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Antioxidant, antimicrobial and anti-proliferative activities of *Solanum tuberosum* L. var. Vitelotte

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ABSTRACT

Solanum tuberosum L. var. Vitelotte is a potato variety widely used for human consumption. The pigments responsible for its attractive color belong to the class of anthocyanins. The objectives of this study were to characterize and measure the concentration of anthocyanins in pigmented potatoes and to evaluate their antioxidant and antimicrobial activities and their anti-proliferative effects in solid and hematological cancer cell lines. Anthocyanins exert anti-bacterial activity against different bacterial strains and a slight activity against three fungal strains. The Gram-positive bacterium *Staphylococcus aureus* and the fungus *Rhyzoctonia solani* were the most affected microorganisms. Antioxidant activities were evaluated by DPPH and FRAP methods; the extract showed a higher reducing capability than anti-radical activity. Moreover, we found that in different cancer cell models the anthocyanins cause inhibition of proliferation and apoptosis in a dose dependent manner. These biological activities are likely due to the high content of malvidin 3-O-*p*-coumaroyl-rutinoside-5-O-glucoside and petunidin 3-O-*p*-coumaroyl-rutinoside-5-O-glucoside.

1. Introduction

Solanum tuberosum L. var. Vitelotte is a potato variety with deep blue skin and violet flesh widely used for human consumption and well appreciated for its good nutritional characteristics. In recent years there has been an increasing interest in red- and purple-fleshed potato varieties because of their color appeal, outstanding taste and "mashability". There has been consumer interest in potatoes with colored flesh for use in salads and novelty crisps, especially since color is retained after cooking or frying (Lewis et al., 1999). The pigments responsible for the attractive color of these potatoes varieties are anthocyanins, whose concentration vary in large ranges and correlates with the degree of pigmentation in potato flesh. The pigments in colored potatoes have been identified as the 5-glucoside-3-rhamnosylglucoside derivatives of all the common anthocyanidins, monoacylated with *p*-coumaric or ferulic acids (Andersen et al., 2002; Harborne, 1960; Lewis et al., 1999). A good correlation between the total anthocyanins and the biological activities of the tubers was found. It is known that these pigments are rapidly adsorbed at the stomach level (Passamonti et al., 2003). Recently, *in vivo* absorption of anthocyanins from an extract of *Ipomea batatas* L. was also demonstrated in rats and humans after their addition to the diet. Acylated anthocyanins, the main constituents of potato, were detected in plasma and urine after ingestion (Harada et al., 2004). Anthocyanins are known to possess different pharmacological properties and are used by humans for therapeutic purpose (Kong et al., 2003). More and more

Abbreviations: AML, Acute Myeloid Leukemia; APL, Acute Promyelocytic Leukemia; COX-2, cyclooxygenase-2; DPPH, Free Radical Scavenging Ability; FAB, French-American-British; FAS, TNF receptor superfamily, member 6; FASL, FAS ligand; FRAP, Ferric Reducing/Antioxidant Power; HPLC-DAD, high performance liquid chromatography-diode array detector; HPLC–ESI–MS, high performance liquid chromatography- electrospray ionization mass spectrometry; MBC, Minimum Bactericidal Concentration; MIC, Minimal inhibitory concentration; NF-κb, Nuclear Factor-kappa B; PML-RAR, Promyelocytic Leukemia-Retinoic Acid Receptor; ROS, Reactive Oxygen Species; TE, Trolox Equivalent; TNF-α, Tumor Necrosis Factor alpha; VEGF, Vascular Endothelial growth factor.

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studies show that anthocyanins have demonstrated ability to protect against a myriad of human diseases such as liver dysfunction, hypertension, vision disorders, microbial infections, and diarrhea (Rice-Evans and Packer, 1998; Smith et al., 2000; Wang et al., 2000). Due to this fact, a high intake of anthocyanin-rich food has been linked to health preventive effects and reduced risk of pathologies such as aged-related macular degeneration, cancer or cardiovascular disorders. Indeed, the consumption of anthocyanins lowers the risk of cardiovascular disease, diabetes, arthritis and cancer due, at least in part, to their anti-oxidant and anti-inflammatory activities (Afaq et al., 2005; Huang et al., 2002; Prior and Wu, 2006; Reddy et al., 2005; Rodrigo et al., 2006). The anticancer action of anthocyanins has also been reported. Pure anthocyanins and anthocyanin-rich extracts from fruits and vegetables have exhibited anti-proliferative activity towards multiple cancer cell types *in vitro* (Rodrigo et al., 2006; Zhang et al., 2005). Cell proliferation was inhibited by the ability of anthocyanins to block various stages of the cell cycle by acting on regulatory proteins (e.g., p53, p21, p27, cyclin D1, cyclin A, etc.). Interestingly, several investigations have compared the antiproliferative effects of anthocyanins on normal vs. cancer cells and found that they selectively inhibit the growth of cancer cells with relatively little or no effect on the growth of normal cells (Galvano et al., 2004; Hakimuddin et al., 2004). Furthermore, anthocyanin-rich extracts from berries and grapes, and several pure anthocyanins and anthocyanidins, have exhibited pro-apoptotic effects in multiple cell types *in vitro* (Afaq et al., 2007; Chen et al., 2005; Martin et al., 2003; Olsson et al., 2004; Reddivari et al., 2007; Seeram et al., 2006), acting via both the mitochondrial and the extrinsic death pathways (Chang et al., 2005; Reddivari et al., 2007). In the intrinsic pathway, anthocyanin treatment alters mitochondrial membrane potential, leading to cytochrome *c* release and modulation of caspase-dependent apoptosis. In the extrinsic pathway, anthocyanins modulate the expression of FAS and FASL (FAS ligand) in cancer cells. In addition, treatment of cancer cells, but not normal cells, with anthocyanins leads to an accumulation of ROS (reactive oxygen species) and subsequent apoptosis, suggesting that the ROS-mediated mitochondrial caspase-independent pathway is important for anthocyanin-induced apoptosis (Feng et al., 2007). Not surprisingly, anthocyanins also exhibit antioxidant and anti-inflammatory effects. Inflammation has been shown to play a role in the promotion of some types of cancer in animals, and probably in humans (Kwon et al., 2007). Abnormal up-regulation of two inflammatory proteins, nuclear factor-kappa B (NF-κB) and cyclo-oxygenase-2 (COX-2), is a common occurrence in many cancers, and inhibitors of these proteins usually exhibit significant chemo-preventive potential (Chang et al., 2005;Martin et al., 2003). Interestingly, through their ability to inhibit the mRNA and/or protein expression levels of COX-2, NF-κB and various interleukins, the anthocyanins have exhibited anti-inflammatory effects in multiple cell types *in vitro* (Afaq et al., 2005; Huang et al., 2002; Reddy et al., 2005;Rodrigo et al., 2006). In addition, the anticancer potential can also be correlated to the anthocyanins anti-angiogenic effects that have been investigated in endothelial (Bagchi et al., 2004), oral cancer (Rodrigo et al., 2006) and mouse epidermal JB6 cells (Huang et al., 2006). Anthocyanins have been shown to suppress angiogenesis through several mechanisms such as: inhibition of H_2O_2-and tumor necrosis factor alpha (TNF-α)-induced VEGF expression in epidermal keratinocytes (Bagchi et al., 2004), and by reducing VEGF and VEGF receptor expression in endothelial cells. Anthocyanin extracts (2.5–100 μM) from different berry types, black rice and eggplant have been evaluated for their ability to inhibit the invasion of multiple cancer cell types in Matrigel (Coates et al., 2007; Nagase et al., 1998). Prevention and treatment of cancer through the induction of cellular differentiation offers a cell-specific approach to cancer prevention and treatment that is likely to be less toxic than standard radio/chemotherapy (Fimognari et al., 2004).

The objectives of this study were to characterize and measure the concentration of anthocyanins in pigmented potatoes and to evaluate their antioxidant, antibacterial and antifungal activities and their anti-proliferative effects in solid and hematological cancer cell lines. Our results indicate the therapeutic value of *S. tuberosum* extracts and its contained anthocyanins as potential antimicrobial, antioxidant and anticancer agents.

2. Materials and methods

2.1. Chemicals

DPPH (1,1-diphenyl-2-picrilhydrazyl), 2,4,6-tris-2,4,6-tripiridyl-2-triazine (TPTZ), ferric chloride dry, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), L-ascorbic acid, tert-butyl-4-hydroxy toluene (BHT), gallic acid, were purchased from Sigma Chemical Co. (St. Louis, MO). Methanol (RPE) was purchased from Carlo Erba (Milano, Italy).

2.2. Plant material

S. tuberosum L. var. Vitelotte (718 g) was collected at Nusco (Avellino) in Southern Italy, on June 2010. All the tubers had pigmented peel and pigmented flesh. They were washed in running tap water, cut into pieces of approximately 0.5 cm, dried in a heated air drier, and then pulverized by the disintegrator. After drying the weight of potatoes was 157 g. Samples were kept at 4 °C.

2.3. Preparation of anthocyanins extract

Several extraction methods have been proposed to obtain extracts rich in anthocyanin, usually based on solvents as methanol, ethanol, acetone, water or mixtures. The addition of a small amount of hydrochloric acid or formic acid is recommended to prevent the degradation of the non-acylated compounds (Kjell and Øyvind, 2005). The optimum condition for anthocyanin extraction of potatoes was determined according to a previous paper (Fan et al., 2008).

Pigmented potato powder was put into a 50 ml conical flask, then added in acid–ethanol (HCl, 1.5 mol/l) with a solid–liquid ratio 1:32 and put in thermostatic water bath at a selected temperature (80 °C) for 60 min, then, centrifuged at 4000 rpm for 15 min. The supernatant was collected and transferred into a 50 ml volumetric flask for the determination of anthocyanins yield. About 1 g of the samples was used for each treatment.

2.4. Antimicrobial activity assays Microorganisms

Nine bacterial strains from the American Type Culture Collection (ATCC; Rockville, MD, USA) were employed. They included Gram-positive (G+) bacteria: *Staphylococcus aureus* (ATCC 13709) and *Enterococcus faecalis* (ATCC 14428), and the following Gram-negative (G−) bacteria: *Proteus mirabilis* (ATCC 7002), *Proteus vulgaris* (ATCC 12454), *Pseudomonas aeruginosa* (ATCC 27853), *Salmonella typhi* (ATCC 19430), *Enterobacter aerogenes* (ATCC 13048), *Enterobacter cloacae* (ATCC 10699), and *Klebsiella pneumoniae* (ATCC 27736). The same bacterial strains, clinically isolated, were used to compare the sensitivity to the extract.

The extract was added with 5×10^{-2} M stock solution in DMSO, and diluted from 0.01 to 1000 μg/ml concentrations in sterile physiological Tris buffer (pH 7.4, 0.05 M) (Ieven et al., 1979) immediately before being used.

2.5. MIC and MBC determination

Bacterial strains were grown on MH agar plates (DIFCO, Detroit, MI) and suspended in MH (Mueller Hinton) broth (DIFCO). The MIC values against bacterial strains were performed using the Ericcson and Sherris (1971) broth-dilution method (MH broth). The inoculum suspensions were prepared from 6 h broth cultures and adjusted to obtain a 0.5 McFarland standard turbidity. The extract was sterilized by filtration through Millipore filters (0.45 μm) and added to MH broth medium. Serial 10-fold dilutions were made for a concentration range between 0.01 and 1000 μg/ml. In the range between the minimum active and the maximum inactive concentrations were tested 2-fold dilutions to obtain a more precise measure of the MIC. The bacterial suspensions were aerobically incubated for 24 h at 37 °C. The MIC was defined as the lowest concentration able to inhibit any visible bacterial growth. Cultures containing only sterile physiological TRIS buffer (pH 7.4, 0.05 M), which did not influence bacterial growth, were used as controls. The MIC values were also determined for tetracycline hydrochloride (Pharmacia, Milano), benzyl penicillin sodium (Cynamid, Catania) and cefotaxime sodium (Roussel Pharma, Milano) in MH broth using standard method.