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Anti-Dermatophyte and Anti-*Malassezia* Activity of Extracts Rich in Polymeric Flavan-3-ols Obtained from *Vitis vinifera* Seeds

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Several human skin diseases are associated with fungi as dermatophytes and *Malassezia.* Skin mycoses are increasing and new alternatives to conventional treatments with improved efficacy and/or safety profiles are desirable. For the first time, the anti-dermatophytes and the anti-*Malassezia* activities of *Vitis vinifera* seed extracts obtained from different table and wine cultivars have been evaluated. Geometric minimal inhibitory concentration ranged from 20 to 97 μg/mL for dermatophytes and from 32 to 161 μg/mL for *Malassezia furfur.* Dried grape seed extracts analyzed by HPLC/DAD/ESI/MS showed different quali–quantitative compositions in terms of monomeric and polymeric flavan-3-ols. The minimal inhibitory concentrations for *Trichophyton mentagrophytes* and for *M. furfur* were inversely correlated with the amount of the polymeric fraction ($r = -0.7639$ and $r = -0.7228$, respectively). Differently, the antifungal activity against *T. mentagrophytes* was not correlated to the content of flavan-3-ol monomers ($r = 0.2920$) and only weakly correlated for *M. furfur* ($r = -0.53604$). These results suggest that extracts rich in polymeric flavan-3-ols, recovered from *V. vinifera* seeds, could be used for the treatment of skin fungal infections. Copyright © 2016 John Wiley & Sons, Ltd.

Keywords: dermatophytes; *Malassezia furfur*; Vitaceae; grape seed extracts; polymeric flavan-3-ols; antifungal activity.

INTRODUCTION

Skin mycoses affect more than 20–25% of the world's population (Havlickova *et al.,* 2008), and frequently, they are associated with dermatophytes (*Trichophyton* and *Microsporum*) and yeasts as *Malassezia* (White *et al.*, 2014). Dermatophytes are a group of pathogenic fungi that cause mostly superficial diseases in humans and other animals. The diseases that result from a dermatophyte infection are known as tineas (White *et al.*, 2014). *Malassezia* spp. are directly responsible for infectious diseases as pityriasis versicolor and folliculitis, and they also act as an exacerbating factor in seborrheic dermatitis and atopic dermatitis (Harada *et al.,* 2015). Although these skin-related problems are not generally life threatening, they represent a common global problem and can become chronic. Furthermore, it should be emphasized that the treatments need long-term therapy and often are not resolutive (Pfaller, 2012). Side effects and resistance are frequently attributed to the current antifungal agents, as the most widely used azole drugs (Pfaller, 2012; Zavrel and White, 2015). Few products showing antifungal activity are being currently developed (Ostrosky-Zeichner *et al*., 2010).

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Plants are important source of potentially antifungal agents and could represent a valuable alternative to synthetic drugs. In the last decades, essential oils have been widely studied and used as antifungal agents for topical treatments (Di Vito *et al*., 2015; Flores *et al*., 2016; Khosravi *et al*., 2016). Plant polyphenols demonstrated several potential healthy properties on humans, mainly as antioxidant, antiallergic, anti-inflammatory, anticancer, antihypertensive, renoprotective, and antimicrobial (Xia *et al.,* 2010; Daglia, 2012; Williamson and Manach, 2005). In particular, grape polyphenols showed antimicrobial activity against *Staphylococcus aureus, Escherichia coli, Salmonella enterica* serovar Typhimurium*, Listeria monocytogenes*, and *Candida albicans* (Xia *et al.,* 2010; Filocamo *et al*., 2015; Pasqua and Simonetti, 2016).

Grape seeds are known to contain a complex mixture of monomeric, oligomeric, and polymeric flavan-3-ols (Cavaliere *et al*., 2010; Liang *et al*., 2012; Ćurko *et al*., 2014; Narduzzi *et al*., 2015). It has been demonstrated that grape seed extracts (GSEs) rich in polymeric flavan-3-ols, obtained from different cultivars of *Vitis vinifera* L. (Vitaceae), showed high antifungal activity in *in vitro* tests, against a broad panel of human fungal pathogens (Pasqua *et al.,* patent, 2010). In addition, the anti-*Candida* activity of GSEs has been shown in a murine model of vaginal candidiasis (Simonetti *et al*., 2014).

It is well known that the concentration of flavan-3-ols in grape seeds varies considerably, depending on several

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Table 1. Antifungal activity of grape seed extracts against dermatophytes

	Trichophyton mentagrophytes						*Microsporum gypseum*			*Microsporum canis*	
	MIC_{80} (μg/mL)										
	PMC 6503	PMC 6509	PMC 6515	PMC 6527	PMC6552	DSM 4870	PMC 7303	PMC 7331	PMC 7342	PMC7426	GM MIC_{80}
MP2009V1N1A*	32	32	32	2	64	16	64	32	16	32	24
MP2010V1N1A	32	32	32	2	64	16	64	4	16	32	20
MP2010V2N1A	32	32	32	2	64	32	64	16	32	32	26
MP2011V1N1A	64	64	32	2	64	64	64	32	64	32	37
MP2011V2N1A	32	64	16	2	64	32	64	32	64	32	30
MP2012A	32	32	16	nd	32	64	nd	nd	nd	nd	28
MP2012B	64	128	32	nd	64	128	nd	nd	nd	nd	72
MP2012C	32	64	32	nd	32	128	nd	nd	nd	nd	45
RG2011V1N1A	64	64	32	4	128	64	128	16	64	64	45
RG2011V2N1A	64	64	32	4	128	64	128	8	32	32	37
Victoria2010A*	64	64	16	4	128	64	128	16	64	32	39
ITA2010V1A*	64	64	16	2	64	32	64	8	32	32	26
ITA2010V2A*	64	64	16	4	64	32	64	2	16	32	26
ITA2010A*	32	32	16	2	64	32	64	16	32	32	23
ITA2011V1A	32	32	32	2	64	32	64	16	32	32	28
ITA2011 V2A	32	32	32	4	64	32	64	16	32	32	24
ITA2012A	32	32	16	nd	32	32	nd	nd	nd	nd	25
ITA2012B	64	64	16	nd	64	128	nd	nd	nd	nd	45
ITA2012C	64	128	16	nd	64	128	nd	nd	nd	nd	57
Pinot2011A	128	256	64	32	128	64	128	128	128	64	97
NEG2011V1N2A	64	64	64	16	64	64	128	128	128	64	69
NEG2011V2N2A	64	64	16	16	64	32	32	128	128	64	48
FLZ	4	4	8	2	4	32	64	8	8	2	7
TRB	0.06	0.5	0.03	0.06	0.03	0.03	0.5	0.5	0.5	0.25	0.13
	MIC_{100} (μg/mL)										
	PMC 6503	PMC 6509	PMC 6515	PMC 6527	PMC 6552	DSM 4870	PMC 7303	PMC 7331	PMC 7342	PMC 7426	GM MIC_{100}
MP2009V1N1A*	64	128	64	32	128	64	256	32	128	64	79
MP2010V1N1A	64	128	64	16	128	64	256	32	64	64	69
MP2010V2N1A	64	64	64	16	128	64	256	64	64	64	69
MP2011V1N1A	128	128	64	16	128	128	256	128	128	64	97
MP2011V2N1A	128	128	128	16	128	128	256	128	128	64	104
MP2012A	128	128	64	nd	128	128	nd	nd	nd	nd	114
MP2012B	256	256	128	nd	256	256	nd	nd	nd	nd	181
MP2012C	256	256	128	nd	256	256	nd	nd	nd	nd	203
RG2011V1N1A	128	128	128	32	256	128	256	16	128	128	104
RG2011V2N1A	64	256	128	16	256	128	512	16	128	128	104

(Continues)

Table 1. (Continued)

	Trichophyton mentagrophytes						*Microsporum gypseum*			*Microsporum canis*	
	MIC_{80} (μg/mL)										
	PMC 6503	PMC 6509	PMC 6515	PMC 6527	PMC6552	DSM 4870	PMC 7303	PMC 7331	PMC 7342	PMC7426	GM MIC_{80}
Victoria2010A*	64	256	128	16	256	128	256	64	128	128	111
ITA2010V1A*	64	256	64	16	128	128	128	64	64	64	79
ITA2010V2A*	64	128	64	16	128	64	256	64	128	64	84
ITA2010A*	64	128	64	16	128	128	256	64	64	64	79
ITA2011V1A	64	128	128	16	128	128	256	64	64	64	84
ITA2011V2A	64	128	128	16	128	128	256	64	64	64	79
ITA2012A	64	128	64	nd	128	128	nd	nd	nd	nd	90
ITA2012B	64	256	64	nd	128	128	nd	nd	nd	nd	128
ITA2012C	64	256	64	nd	128	128	nd	nd	nd	nd	114
Pinot2011A	256	256	256	64	256	256	256	256	256	256	223
NEG2011V1N2A	128	128	128	32	256	128	256	256	256	128	147
NEG2011V2N2A	128	128	128	32	256	128	128	256	256	128	137
FLZ	64	32	64	16	32	64	256	32	32	8	39
TRB	0.06	0.5	0.06	0.06	0.03	0.06	0.5	0.5	0.5	0.5	0.16

Antifungal activity against *T. mentagrophytes* and *M. gypseum*, and *M. canis* was determined according to Clinical and Laboratory Standards Institute guidelines (CLSI document M38-A2, 2008). MIC_{80}, and MIC_{100} = lowest drug concentration that prevented 80% and 100% of growth with respect to the untreated control. The values shown are the median from three independent determinations. FLZ, fluconazole; ITA, Italia; GM, geometric mean; MIC, minimal inhibitory concentration; MP, Michele Palieri; NEG, Negroamaro; PMC, Pharmaceutical Microbiology Culture Collection; RG Red Globe; TRB, terbinafine.

*Grape seed extracts not chemically characterized. *A* = AEtOH/H_2O (7:3 v/v), *B* = EtOH, *C* = MeOH.

factors: mainly cultivar, irrigation, nitrogen fertilization, delayed harvest, and storage conditions (Cavaliere *et al*., 2010; Simonetti *et al*., 2014). Polar solvents as ethanol, methanol, or their water mixtures are suitable to efficiently recover both oligomeric and polymeric flavan-3-ols from grape seeds (Pinelo *et al*., 2005). To date, different analytical methods have been suggested for their qualitative and quantitative determination (Fontana *et al*., 2013).

In the present study, GSEs, coming from several table and wine *V. vinifera* cultivars, against collection strains of *Trichophyton mentagrophytes*, *Microsporum gypseum, Microsporum canis,* and *Malassezia furfur* have been tested. A relationship between fungal inhibition and chemical composition, in terms of monomeric and polymeric flavan-3-ols, has been found.

MATERIALS AND METHODS

Plant material. Mature grapes, grown as described in Simonetti and collaborators (2014) during vintages 2009–2012, were collected from different cultivars of *V. vinifera*: Michele Palieri (MP), Italia (ITA), Red Globe, Victoria, Negroamaro, and Pinot. The vines were grown in the experimental farm of CREA-UTV in Turi (Bari, Italy). The same cultural practices were carried out in this vineyard all the time (Simonetti *et al.*, 2014). The vines were treated with reduced irrigation volume per hectare (1200 m^3) (V1) or (2000 m^3) (V2) and with reduced nitrogen fertilization (120 kg/ha) (N1) or with 180 kg/ha (N2), which is the quantity generally used in the growing area. The grapes were harvested at technological maturation and put in small boxes (30 × 40 cm), 5 kg of weight each. The seeds were separated from the flesh and the skin, weighed and immediately frozen, and stored at −20 °C. For each cultivar, about 10 kg of grapes were used and the yields in fresh seeds were close to 0.69 kg.

Extraction and HPLC/DAD/ESI/MS analysis. Grape seed extracts were obtained in a solid–liquid process. The seeds (vintages 2009–2012) were separated from the flesh and the skin, weighed, put in liquid nitrogen

Table 2. Chemical composition of grape seed extracts obtained, from Michele Palieri (MP) and Italia (ITA) cultivars (vintage 2012), using different extractive solvents

	MP2012A*	MP2012B*	MP2012C*	ITA2012A*	ITA2012B*	ITA2012C*
mg/g dried extract						
Procyanidin dimer	4.7	2.5	2.6	3.4	2.0	2.3
Catechin	30.7	20.0	19.8	11.9	7.8	8.5
Procyanidin B2	7.7	5.0	5.2	5.3	3.1	3.9
Epicatechin	29.5	19.0	18.4	13.0	8.2	9.6
Epicatechin gallate	0.0	0.0	0.0	8.3	5.3	6.3
Polymer 1	240.5	148.0	156.0	268.4	166.1	166.5
Polymer 2	70.3	77.0	86.2	152.4	106.1	97.3
Total flavan-3-ols	383.4	272.0	288.2	462.8	298.5	294.4

The data are expressed in terms of flavan-3-ols defined by HPLC/DAD/ESI/MS. Polymer 1 and Polymer 2 (polymeric flavan-3-ols with degree of polymerization ≥4, determined by mass spectrometry).
*A = AEtOH/H_2O (7:3 v/v), B = EtOH, C = MeOH.

Table 3. Anti-*Malassezia* activity of grape seed extracts obtained, from Michele Palieri (MP) and Italia (ITA) cultivars (vintage 2012), using different extraction solvents

	Malassezia furfur							
	MIC_{50} (μg/mL)				MIC_{100} (μg/mL)			
	DSM6170	IMRMC529	IMRMC227	GM MIC_{50}	DSM6170	IMRMC529	IMRMC227	GM MIC_{100}
MP 2012 A*	16	32	64	32	128	32	64	64
MP 2012 B*	64	256	256	161	256	256	256	256
MP 2012 C*	32	32	128	51	128	64	128	102
ITA 2012 A*	16	32	64	32	128	32	128	81
ITA 2012 B*	32	128	128	81	256	256	256	256
ITA 2012 C*	16	128	256	81	128	128	512	203
FLZ	8	8	32	14	32	32	>64	55

Antifungal activity was determined according to Clinical and Laboratory Standards Institute guidelines (CLSI document M38-A2, 2008). The values shown are the median from three independent determinations. GM of minimum inhibitory concentration. MIC_{50} and MIC_{100} = lowest drug concentration that prevented 50% and 100% of growth with respect to the untreated control FLZ. FLZ, fluconazole; GM, geometric mean; MIC, minimal inhibitory concentration.
*A = AEtOH/H_2O (7:3 v/v), B = EtOH, C = MeOH.

in a porcelain mortar, and ground to obtain a fine powder. They were extracted three times (24 h for each extraction) with EtOH/H_2O (7:3 v/v) acidified with formic acid at pH 3; the ratio matrix/solvent was 1 g/10 mL. The extracts were dried by a rotavapor at 30 °C, then redissolved in exact volumes of EtOH/H_2O (7:3 v/v), centrifuged at 12,000 rpm for 5 min and the supernatant analysed by HPLC (Simonetti *et al.,* 2014). The seeds of MP and ITA cultivars (vintage 2012) were extracted with EtOH (*B*) or MeOH (*C*) as well as with EtOH/H_2O (*A*), applying a matrix/solvent ratio of 1 g fresh weight/10 mL. The use of alcohols, without the presence of water during the extraction can guarantee a more rapid drying process useful for industrial applications.

The HPLC/DAD/ESI/MS analysis was carried out using a liquid chromatographic HP 1100 L equipped with a diode array detector (DAD) detector and an electrospray (ESI) HP 1100 Mass Detector Spectrometer (MDS) mass detector with an API (Agilent Technologies; Santa Clara-California-USA).

The operative conditions of the mass spectrometer were as follows: gas temperature of 350 °C, nitrogen flux 10 L/min, nebulizer pressure 30 psi, quadrupole temperature 30 °C, capillary voltage 3000–4000 V, negative and positive ionization with fragments 80–220 V. The column was a Poroshell C_{18}, 3 mm ID × 150 mm, 2.7 µm (Agilent Technologies; Santa Clara-California-USA) and the gradient elution was in keeping with our previous work (Simonetti *et al.* 2014).

The quantitative data for monomer, oligomers, and polymers were obtained by DAD at 280 nm using procyanidin B2 as external standard in a concentration range 0.2–5.7 µg, to build a five-point calibration curve with $r^2 = 0.999$. The pure catechin, epicatechin, gallate-epicatechin, procyanidin B1, and procyanidin B2 were purchased from Extrasynthese Genee (France) and used for the flavan 3-ols identification.

Fungal strains. The evaluation of the antifungal activity was carried out with strains coming from: German Collection of Microorganisms (DSMZ, Braunschweig, Germany), the Pharmaceutical Microbiology Culture Collection (PMC, Sapienza University, Rome, Italy) and Instituto de Medicina Regional, Micologia Culture Collection (IMRMC, Departamento de Micologia, Universidad Nacional del Nordest, Argentina). The strains Deutsche Sammlung von Mikroorganismen (DSM) coming from DSMZ were *T. mentagrophytes* DSM 4870 and *M. furfur* DSM 6170. The strains coming from PMC were *T. mentagrophytes* (PMC 6503, PMC 6509, PMC 6515, PMC 6627, PMC 6552), *M. canis* (PMC 7426), and *M. gypseum* (PMC 7303, PMC 7331, PMC 7342). The strains coming from Instituto Medicina Regional - Micologia Collection (IMRMC) were *M. furfur* (IMRMC 529 and IMRMC 227).

Antifungal susceptibility testing. To evaluate the minimal inhibitory concentration (MIC), the susceptibility *in vitro* assay was performed on dermatophytes

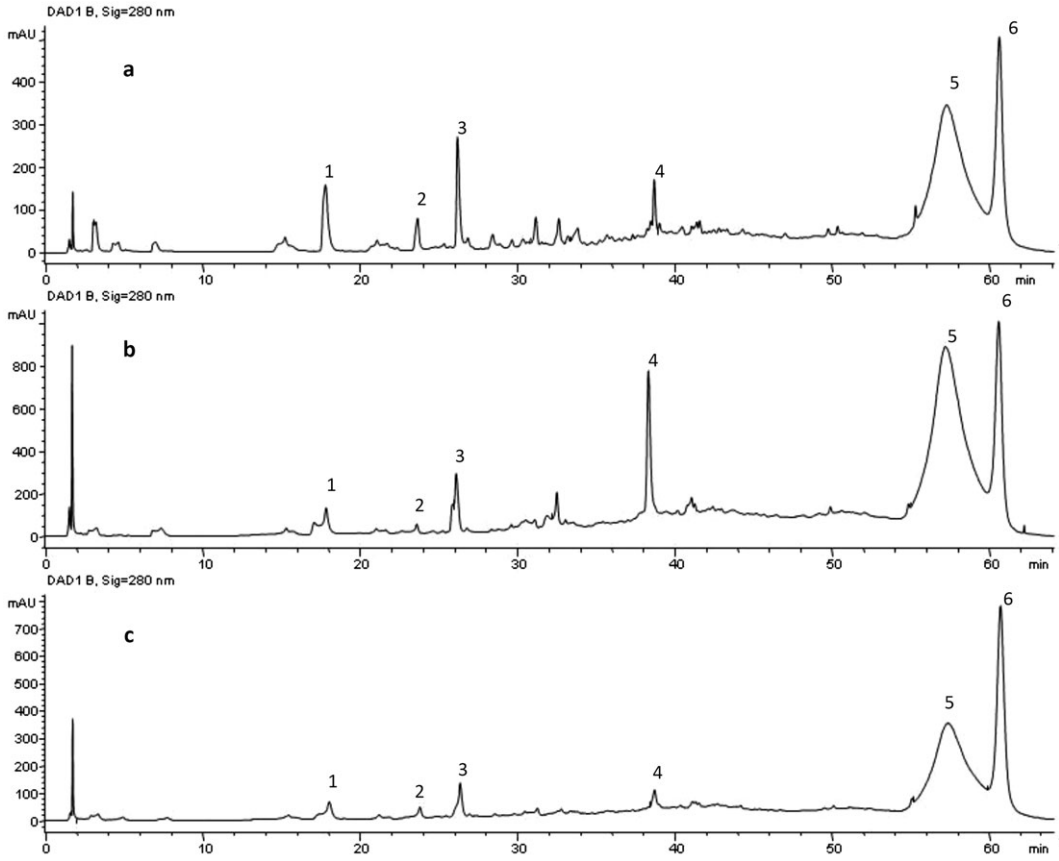

Figure 1. Chromatographic profiles at 280 nm of grape seed extracts from MP2011V2N1A (a), and RG2011V2N1A (b), and ITA2012A (c): 1, (+)–catechin; 2, procyanidin B2; 3, (−)epicatechin; 4, epicatechin gallate; 5, Polymer 1, and 6, Polymer 2 (polymeric flavan-3-ols with degree of polymerization ≥4, determined by mass spectrometry).

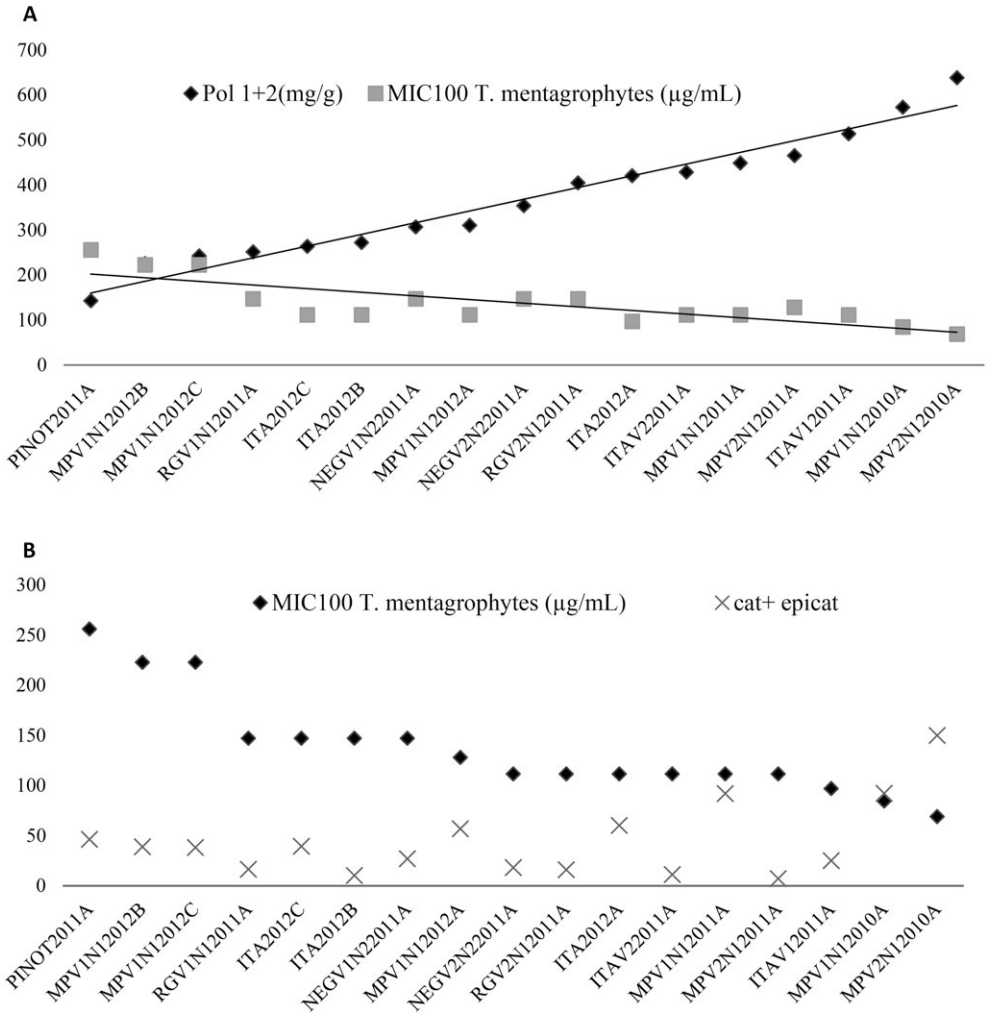

Figure 2. A) GM MIC_{100} values of 17 grape seed extracts from *Vitis vinifera* (2010–2012 vintages) tested against five *Trichophyton mentagrophytes* strains (PMC6503, PMC6515, PMC6552, PMC6509, DSM4870) and their correlation with polymeric content. Pol 1 + 2 = Polymer 1 and Polymer 2 (polymeric flavan-3-ols with degree of polymerization ≥4, determined by mass spectrometry). GM MIC_{100} = geometric mean of lowest drug concentration that prevented 100% of growth with respect to the untreated control. H_2O volume per hectare: V1 = 1200 m^3; V2 = 2000 m^3. Nitrogen fertilization: N1 = 120 kg/ha; N2 = 180 kg/ha. B) GM MIC_{100} values related to the catechin and epicatechin amounts in 17 grape seed extracts against five *T. mentagrophytes* strains (PMC6503, PMC6515, PMC6552, PMC6509, DSM4870). GM MIC_{100} = geometric mean of lowest drug concentration that prevented 100% of growth with respect to the untreated control. H_2O volume per hectare: V1 = 1200 m^3; V2 = 2000 m^3. Nitrogen fertilization: N1 = 120 kg/ha; N2 = 180 kg/ha. GM, geometric mean; ITA, Italia; MIC, minimal inhibitory concentration; MP, Michele Palieri; NEG, Negroamaro; RG Red Globe.

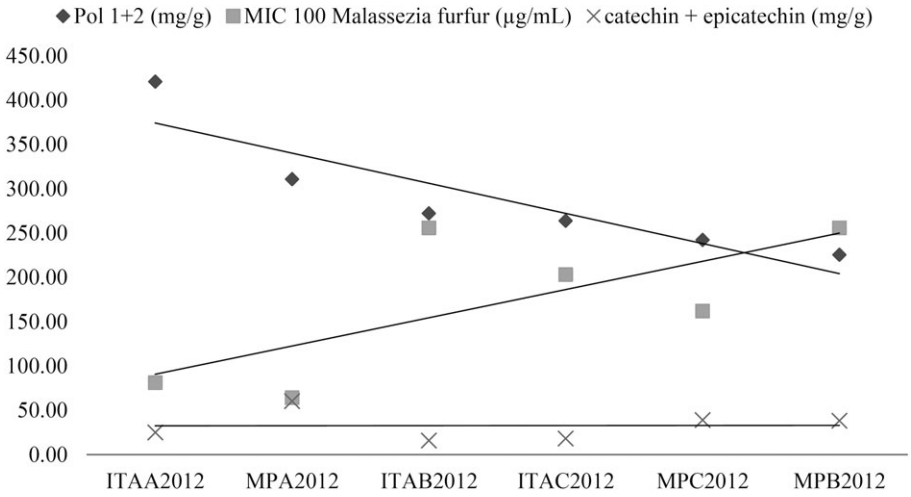

Figure 3. The GM MIC_{100} values of six grape seed extracts from *Vitis vinifera* (2012 vintage) tested against three *Malassezia furfur* strains (DSM6170, IMRMC529, IMRMC227) and their correlation with polymeric content. Pol 1 + 2 = Polymer 1 and Polymer 2 (polymeric flavan-3-ols with degree of polymerization ≥4, determined by mass spectrometry) and with catechin and epicatechin amounts. GM MIC_{100} = geometric mean of lowest drug concentration that prevented 100% of growth with respect to the untreated control. GM, geometric mean; ITA, Italia; MIC, minimal inhibitory concentration; MP, Michele Palieri.

according to standardized methods for filamentous fungi (CLSI. M38-A2, 2008).

Antifungal susceptibility of *M. furfur* was determined according to standardized methods for yeast using the broth microdilution method with some modifications (CLSI M27-A3, 2008; CLSI, 2012; Simonetti *et al.,* 2016). Dermatophytes were grown on potato dextrose agar (Sigma Aldrich, St. Louis, Missouri, USA) at 28–30 °C until good conidial growth was present. The conidia suspension was prepared at final concentration of 10^3–3×10^3 CFU/mL (CLSI. M38-A2, 2008). *M. furfur* strains were grown on modified Dixon agar at 32 °C for 4 days. The final concentration of the inoculum was 1–5×10^6 CFU/mL. The *in vitro* antifungal susceptibility was evaluated using GSEs and, as reference drugs, fluconazole, and terbinafine (Sigma Aldrich, St. Louis, Missouri, USA). The concentration of GSEs ranged from 512 to 0.5 μg/mL. The concentration of fluconazole and terbinafine ranged from 128 to 0.125 μg/mL and from 8 to 0.0078 μg/mL, respectively. The MIC_{50} was the lowest concentration of GSEs or reference drugs that caused ≥50% growth inhibition; the MIC_{80} was the lowest concentration that caused 80% growth inhibition, and the MIC_{100} was the lowest drug concentration that inhibited 100% of growth.

Statistical analysis. The antifungal activity shown is the result of three independent experiments performed in duplicate. The data were presented as median and geometric means.

In order to relate the phenolic content and, the MIC values of different dried GSEs, obtained from selected cultivars of *V. vinifera*, Pearson's correlation coefficient (r) was determined. The correlation coefficient close to one indicates that the variables are positively and linearly related; on the opposite −1 indicates variables negatively related. The zero value indicates weak relationship between the variables, a correlation less than 0.5 is weak.

RESULTS AND DISCUSSION

Grape seed extracts, coming from several wine and table *V. vinifera* cultivars, against collection strains of *T. mentagrophytes*, *M. gypseum, M. canis,* and *M. furfur* have been tested.

Regarding the anti-dermatophyte activities, the results showed that the GM MIC_{80} values ranged from 20 to 97 μg/mL (Table 1), and that the activity of the extracts was lower than terbinafine but comparable with that of fluconazole. Among the examined samples, MP and ITA extracts showed the highest and reproducible antifungal activity across the time as compared with the other cultivars with GM MIC_{80} values from 20 to 37 μg/mL and from 23 to 28 μg/mL, respectively (Table 1). On the contrary, the lowest activity was observed for the Pinot extract (GM MIC_{80} = 97 μg/mL).

With the aim to obtain dry samples to be used in future topical formulations, MP and ITA seeds, which showed the best antifungal activity against dermatophytes, were selected to further experiments and extracted with ethanol (B) or methanol (C) as well as with EtOH/H_2O (A). Even if the chemical profiles obtained with the three solvents were qualitatively very similar, ethanol/water (A) was the most effective to recover the polymeric flavan-3-ols both in MP and ITA extracts (Table 2). The extracts obtained with ethanol/water (A) were more active against dermatophytes than those obtained with ethanol or methanol (Table 1). The same results were observed against *M. furfur* showing the best activities with MP2012A and ITA2012A extracts (GM MIC_{50} = 32 μg/mL) (Table 3). The different activities could be attributed to the different content in flavan-3-ols of the extracts as previously reported for *Candida* spp. In the same study, we demonstrated that the antifungal activity of GSEs was mostly related to the presence of flavan-3-ols with a polymerization degree ≥4, and that this result was influenced by reduced irrigation and reduced nitrogen fertilization (Simonetti *et al*., 2014). To evaluate the differences among the GSEs and to separate flavan-3-ol monomers from polymers, the poroshell column was used, in keeping with our previous work (Simonetti *et al*., 2014). Chromatographic profiles of MP2011V2N1A, RG2011V2N1A, and ITA2012A extracts are shown in Fig. 1. The MIC_{100} values of all extracts tested against *T. mentagrophytes* and *M. furfur* have been correlated with the content of monomeric and polymeric flavan-3-ols. A negative correlation coefficient between polymeric flavan-3-ols and GM MIC_{100} has been demonstrated for *T. mentagrophytes* (r = −0.7639) (Fig. 2A) and for *M. furfur* (r = −0.72286) (Fig. 3), highlighting that these compounds exert a positive action on the antifungal activity. Differently, the antifungal activity against *T. mentagrophytes* was not correlated to the content of flavan-3-ol monomers (r = 0.2920) (Fig. 2B) and only weakly correlated for *M. furfur* (r = −0.53604) (Fig. 3).

It should be emphasized that extracts from grapes are included in several topical cosmetic formulations acting as potent antioxidants (Soto *et al*., 2015). Moreover, they stabilize collagen and elastin, thereby improving the elasticity, flexibility, and appearance of the skin (Madhan *et al*., 2005). GSEs are generally recognized as safe, approved by Food and Drug Administration. Moreover, MP2009V1N1A extract did not exhibit any cytotoxic activity on human monocytic cells (U937) (CC50 > 500 μg/mL) (Pasqua *et al*., 2010). All these properties, together with the high antifungal activity and lack of toxicity (Fiume *et al*., 2014) support the use of grape seed extracts, rich polymeric flavan-3-ols, in the treatment of skin infections caused by dermatophytes and *Malassezia*.

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Conflict of Interest

The authors have declared that there is no conflict of interest.

REFERENCES

Cavaliere C, Foglia P, Marini F, Samperi R, Antonacci D, Laganà A. 2010. The interactive effects of irrigation, nitrogen fertilization rate, delayed harvest and storage on the polyphenol content in red grape (*V. vinifera*) berries: a factorial experimental design. *Food Chem* **122**: 1176–1184.

CLSI. Clinical and Laboratory Standards Institute. 2008.Reference method for broth dilution antifungal susceptibility testing of filamentous fungi; Approved Standard—Second Edition M38–A2.

CLSI. Clinical and Laboratory Standards Institute. 2008.Reference method or broth dilution antifungal susceptibility testing of yeasts; Approved Standard—Third Edition M27-A3.

CLSI. Clinical and Laboratory Standards Institute. 2012.Reference method for broth dilution antifungal susceptibility testing of yeasts; Fourth Informational Supplement M27–S4.

Ćurko N, Ganić KK, Gracin L, Dapić M, Jourdes M, Teissedre PL. 2014. Characterization of seed and skin polyphenolic extracts of two red grape cultivars grown in Croatia and their sensory perception in a wine model medium. *Food Chem* **145**: 15–22.

Daglia M. 2012. Polyphenols as antimicrobial agents. *Curr Opin Biotech* **23**: 174–181.

Di Vito M, Mattarelli P, Modesto M, *et al.* 2015. *In vitro* activity of tea tree oil vaginal suppositories against *Candida* spp. and probiotic vaginal microbiota. *Phytother Res* **29**: 1628–1633.

Filocamo A, Bisignano C, Mandalari G, Navarra M. 2015. *In vitro* antimicrobial activity and effect on biofilm production of a white grape juice (*Vitis vinifera*) extract. *Evid Based Complement Alternat Med* **2015**: ID 856243. doi:10.1155/2015/856243

Fiume MM, Bergfeld WF, Belsito DV, *et al.* 2014. Safety assessment of *Vitis vinifera* (Grape)-derived ingredients as used in cosmetics. *Int J Toxicol* **33**: 48S–83S.

Flores FC, Beck RCR, da Silva CB. 2016. Essential oils for treatment for onychomycosis: a mini-review. *Mycopathologia* **181**: 9–15.

Fontana AR, Antoniolli A, Bottini R. 2013. Grape pomace as a sustainable source of bioactive compounds: extraction, characterization, and biotechnological applications of phenolics. *J Agric Food Chem* **61**: 8987–9003.

Harada K, Saito M, Sugita T, Tsuboi R. 2015. *Malassezia* species and their associated skin diseases. *J Dermatol* **42**: 250–257.

Havlickova B, Czaika VA, Friedrich M. 2008. Epidemiological trends in skin mycoses worldwide. *Mycoses* **51**: 2–15.

Khosravi AR, Shokri H, Fahimirad S. 2016. Efficacy of medicinal essential oils against pathogenic *Malassezia* sp. isolates. *J Mycol Med* **26**: 28–34.

Liang Z, Yang Y, Cheng L, Zhong GY. 2012. Polyphenolic composition and content in the ripe berries of wild *Vitis* species. *Food Chem* **132**: 730–738.

Madhan B, Subramanian V, Rao JR, Nair BU, Ramasami T. 2005. Stabilization of collagen using plant polyphenol: role of catechin. *Int J Biol Macromol* **37**: 47–53.

Narduzzi L, Stanstrup J, Mattivi F. 2015. Comparing wild american grapes with *Vitis vinifera*: a metabolomics study of grape composition. *J Agric Food Chem* **63**: 6823–6834.

Ostrosky-Zeichner L, Casadevall A, Galgiani JN, Odds FC, Rex JH. 2010. An insight into the antifungal pipeline: selected new molecules and beyond. *Nat Rev Drug Discov* **9**: 719–727.

Pasqua G, Simonetti G, D'Auria FD, Santamaria AR, Antonacci D. 2010.Estratti ottenuti da semi e/o vinacce di *Vitis vinifera* e relativi impieghi come agenti antifungini. Patent ITRM20100636

Pasqua G, Simonetti G. 2016. Antimicrobial and antiviral activities of grape seed extracts. In Grape Seeds: Nutrient Content, Antioxidant Properties and Health Benefits, Rodríguez JML, Ruiz DF (eds). Nova Science Publishers: New York; 211–224.

Pfaller MA. 2012. Antifungal drug resistance: mechanisms, epidemiology, and consequences for treatment. *Am J Med* **125**: S3–S13.

Pinelo M, Rubilar M, Jerez M, Sineiro J, Núñez MJ. 2005. Effect of solvent, temperature, and solvent-to-solid ratio on the total phenolic content and antiradical activity of extracts from different components of grape pomace. *J Agric Food Chem* **53**: 2111–2117.

Simonetti G, Santamaria AR, D'Auria FD, *et al.* 2014. Evaluation of anti-*Candida* activity of *Vitis vinifera* seed extracts obtained from wine and table cultivars. *BioMed Res Int* **2014**: ID 127021. doi:10.1155/2014/127021

Simonetti G, Tocci N, Valletta A, *et al.* 2016. *In vitro* antifungal activity of extracts obtained from *Hypericum perforatum* adventitious roots cultured in a mist bioreactor against planktonic cells and biofilm of *Malassezia furfur*. *Nat Prod Res* **30**: 544–550.

Soto ML, Falqué E, Domínguez H. 2015. Relevance of natural phenolics from grape and derivative products in the formulation of cosmetics. *Cosmetics* **2**: 259–276.

Xia EQ, Deng GF, Guo YJ, Li HB. 2010. Biological activities of polyphenols from grapes. *Int J Mol Sciences* **11**: 622–646.

Williamson G, Manach C. 2005. Bioavailability and bioefficacy of polyphenols in humans. II. review of 93 intervention studies1– 4. *Am J Clin Nutr* **81**: 243S–255S.

White TC, Findley K, Dawson TL, *et al.* 2014. Fungi on the skin: dermatophytes and *malassezia*. *Cold Spring Harb Perspect Med* **4**: a019802. doi:10.1101/cshperspect.a019802

Zavrel M, White TC. 2015. Medically important fungi respond to azole drugs: an update. *Future Microbiol* **10**: 1355–1373.