Exploring the powerful phytoarsenal of white grape marc against bacteria and parasites causing significant diseases

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Plant extracts with high polyphenolic contents have shown to have antibacterial, antiparasitic and fungicidal activities. The present work describes the physicochemical features and antimicrobial capacities of bioactive extracts obtained from the white winemaking byproducts to give them a new use and valorisation. The white grape marc is rich in polyphenols. The health promoting and disease preventing benefits of different types of grape polyphenols are well documented (Simoes, 2009; Yadav, 2015). Phenolic acids, flavan-3-ols and their gallates, and flavonols and their glycosides, are the main phenolic constituents of white grape marc (Álvarez-Casas, 2014). Antioxidant dietary fibre as well as extractable and non-extractable polyphenol content in bagasse obtained during winemaking usually ranges from 50 to 75 %, 1 to 9 % and 15 to 30 % by dry matter, respectively. Due to this complex composition, grape marc extracts have a great potential to display extensive uses in agricultural, pharmaceutical and cosmetic industries among others (Bargiacchi, 2017; Placha, 2013; de O Ribeiro, 2018; Tayengwa, 2018). Bioactive polyphenols are extracted with hydro-organic mixtures from the byproduct of the production of high quality Albariño white wines (Galicia, NW Spain). In this work, we evaluated the "*in vitro*" antimicrobial activity of two extracts from Albariño bagasse using two different hydroorganic mixtures (HO_L & HO_P). Extracts were used against relevant microorganisms, including Gram positive and negative bacteria, two Apicomplexan parasite species and one Oomycota parasite. The microbial species studied in the present work are involved in several diseases in humans and animals, such as foodborne illness (*Bacillus cereus*, *Eschericha coli*, *Salmonella enterica*), skin infections (*Staphylococcus aureus*), mastitis (*Streptococcus uberis*), parasite infections as Malaria (*Plasmodium falciparum*) or Toxoplasmosis (*Toxoplasma gondii*), and plant infections as "chestnut ink" in chestnuts or "root rot" in avocado, both diseases caused by *Phytophthora cinnamomi*.

Material and methods:

Extracts production and polyphenolic evaluation: The extraction procedure is a green and straightforward process with few steps, under gentle conditions and using non-contaminating materials, while preventing the obtained eluates from containing suspended solids. Raw material is white grape marc from *Vitis vinifera* var. Albariño. Extract and process are patent-protected (Lores, 2014: ES 2 443 547; WO 2014/013122 A1) and can be obtained on lab, pilot or industrial scales.

Anti-bacterial assays: a cellular suspension of the microorganisms to test was incubated in different extract concentrations at 20, 10, 5, 2.5,1,25 and 0% at 37º for different times depending on the species. Time was adjusted to 3h for *E. coli* and *S. enterica*, to 2h for *B. cereus* and to 1,5h for *S. uberis* and *S. aureus.* Subsequently, colony forming units (CFU) were determined to calculate IC_{50} values.

Anti-parasitic assays: the growth inhibition of three species has been evaluated; one plant parasite, *P. cinnamomi* and two human and animal parasites, *T. gondii* and *P. falciparum*. ***P. cinnamomi*:** was grown on potato-dextrose-agar medium (PDA) with the different extract's concentrations at 10, 4 and 0% for 10 days at 22°C in the dark. The antifungal capacity has been determined according to the growth inhibition respect to the untreated cultures. ***T. gondii*:** Human foreskin fibroblasts (HFF) cells were infected with red-fluorescent *T. gondii* tachyzoites in culture medium DMEM. The extract concentrations used were 1, 0.5, 0.25, 0.125, 0.065 and 0% (v/v drug/medium) and controls of uninfected cells and the solvent were included. Cultures were incubated for 7 days at 37°C and fluorescence was read daily at 540 nm/590 nm ex/em. ***P. falciparum*:** *P. falciparum* 3D7 was cultured in RPMI 1640 in human red blood cells (RBCs). Parasites were cultured at 0.3% parasitemia and 2.5% haematocrit for 72h at 37ºC in culturing chambers with reduced oxygen. Infected RBCs were exposed to 1:2 dilutions of the extracts from a concentration of 2% to 0.004% (v/v) including no drug controls and solvent-only controls. SYBR green was used to measure viability and fluorescent signal was acquired in a plate reader using a 485-520nm filter.

Results and discussion:

Table 1 shows the composition of the polyphenols present in both extracts. Many polyphenols are shared in both extracts, but their total composition and concentration present significant differences that may be related to their

dissimilar behavior in the antimicrobial tests performed (Table 2). As indicated in table 2, for the majority of the microorganism species analyzed, the IC_{50} is lower than 2%.

Table 1. Main Polyphenols in white grape marc extracts. Concentration expressed in mg polyphenol/L extract (Testing method: LC-MS/MS Analysis).

Polyphenolic Compound	Extract HO_L (mg/L)	Extract HO_P (mg/L)	Polyphenolic Compound	Extract HO_L (mg/L)	Extract HO_P (mg/L)
Catechin	100.1	64.6	Procyanidine (total)	40.0	38.3
Epicatechin	54.9	38.3	Procyanidine B2	8.2	10.4
Epicatechin-gallate	13.0	10.4	Quercetin	2.9	0.15
Epigallocatechin-gallate	0.31	0.15	Isoquercetin	63.5	7.3
Gallic acid	4.7	7.3	Miquelianin	44.7	64.6
Cafeic acid	0.007	0.02	Rutin	3.0	38.3
Caftaric acid		21.6	Kaempferol	4.0	10.4
Phloroglucinic acid	35.7	64.6			

Table 2. Inhibitory concentration 50% (IC_{50}). Concentration expressed in % (v/v).

Species	IC_{50} (Extract HO_1)	IC_{50} (Extract HO_2)
Toxoplasma gondii	1,23	0,57
Plasmodium falciparum	1,07	0,26
Phytophthora cinnamomi	3,17	20,23
Staphylococcus aureus	<1,25	<1,25
Bacillus cereus	<1,25	<1,25
Eschericha coli	<1,25	<1,25
Streptococcus uberis	<1,25	1,980
Salmonella enterica subsp. enterica	<1,25	<1,25

Average values for triplicates were used to calculate IC_{50} concentrations using the software GraphPad Prism 5.0

Conclusions:

Both extracts are very rich in polyphenols and show high antimicrobial potential, being effective for several kinds of bacteria and parasites belonging to different clades, all of them of economical and global health importance. These results open up promising ways to valorise white grape marc, a by-product of wineries activity, by the potential application of the target extracts as preservatives in cosmetic and food industry, sanitizing agents and phytosanitary. In addition, it allows us to dream with a plant phenolics-based therapy against very concerning human diseases.

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