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Phenolic content and biological activity of extracts of blackcurrant fruit and leaves

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ABSTRACT

The studies were designed to determine the polyphenolic composition and biological activity of extracts from fruit and leaves of blackcurrant in relation to biological and lipid membranes. A detailed quantitative and qualitative analysis of extracts was conducted, using the UPLC-DAD and UPLC-PDA–Q/TOF-MS methods. Furthermore, stability of the phenolic content in the extracts was determined using the Folin–Ciocalteu method. The antioxidant activity of the extracts in relation to the membrane of erythrocytes and lipids extracted from red blood cell membranes (RBCL) exposed to chemical oxidizing agents (AAPH) was determined, and the effects of blackcurrant extracts on the properties of the membrane of erythrocytes and liposomes (DPPC and RBCL) were examined. Antioxidant activity of the extracts was studied fluorometrically, while effects of the extracts on the properties of membranes were examined using calorimetric, IR spectroscopy and fluorimetric methods. In particular, the effects of the extracts on packing order, membrane fluidity and the main phase transition temperature were determined. Additionally, the affinity of extracts to organic or aqueous media was determined on the basis of their partition coefficient between octanol and phosphate buffer. The results showed that the tested extracts are rich sources of polyphenols, primarily from the group of flavonoids; in leaves flavonols dominate, while in fruit anthocyanins dominate. Their polyphenolic content was quite stable and only slightly changed within 12 months. The substances used markedly protect the membranes against oxidation and to varying degrees modify properties of the hydrophilic regions of membranes. They localize mainly in the area of lipid polar heads of membrane, changing their arrangement, which is consistent with their disclosed hydrophilic character. Due to the rich polyphenolic composition and high antioxidant activity, extracts from the leaves and fruits of blackcurrant protect the organism or food products from the harmful effects of free radicals. In addition, they slightly change the properties of biomembrane lipids and lipid vesicles once they incorporate these extracts. This kind of knowledge is not only of pro-health importance, but can also be used in the food, pharmaceuticals or cosmetics industry, for designing liposomal structures as carriers of drugs, dietary supplements and extracts as biologically active substances.

1. Introduction

Modern medicine cannot provide effective treatment of various dangerous diseases, including civilizational ones. Scientific studies show that a cause of serious diseases, including Alzheimer's disease, Parkinson's disease, cancer and others, is the oxidative stress caused by an excess of free radicals in the organism (Bullon, Newman, & Battino, 2014; Montezano & Touyz, 2014; Pitocco, Tesauro, Alessandro, Ghirlanda, & Cardillo, 2013). The radicals arise in physiological processes and as a result of physicochemical factors of the body. Therefore, intensive research is focused on the search for new efficient protective substances, of plant origin in particular. Numerous studies have shown that plant polyphenols are effective scavengers of free radicals. Another advantage of plant substances is the fact that they have high biological activity (Choudhary & Swarnkar, 2011; Zhang et al., 2011).

Abbreviations: AA, L(+) ascorbic acid; AAPH, 2,2′-azobis (2-amidinopropane) dihydrochloride; BCFs, blackcurrant fruits; BCLs, blackcurrant leaves; BHA, butylated hydroxyanisole; DPH, 1,6-diphenyl-1,3,5-hexatriene; DPPC, 1,2-dipalmitoyl-sn-glycero-3-phosphatidylcholine; DSC, differential scanning calorimetry; FTIR, Fourier transform infrared spectroscopy; GAE, gallic acid; Laurdan, 6-dodecanoyl-2-dimethylaminonaphthalene; MLVs, multilamellar liposomes; TMA-DPH, 1-(4-trimethylammonium-phenyl)-6-phenyl-1,3,5-hexatriene p-toluenesulfonate; Prodan, 6-propionyl-2-dimethylaminonaphthalene; RBCL, lipids extracted from erythrocyte membrane; SUVs, small unilamellar liposomes.

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These substances also exhibit, apart from antioxidant activity, a number of health-supporting properties: anti-inflammatory, anticancer, and antimycosis (Betts, Wareham, Haswell, & Kelly, 2013; Gonzalez-Vallinas, Gonzalez-Castejon, Rodriguez-Casado, & Ramirez de Molina, 2013). Thus they exhibit a range of protective properties, which allows them to be classified as a group of modern medicines (Kashani, Hoseini, Nikzad, & Aarabi, 2012). The mechanism responsible for this health protective effect of polyphenolic compounds is not yet fully understood; hence intensive studies are being conducted in this field worldwide. In order to clarify the mechanism of action of polyphenolic compounds at the molecular and cell level, it is necessary to conduct research also on simple models of well-known structure (Virgili & Marino, 2008). The first and main place of impact of various physico-chemical factors on the organism is the cell membrane and, therefore, it is important to determine the effects of polyphenolic compounds on membranes. Test results indicate that polyphenols can alter the properties of biological membranes by changing both the protein and lipid phase (Hendrich, 2006; Pawlikowska-Pawlęga et al., 2007). These compounds interacting with cellular membranes exhibit protective effects in relation to cells, e.g. erythrocytes (Cyboran, Oszmiański, & Kleszczyńska, 2013), hepatocytes (Kim et al., 2012), human colon epithelium and myofibroblast cells (Tomczyk et al., 2013). Modification of membrane properties by these substances can also lead to impaired functioning of cells, including inhibition of activity of mitochondrial enzymes (Hodnick, Duval, & Pardini, 1994), aggregation of erythrocyte membrane proteins (Chen, Wang, Zhang, Ren, & Zeng, 2011), and DNA damage in mouse spleen cells (Fan & Lou, 2008). Therefore, it is important to determine the molecular mechanism of interaction of these substances with organisms, which will allow their safe and rational use.

Especially blackcurrant is of great interest among scientists compared to other plant substances, due to its content of polyphenolic compounds that exhibit a variety of healthy properties. The blackcurrant shrub is commonly cultivated in temperate climates around the world. Its fruits are a rich source of vitamins, macro- and microelements, organic acids, pectins and essential oils, as well as a range of polyphenolic compounds showing positive action on the organism (Brangoulo & Molan, 2011; Mattila et al., 2011; Molan, Liu, & Kruger, 2010; Tabart et al., 2012). The polyphenolic substances contained in fruits and leaves of blackcurrant have a protective action and support the treatment of many diseases. They exhibit anti-inflammatory, antifungal, antioxidant, probiotic and anticancer effects, among others (Bishayee et al., 2011; Cyboran, Bonarska-Kujawa, Oszmiański, & Kleszczyńska, 2011; Jia, Kong, Liu, Diao, & Xia, 2012; Kirsch, Ordogh, Galgoczy, Papp, & Vagvolgi, 2009; Molan et al., 2010; Sivam, Sun-Waterhouse, Perera, & Waterhouse, 2012; Szachowicz-Petelska, Dobrzyńska, Skrzydlewska, & Figaszewski, 2012). In addition, it has been documented that they improve the metabolism, regulate oxygen economy and improve vision (Matsumoto, Nakamura, Iida, Ito, & Ohguro, 2006; Matsumoto et al., 2005; McDougall, Kulkarni, & Stewart, 2009). In our previous work (Bonarska-Kujawa, Cyboran, Żyłka, Oszmiański, & Kleszczyńska, 2014; Cyboran et al., 2011) we have shown that extracts from the leaves and fruits of blackcurrant in varying degrees modify cells and the membranes of red blood cells. Compounds contained in them do not damage the membrane of erythrocytes but strengthen it. In addition, the compounds contained in the extracts induce changes in the shape of erythrocytes, becoming located primarily in the outer monolayer of the erythrocyte membrane. We have shown also that they effectively protect the membranes of red blood cells against free radicals induced by UVC radiation and AAPH compound. In order to better understand the mechanism responsible for the observed effects on the cells and membranes, in this study we carried out biophysical tests aiming to determine the effect of the extracts mainly on the lipid phase of membrane. Understanding this mechanism will make it possible to extend the range of their activities and the use of the substances both in medicine and in the food industry and cosmetics.

In the present study we conducted an analysis of the extracts and research was performed defining the biological activity of phenolic compounds contained in the leaves and fruits of blackcurrant in respect of biological membranes and lipid membrane models. Stability of compounds contained therein, and their physicochemical properties, are also specified. The main aim of the study was to determine their influence on the different model membranes, and in particular on the membrane lipid phase. Additionally, the antioxidant activity of the extracts was determined. This research was conducted on the membranes of red blood cells and on model membranes, i.e. liposomes created from DPPC and lipids extracted from erythrocyte membranes. Using chromatography, spectrophotometry, calorimetry and fluorimetry, the biological activity of the extracts, including antioxidative activity, was determined on the basis of the effects of the substances contained therein on the membranes.

2. Materials

Blackcurrant (*Ribes nigrum* L.) leaves and fruits were harvested from an experimental field of the Garden of Medicinal Plants herbarium of the Medical University of Wroclaw, Poland. Polyphenols were isolated from fruits and leaves by extraction with water containing 200 ppm of SO_2, the ratio of solvent to fruits (or leaves) being 3:1. The extracts were absorbed on Purolite AP 400 (UK) for further purification. The polyphenols were then eluted out with 80% ethanol, concentrated and freeze-dried. By means of the above method a mixture of polyphenols was obtained using the method described by Gąsiorowski et al. (1997).

The studies were conducted on isolated erythrocyte membranes (RBC), small unilamellar liposomes (SUVs) and multilamellar liposomes (MLVs). Pig erythrocyte membranes were obtained from fresh blood using the method described by Dodge, Mitchell, and Hanahan (1963). The content of erythrocyte membranes in the samples was determined on the basis of protein concentration, which was assayed using the Bradford method (1976), and it was 100 mg/ml. The choice of pig erythrocytes was dictated by the fact that this cell's percentage content of lipids is closest to that of the human erythrocyte, and the blood was readily available. Fresh blood was taken each time to a physiological solution of sodium chloride with heparin added.

Small unilamellar liposomes (SUVs) were composed of lipids extracted from erythrocyte membranes (RBCL) according to the method described by Maddy, Dunn, and Kelly (1972), and of DPPC purchased from Sigma Aldrich, Steinheim, Germany. All lipids were evaporated to dryness under nitrogen. Subsequently, a phosphate buffer of pH 7.4 was added and multilamellar liposomes (MLVs) were formed by mechanical shaking. Then SUVs were formed using a Sonics VCX750 sonicator (Sonics & Materials, Inc.).

The fluorescent probes Laurdan (6-dodecanoyl-2-dimethylaminonaphthalene), Prodan (6-propionyl-2-dimethylaminonaphthalene), DPH (1,6-diphenyl-1,3,5-hexatriene) and TMA-DPH (1-(4-trimethylammoniumphenyl)-6-phenyl-1,3,5-hexatriene p-toluenesulfonate) were purchased from Molecular Probes, Eugene, Oregon, USA. The Folin–Ciocalteu phenol reagent, 2,2′-diazobis (2-amidinopropane) dihydrochloride (AAPH), butylated hydroxyanisole (BHA), gallic acid (GAE) and L(+) ascorbic acid (AA) were purchased from Sigma-Aldrich, Inc., Steinheim, Germany.

3. Methods

3.1. UPLC/DAD and UPLC-PDA–Q/TOF-MS methods

The content of polyphenols in the extracts of blackcurrant leaves (BCL) and fruits (BCF) was determined by means of liquid chromatography (UPLC/DAD) and the method of UPLC-PDA–Q/TOF-MS analysis described by Oszmiański, Kolniak-Ostek, and Wojdyło (2013).

Identification of polyphenol in the extracts was carried out using the ACQUITY ultra-performance LC (UPLC) system with binary solvent