ANTIOXIDANT AND ANTIPROLIFERATIVE POTENTIAL OF ANTHOCYANIN-RICH FRACTION OBTAINED FROM COMERCIAL JUICES

POTENȚIALUL ANTIOXIDANT ŞI ANTIPROLIFERATIV AL FRACȚIEI BOGATE ÎN ANTOCIANI OBȚINUTĂ DIN SUCURI COMERCIALE

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Abstract. *The present study was performed to determine antiproliferative potential of anthocyanin-rich fraction obtained from commercial available juices on A2780 ovarian cancer line. Regarding antioxidant activity of analyzed juices against DPPH radical, chokeberry juice had stronger antioxidant activity than elderberry and beetroot juices. Also it was found that purified glycosilated cyanidin from elderberry and chokeberry could suppressed the A2780 cellular survival by 50% after a 24-h exposure. Purified betalanins from beetroot juice inhibited the proliferation of A2780 cells in a dose dependent manner. This effect was associated with bioactive photochemicals and antioxidant potential of anthocyanins and betalanins, compounds presents in our purified extract.*
Key words: *berries juice, antioxidant activity, cell viability, ovarian cancer*

REZUMAT. *Scopul acestui studiu a fost de a determina potențialul antiproliferativ al fracțiilor bogate în antociani obținute din sucuri comerciale, pe linia de celulară tumorală A2780 (cancer ovarian). În ceea ce privește activitatea antioxidantă a sucurilor analizate împotriva radicalului DPPH, sucul de aronia a demonstrat activitate antioxidantă mai puternică decât sucul de soc şi sfeclă roşie. De asemenea, s-a observat că cianidinele glicozilate purificate din soc şi aronia au suprimat de rata de supraviețuire a celulelor tumorale A2780 cu 50%, după o expunere a acestora la tratament pentru 24 de ore. Betalaninele purificate din sucul de sfeclă roşie inhibă proliferarea celulelor A2780 într-o manieră dependent de doză. Aceste efecte a fost asociate cu moleculele bioactive şi potențialul antioxidant al antocianilor şi betalaninelor, compuşi prezenți în sucurile purificate.*
Cuvinte cheie: *sucuri, activitate antioxidantă, viabilitate celulară, cancer ovarian*

INTRODUCTION

Antioxidants can be definite as molecules which can donate a free electron or hydrogen atoms to reactive free radicals. In a recent study Record et al., 2001 explained very well that the compounds, easier to oxidize are often the best antioxidants. Also this great propriety contributes to the fruit and the vegetable protective effect against degenerative and chronic diseases (Kumpulainen and Salonen, 1998).

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The phytochemical substances which are involved in antioxidant activity are the phenolic compounds (anthocyanins), nitrogenous compounds (chlorophyll derivatives), tocopherols, carotenoids and ascorbic acid (Nichenametla et al., 2006; Fleschhut et al., 2006). Bagchi et al., 2004 revealed that vitamins C and E have a lower antioxidant activity than anthocyanidins and anthocyanins. The mechanism of those compounds is related to their ability to capture free radicals by donation of phenolic hydrogen atoms (Chen et al., 2005). Several studies reported a positive correlation between the values of the antioxidant activity and the anthocyanins content in edible plants (Heinonen et al., 1998). There is a population-based survey hinting the potential activity of anthocyanins on cancer prevention.

It has been suggested that the consumption of colored fruits and vegetables are associated with a reduced risk of human breast cancer (Adlercreutz, 1998), human melanoma cancer (Huang et al., 2008), human ovarian cancer (Abdah et al., 2011). Flavonoids modulates a number of key elements in cellular signal transduction pathways of the apoptotic process, but the specific mechanism for apoptosis induction is unclear (Ramos, 2007).

An comparative study, between berries based diet of elderly individuals, demonstrate that the consumtion of large amounts of strawberries reduce cancer developing (Colditz et al., 1985). The chemopreventive effects of anthocyanins reagarding their supposed action mechanisms in animal and human models are reviewed in several recents studies (Jing and Giusti, 2011; Winny and Valerie, 2011). However, this paper focuses on chemoprotective potential of the anthocyanin-rich fraction from commercial available juices on two different tumor cell lines.

MATERIAL AND METHOD

Anthocyanin HPLC quantification was performed using cyanidin-3-*O*-sambubioside standard. The Equipment used was an Shimadzu HPLC system equipped with a binary pump delivery system LC-20 AT (Prominence), a degasser DGU-20 A3 (Prominence), diode-array SPDM20 A UV-VIS detector (DAD) and a Luna Phenomenex C-18 column (5µm, 25 cm x 4.6 mm). Formic acid (4.5%) in bidistilled water and acetonitrile were used as mobile phase. The gradient elution system was: 10% B, 0-9 min; 12% B, 9-17 min; 25% B 17-30 min; 90% B, 30-50 min; 10% B, 50-55 min. The flow rate was 0.8 ml/min and the analyses were performed at 35°C. The chromatograms were recorded at 520 nm.

Determination of antioxidant activity. The 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging activity was evaluated determined using Brand-Williams *et al.,* (1995) method. The procedure is briefly presented below: DPPH methanolic solution (80 µM) was freshly prepared. A volume of 250 µl of this solution was allowed to react with 35 µl sample and the absorbance was measured at 515 nm, for 30 minutes. The percentage of scavenging effect of purified commercial available juices against DPPH radicals was calculated using the following equation:

DPPH scavenging effect (%) = $[(A_0 - A_s) \times 100]/A_0$, Where, A_0 is absorbance of the blank, and A_s is absorbance of the samples at 515 nm.

Cell culture. Adherent epithelial human ovarian tumor cells A2780 were maintained in RPMI medium supplemented with 10% fetal serum, at 37^0C, 5%, CO2, 90% humidity. Cells, $5x10^3$ cells/well were placed on 96-well microplate and allowed to attach

for 24h. Cells growth medium was replaced next day with complete medium containing different concentrations from 0 to 25 µl/ml purified extracts.

MTT proliferation assay. Cells proliferation was determined using MTT reagent (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide). Growth medium was removed from each well including control, and the cells were whased three times with PBS. This step was followed by 1h of incubation with MTT solution (0.5 mg/ml) in DMEM without phenol red. The formazan particles were solubilized with DMSO. The obtained results were expressed as survival percent comparing to an untreated control.

RESULTS AND DISCUSSIONS

In order to evaluate antioxidant activity of chosen red fruit juices, DPPH assay was used. The DPPH scavenging activity, expressed in % inhibition of analyzed juices is presented in Fig. 1. The obtained results showed no significant differences between all the juices analyzed. The highest antioxidant capacity was determined for (49.1%) follows by elderberry juice (45.6%) and beetroot juice (31.3%). Additionally, statistical differences were not observed between elderberry and chokeberry juice antioxidant capacity (49.1 pmol TE/ml, 645.6 pmol TE/ml respectively). Antioxidant activity of chokeberry juice against DPPH radical was stronger than antioxidant activity of beetroot and elderberry juice. The present data are in agreement with published data (Slatnar et al., 2012) and lower than values obtained by Lidija Jakob et al., 2007, Adriana Bramorski et al., 2012.

Treatment of A2780 cells with berries extracts had effect on cell morphology as cell shrinkage and they became irregular in shape and size. Contrary under normal growth conditions (untreated cells) no any morphological changes were observed (Fig. 3). The treatment with chokeberry anthocyanin-rich fraction inhibited the proliferation of A2780 cells by 42% at the highest concentration (Fig. 2A). We observed that cell viability decreased in a dose dependent manners. Same effects were observed at the treatment with elderberry and beetroot anthocyanin-rich fractions.

Regarding the antiproliferative effects of edible berry juice, in a recent study Boivin et al., 2007, observed a significant inhibition of cell growth for the raspberry, lowbush blueberry and cranberry juices. The tumor cell lines use in this experiment were stomach, prostate, intestine and breast. Khanh Dang Vu et al. 2012 demonstrate that cranberry juice can inhibit growth of two colon cancer (HT-29 and LS-513 cell lines). Moreover, Magdalena Kędzierska et al., 2013 evaluate the protective effects of chokeberry commercial extract. They observed the obtained that the commercial extract from *A. melanocarpa* berries significantly reduced, in *in vitro* system, the oxidative/nitrative stress and hemostasis changes in plasma from breast cancer patient.

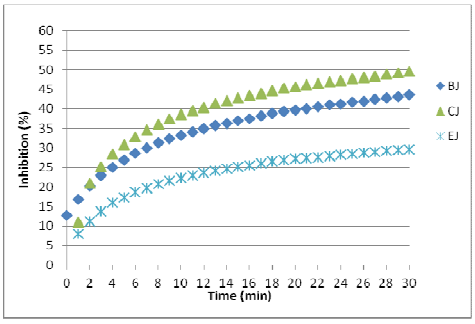

Fig. 1 - Antioxidant activity of blueberry extracts using DPPH method. The inhibition percentage represents the antioxidant activity

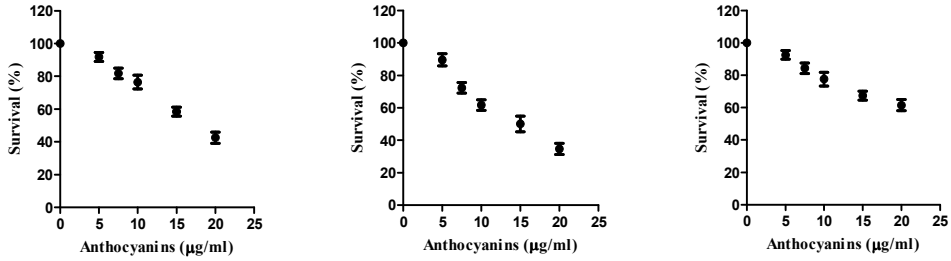

Fig. 2 - Results of MTT proliferation assay (proliferation %) of A2780 human ovarian cancer cells -treated by anthocyanin-rich fraction obtained from comercial juices

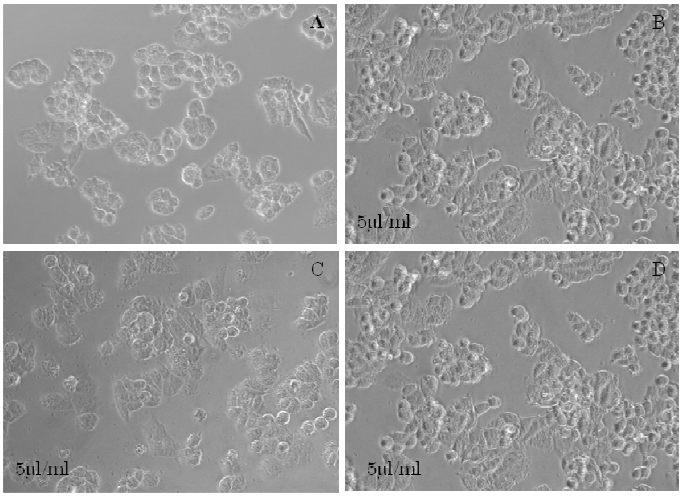

Fig. 3 - Comparative morphology of A2780 cells non-treated and treated with anthocyanin-rich fraction (A)- control, (B)-ckokeberry, (C)-elderberry, (D)-beetroot.

CONCLUSIONS

1. Edible berries are presently becoming very popular for their health benefits.

2. This study demonstrated the high antioxidant power and chemopreventive potential of anthocyanins from commercial juices

3. Is recommended that more research to be done in order to evaluate the benefits of berries consumption because these shows to be a new alternative for the prevention of many types of cancers.

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