcs were subjected to an acid treatment followed by a steam explosion. The initial concentration of reducing and total sugars was very low for all grape marcs (values less than 1.0 g L⁻¹), but after acid hydrolysis treatment, an increase of sugars was induced in the hydrolysates. As result, high values of sugars were obtained in the skin hydrolysates of PGM and WGM. These findings could be explained because pressure causes the explosion of cell wall polysaccharides, making the polymers more accessible to the action of acid treatment. In addition, the type of cultivar and the oenological technique influences the sugar concentration of hydrolysates. The PGM and WGM samples corresponded to byproducts of ‘Grenache’ and ‘Sauvignon blanc’, respectively. Generally, these varieties are used in the processing of pink and white wines, and byproducts are generated before alcoholic fermentation, thus preserving the sugar contents (Ribeiro et al., 2018). Conversely, the byproducts generated during the production of red wines contain a low sugar content.

Phenolic compounds, flavonoids and antioxidant activity

Hydrolysates of grape marcs were characterized analyzing total phenolic compounds, flavonoids and antioxidant activities (Table 1). A significant increase of total phenols was found mainly in hydrolysates of seeds rather than in skins. The seed hydrolysates of WGM (0.52 ± 0.1 mg GAE mL⁻¹) obtained a high concentration of phenols compared to RGM and PGM (0.10 ± 0.1 and 0.18 ± 0.1 mg GAE mL⁻¹, respectively). Skin hydrolysates of PGM and WGM showed concentrations of 0.17 mg GAE mL⁻¹ (Table 1). Conversely, Ribeiro et al. (2018) obtained low concentrations of phenolic compounds in seeds of white grapes (‘Sauvignon blanc’) and high concentrations in the pink wine grapes (‘Grenache’). Meanwhile, several studies have reported the highest concentrations of phenols in seed extracts of red grapes (Rockenbach et al., 2011; Apolinar-Valiente et al., 2015; Peixoto et al., 2018). Multiple factors have been shown to affect the content of phenolic compounds, including grape cultivar, climate, wine processing technique and storage conditions of the grape marcs (Gutiérrez-Gamboa and Moreno-Simunovic, 2018; Gutiérrez-Gamboa et al., 2018). In addition, the hydrolysis method is a factor that might cause the partial degradation of phenols. Therefore, our results confirm that differences in the phenolic compounds of grape marcs depended on grape variety and suggest an increase of phenols in seeds due to hydrolysis treatment.

Table 1. Total phenols, total flavonoids and antioxidant capacity of hydrolysates of seeds and skins of red (RGM), pink (PGM) and white grape marcs (WGM).

| Hydrolysate | Total phenols | | Total flavonoids | | Antioxidant activity ¹ | |
|-------------|-------------------------------------|------------------|------------------------------------|------------------|-----------------------------------|----------------|
| | Skin | Seed | Skin | Seed | Skin | Seed |
| | mg GAE mL ⁻¹ hydrolysate | | mg QE mL ⁻¹ hydrolysate | | % | |
| RGM | $0.08 \pm 0.00a$ | $0.10 \pm 0.01a$ | $0.40 \pm 0.01b$ | $0.46 \pm 0.00a$ | $96 \pm 0.61c$ | $34 \pm 1.49a$ |
| PGM | $0.17 \pm 0.00b$ | $0.18 \pm 0.01b$ | $0.41 \pm 0.01b$ | $0.47 \pm 0.01a$ | $20 \pm 1.06b$ | $33 \pm 0.66a$ |
| WGM | $0.17 \pm 0.00b$ | $0.52 \pm 0.10c$ | $0.37 \pm 0.02a$ | $0.61 \pm 0.02b$ | $16 \pm 0.22a$ | $59 \pm 0.56b$ |

Values are expressed as means \pm standard deviation (n = 3). Means in the same column with different letters are significantly different according to Tukey’s test ($P \leq 0.05$).

GAE: Gallic acid equivalents; QE: quercetin equivalents.

¹Antioxidant activity is expressed as 1,1-diphenyl-2-carboxylic acid (DPPH) radical scavenging activity.

Total flavonoids were also determined in this study (Table 1). Similarly, the flavonoid concentration was higher in hydrolysates of seeds than skins. Interestingly, the seed hydrolysates of WGM showed the highest concentration (0.61 ± 0.02 mg QE mL⁻¹), followed by RGM and PGM (0.46 and 0.47 mg QE mL⁻¹, respectively). In agreement with these results, flavonoids were the most abundant group of phenolic compounds in the plants. In addition, the content of flavonoids has been reported to increase with acid extraction methods (Putnik et al., 2016). The antioxidant activities of seed and skin hydrolysates were determined as DPPH radical scavenging activity (Table 1). This assay is based on hydrogen atom transfer reactions between antioxidant compounds and the DPPH radicals (Agustin-Salazar et al., 2014). According to the results, the seed hydrolysates of PGM and WGM showed high values of antioxidant activity ($33 \pm 0.66\%$ and $59 \pm 0.56\%$, respectively) compared with skin hydrolysates. However, the highest antioxidant activity was found for RGM skin ($96 \pm 0.61\%$). These findings could be explained by the presence of individual phenolic compounds. As shown in Table 2, the hydrolysates showed different profiles of phenolic compounds. In the case of RGM, the most abundant phenolic compound was the vanillic acid ($159 \mu\text{g mL}^{-1}$) followed by *p*-coumaric acid ($34 \mu\text{g mL}^{-1}$) and gallic acid ($23 \mu\text{g mL}^{-1}$). Meanwhile, gallic acid ($139 \mu\text{g mL}^{-1}$) and *p*-coumaric acid ($9 \mu\text{g mL}^{-1}$) were identified in the hydrolysates of PGM. A different trend was observed for WGM, where vanillic acid ($90 \mu\text{g mL}^{-1}$) was the most abundant phenolic compound. According to previous studies, three main groups of phenols have been reported: phenolic acids, flavonoids and proanthocyanidins (Peixoto et al., 2018). In addition, phenolic acids have been shown to be abundant and related to antioxidant activity in grapes, mainly gallic acid (Verma et al., 2013) and derivatives of *p*-hydroxybenzoic acid (Farhoosh et al., 2016). Therefore, our findings suggest that the presence of phenolic acids, such as vanillic acid, influenced the high antioxidant activity of the hydrolysates of red grape marc compared with pink and white grape marcs.

Antifungal activity of hydrolysates

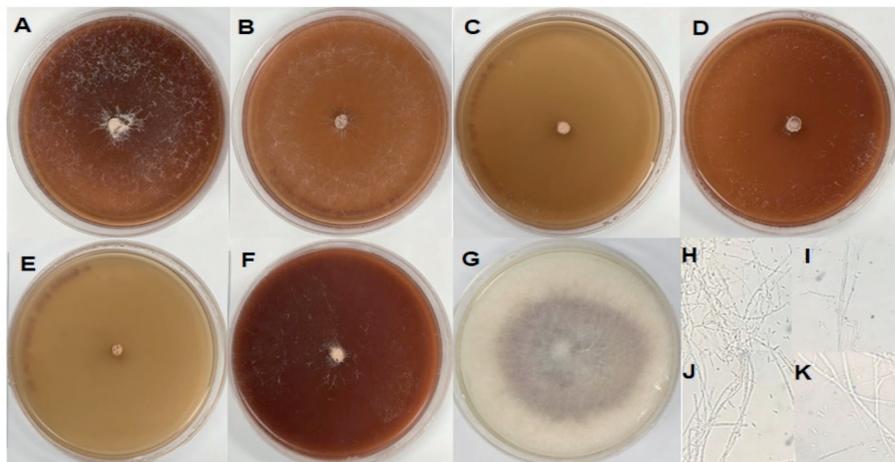
The antifungal activity of hydrolysates on mycelial growth and spore viability was evaluated in this study. Results showed that all hydrolysates from seeds and skins of the three grape marcs were effective against *F. oxysporum* (Figure 2). A high inhibition of mycelial growth was mainly observed for RGM seeds, PGM seeds and WGM seeds compared to the control. These findings were in agreement with the high concentrations of phenols and flavonoids previously showed in the hydrolysates. In addition, damages to the mycelial structure of *F. oxysporum* exposed to hydrolysates of RGM seeds, PGM seeds and WGM seeds were observed using microscopic examinations (Figure 2). According to previous reports, phenolic compounds including simple molecules (phenolic acids) and complex structures (flavanols, flavonols and anthocyanins) have been reported to be directly involved in the defence mechanisms of plants against pathogenic fungi (Ahmed et al., 2017; Nechita et al., 2019). In addition, Luo et al. (2016) confirmed that phenolic compounds could increase the cell permeability, thus reducing fungal growth. Similarly, Gauthier et al. (2016) demonstrated that chlorogenic acid is a key metabolite in cereals to counteract *F. graminearum* and its production of mycotoxins. Wang et al. (2010) reported morphological changes to the mycelial hyphae of *Botrytis cinerea* due to the antifungal activity of eugenol, leading to alteration of cytoplasmic membrane permeability. In our study, the antifungal activity of hydrolysates against *F. oxysporum* could be explained by the presence of the phenolic acids such as gallic acid, hydroxybenzoic acid, vanillic acid and *p*-coumaric acid (Table 2). These phenolic acids probably caused damages to the mycelial structure of *F. oxysporum*, as previously mentioned, and reduced its growth.

Table 2. Identification of phenolic compounds in hydrolysates obtained from red (RGM), pink (PGM) and white grape marcs (WGM).

| Phenolic compounds | RWM | PWM | WWM |
|-------------------------|-----------------------|-----|-----|
| | $\mu\text{g mL}^{-1}$ | | |
| Gallic acid | 23 | 139 | 84 |
| Hydroxybenzoic acid | nd | nd | 20 |
| Vanillic acid | 159 | nd | 90 |
| Rutin | nd | nd | nd |
| <i>p</i> -Coumaric acid | 34 | 9 | 14 |
| Resveratrol | nd | nd | nd |

Standard deviation values are not shown due to values are very low.
nd: Not detected.

Figure 2. Antifungal activity of hydrolysates obtained from seeds and skins of red (RGM), pink (PGM) and white grape marcs (WGM) against *Fusarium oxysporum*: RGM skin (A), RGM seed (B), PGM skin (C), PGM seed (D), WGM skin (E), WGM seed (F), control (G). Microscopic examination with 40× magnification of *F. oxysporum* exposed to hydrolysates of RGM seed (H), PGM seed (I), WGM seed (J), and control (K).



Other studies have highlighted that phytopathogen inhibition mechanisms vary depending on phenolic compounds and the fungi tested (Nechita et al., 2019; Joaquín-Ramos et al., 2020). In this study, a different trend was observed for *Alternaria* spp.; the hydrolysates of RGM seed, PGM seed and RGM skin showed a high inhibition of mycelial growth compared to the control (Figure 3). However, structural changes in the hyphae and conidiophores were not observed from *Alternaria* spp. exposed to these hydrolysates (Figure 3). Therefore, the spore viability of pathogenic fungi exposed to hydrolysates was evaluated in this study. As shown in Table 3, the viability of *F. oxysporum* spores was completely reduced using the hydrolysates of RGM skin and seed WGM (0% viability). Conversely, the spores of *Alternaria* spp. were mainly affected when exposed to the hydrolysate of PGM seed ($42 \pm 6.7\%$ viability). According to previous reports, Pane et al. (2016) demonstrated the antifungal effect of pepper extracts due to the presence of phenolic acids and suggested a synergism among the phenolic compounds and other metabolites of the extracts to control of *A. alternata*. Our findings indicate that the hydrolysates were mainly effective at reducing the mycelial growth and spore viability of *F. oxysporum* due to the presence of the phenolic acids; however, for *Alternaria* spp., the outcome was different. Therefore, future studies focused on studying the biochemical and molecular mechanisms are necessary to understand the control of phytopathogen fungi.

Figure 3. Antifungal activity of hydrolysates obtained from seeds and skins of red (RGM), pink (PGM) and white grape marcs (WGM) against *Alternaria* spp.: RGM skin (A), RGM seed (B), PGM skin (C), PGM seed (D), WGM skin (E), WGM seed (F), control (G). Microscopic examination with 40× magnification of *Alternaria* spp. exposed to hydrolysates of RGM seed (H), PGM seed (I), RGM skin (J), and control (K).

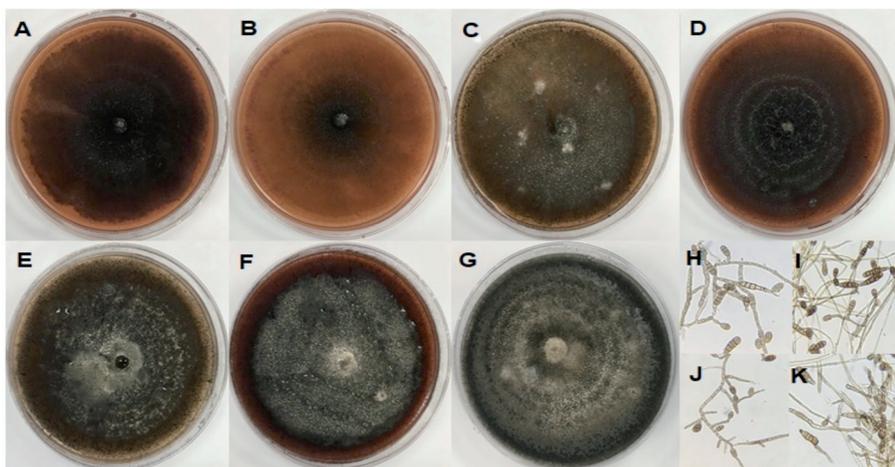


Table 3. Assays of spore viability of *Fusarium oxysporum* and *Alternaria* spp. exposed to hydrolysates of seeds and skins of red (RGM), pink (PGM) and white grape marcs (WGM).

| Hydrolysate | Spore viability | |
|-------------|---------------------|------------------------|
| | <i>F. oxysporum</i> | <i>Alternaria</i> spp. |
| | % | |
| Control | 100.0 ± 0.0e | 100.0 ± 0.0f |
| RGM skin | 0.0 ± 0.0a | 68.0 ± 5.6e |
| RGM seed | 1.0 ± 0.3b | 67.0 ± 5.6d |
| PGM skin | 13.0 ± 0.1d | 58.0 ± 7.3c |
| PGM seed | 2.0 ± 0.7b | 42.0 ± 6.7a |
| WGM skin | 5.0 ± 2.7c | 55.0 ± 18.9b |
| WGM seed | 0.0 ± 0.0a | 100.0f |

Values are expressed as means ± standard deviation (n = 3).

Means in the same column with different letters are significantly different according to Tukey's test ($P \leq 0.05$).

CONCLUSIONS

This research highlights the valorization of bioactive compounds contained in the hydrolysates of wine grape marc that could be used for the control of phytopathogenic fungi. Our findings revealed the antifungal activity of the hydrolysates against phytopathogenic fungi showing a marked inhibition of the mycelial growth and spore viability of *Fusarium oxysporum* compared to *Alternaria* spp. due to the presence of phenolic acids. It is important to mention that after the acid hydrolysis treatment of wine residues, increased concentrations of reducing and total sugars and phenols were obtained in the hydrolysates. In addition, the high antioxidant activity of the seed hydrolysates of red grape marc compared with pink and white grape marcs was demonstrated. Therefore, the results obtained in this study suggest that the hydrolysates from the seeds and skins of grape marc could be used as fungicides for the management of *F. oxysporum*.

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