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Effect of extraction conditions of paprika oleoresins on their free radical scavenging and anticancer activity

Research Article

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Abstract: Ground spice paprika was extracted with hexane, by conventional Soxhlet procedure (SX oleoresin), and with supercritical carbon dioxide at three different pressures - 20, 30 and 40 MPa (SF20, SF30 and SF40 oleoresins). The effect of extraction method and conditions on the colour intesity of paprika oleoresins, content of α-tocopherol, as well as antioxidant and antiproliferative activity was examined. Hexane showed highest selectivity for paprika pigments (886.02 ASTA), while α-tocopherol showed highest solubility (3846.9 mg kg^{-1}) in supercritical carbon dioxide at 20 MPa. All paprika oleoresins exhibited good superoxide anion radical scavenging activity SF30 being the best superoxide anion radical scavenger. Cell growth activity was evaluated in vitro in human cell lines:cervix epitheloid carcinoma (HeLa), breast adenocarcinoma (MCF7) and colon adenocarcinoma (HT-29). The highest antiproliferative activity was exhibited by SX in MCF7 cell line (IC_{50}=14.28 mg mL^{-1}). Extract SF40 produced significant and selective antiproliferative action towards HeLa cell line. These results suggest that paprika oleoresins, due to high antiradical and tumor cell-inhibiting activity, can be regarded as functional food ingredients.

Keywords: *Paprika oleoresin • Extraction • α-tocopherol • Antioxidant activity • Antiproliferative activity*

1. Introduction

Oxyradicals have the potential to damage cellular lipids, nucleic acids, proteins and carbohydrates; the result is wide-ranging impairment in cellular function and integrity [1]. Oxidative stress reduction through the dietary intake of antioxidants from fruit and vegetables has been suggested to reduce such oxidative damage [2]. There are numerous antioxidants in plants consumed in the diet, and this network of antioxidants with different chemical properties may be needed for proper protection against oxidative damage [3].

Paprika, the dehydrated and milled fruit of certain varieties of red pepper (*Capsicum annuum* L.), is widely used for modifying the colour and taste of foods [4]. Peppers are a good source of antioxidant bioactive compounds which are intestinally bioaccessible, particularly extractable polyphenols, β-carotene and zeaxanthin, vitamins C and E [5,6]. According to Halvorsen *et al*. [7] paprika is among the top 50 foods

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with the highest antioxidant content (ranked on the twelfth place) with 8.601 mmol per 100 g of antioxidants - higher than blueberries (2.154 mmol per 100 g), red wine (2.135 mmol per 100 g) and many other well known antioxidant rich foods. Due to the favorable weather, northern parts of Serbian province Vojvodina are well known for its production and processing of paprika. Also, Vojvodina cookery is famous for the use of spice paprika, especially hot varieties, which is part of the Hungarian legacy.

Spice paprika can be used as dried whole fruit and as ground powder. These forms usually don't meet the quality requirements for uniform, consistent, sterile and sustainable product. However, it is known to be a significant source of microorganisms, due to the high microbial contamination caused by poor sanitary conditions during growing and harvesting [8]. Disinfection of paprika is normally achieved by applying high temperatures which lead to loss of colour (due to the thermal degradation of carotenoids), loss of aroma and reduction of stability [9]. This has led to the use of their extracts, oleoresins, which offer several advantages: uniformity of flavour, better stability during storage, little or no microbiological contamination, easier storage, cost-effectiveness and efficiency [10].

Paprika oleoresin, obtained by extraction with organic solvents, is a concentrated form of red pigments and the flavour, dissolved in triacylglycerols present naturally in red paprika [11,12]. Paprika extract/oleoresin is listed by the European Community as a natural colour which is permitted for use in foods, and it has been assigned the number E160(c) [12].

Generally, extraction with organic solvents has limitations in obtaining solvent-free extract [13]. Over the past three decades, supercritical CO_2 has been used for the extraction and isolation of valuable compounds from natural products [14,15]. Paprika was also successfully extracted by supercritical carbon dioxide [16], and the recovery of total pigment and/or capsaicinoids has been emphasized [17,18]. CO_2 is inert, non-toxic, readily available, inexpensive, designated as GRAS (generally recognized as safe) by FDA (Food and Drug Administration), and therefore, highly acceptable for the use in food industry.

Although a lot has been reported on the chemical characteristics of antioxidant phytoconstituents in oleoresin from *Capsicum annuum* varieties, there is very little information on the biological activity of paprika oleoresin (*in vitro* and *in vivo*), especially Serbian cultivars. This study reports biological activity – the effect on superoxide anion radicals and human tumor cell lines of paprika oleoresins obtained by conventional extraction (with hexane) and by supercritical fluid extraction (with carbon-dioxide) at three different pressures, as well as their colour quality and α-tocopherol content.

2. Experimental procedure

2.1. Material and extraction procedures

Commercial ground paprika, "Aleva N.K." variety was obtained from the Aleva a.d. company from Novi Kneževac, the most important producer of ground pepper in Serbia. The mean diameter of the particles was 0.224 mm. Soxhlet oleoresin (SX) of paprika was obtained using technical grade hexane. Ground pepper was placed into the thimble in the middle portion of the Soxhlet apparatus, the solvent was then added and the process was continued until complete discoloration of sample was achieved [19]. The solvent was evaporated from extract under vacuum. Supercritical fluid oleoresins (SF) were obtained with commercial carbon-dioxide (Tehno-gas, Novi Sad, Serbia) using a laboratory scale high-pressure extraction plant (NOVA-Swiss, Effretikon, Switzerland) at 40°C and pressures of 20 (SF20), 30 (SF30) and 40 (SF40) MPa, with the carbon-dioxide flow rate 3.59 g min^{-1}, as previously described [19].

2.2. HPLC analysis of α-tocopherol content

α-Tocopherol content in oleoresins was analysed by HPLC method described by Viñas *et al.* [20]. Prior to analysis, oleoresin samples were diluted with methanol and filtered through 0.45 μm PTFE membrane filters (Millipore, Bedford, MA, USA). α-Tocopherol content in samples was determined using a Hewlett-Packard Liquid Chromatograph HP 1090 (Hewlett-Packard Company, Avondale, PA, USA) equipped with Diode Array Detector (DAD). α-Tocopherol separation was performed on a reversed-phase column (Zorbax SB-C18, 5 μm, 3.0×250 mm) with an isocratic elution using methanol (J. T. Baker, Deventer, Holland); the solvent flow rate was maintained at 0.4 mL min^{-1} with column temperature of 24°C. The sample injection volume was 10 μL, and the injection was performed manually. The chromatograms were acquired in the range 294±4 nm by DAD detector, with reference wavelength at 550/100 nm. α-Tocopherol content in oleoresin samples was identified by comparison of the retention time with that of α-tocopherol standard (Supelco, Bellefonte, PA, USA) and quantified by an external standard method. Linear calibration curve, obtained with a series of α-tocopherol standard dilutions, was constructed: $A = 6624.7\ c + 2.5054$. Results are expressed as mg of α-tocopherol per 100 g of oleoresin. All reagents used were HPLC grade.

2.3. Extractable colour determination

Extractable colour (ASTA 20.1) measurement was carried out by the American Spice Trade Association method [21]. Oleoresin sample (0.07-0.1 g) was weighed into a 100 mL measuring flask and filled with acetone. The glass was shaken and left in the dark for 4 h. A portion of extract was used for the spectrophotometric measurement at 460 nm with an acetone blank. The ASTA units were calculated by Eq. 1:

$$ASTA = (A \times 16.4) / m \times I_f \quad (1)$$

where A is the absorbance of acetone extract, I_f is the correction factor of the instrument, calculated from a standard solution of potassium dichromate, ammonium and cobalt sulphate and, m is the mass of sample weighed.

2.4. Superoxide anion radical scavenging activity

Free radical scavenging activity on superoxide anion radicals was measured according to [22]. A solution containing superoxide anion radicals was prepared by dissolving KO_2/crown ether (10 mM/20 mM; Merck Schuchardt OHG, Hohenbrunn, Germany) in dry dimethylsulfoxide (DMSO; J.T. Baker, Deventer, Netherlands). A 5 µL sample of this solution was added to 500 µL of dry DMSO and 5 µL of a DMPO (5,5-dimethyl-1-pyrroline-N-oxide, 2M; Sigma Chemical Co., St. Louis, MO, USA) spin trap solution. The influence of paprika oleoresins on the formation of DMPO/$O_2^{\cdot-}$ adducts was studied by adding the DMF (N,N-dimethylformamide; J.T. Baker, Deventer, Netherlands) solution of oleoresin to the superoxide anion reaction system at the final mass concentration range of 0.25 – 10 mg mL^{-1}. Then, the solution was transferred to a quartz flat cell ER-160FT, and 2 min after mixing, the spectrum was recorded on an ESR (electron spin resonance) spectrometer (Bruker, Rheinstetten, Germany). The following instrument settings were used: field modulation 100 kHz, modulation amplitude 4.00 G, receiver gain 1×10^4, time constant 327.68 ms, conversion time 40.96 ms, center field 3440.00 G, sweep width 100.00 G, x-band frequency 9.64 GHz, power 20 mW, temperature 23°C. The extent of scavenging by oleoresins was expressed as EC_{50} values, *i.e.*, the amount of paprika oleoresin necessary to decrease by 50% the initial superoxide anion radical concentration. EC_{50} values were calculated from dose response curves of superoxide anion scavenging activity ($SAo_2^{\bullet-}$) vs. oleoresin concentration. $SAo_2^{\bullet-}$ value was defined with Eq. 2:

$$SAo_2^{\bullet-}=100\times(h_0 - h_x)/h_0\ [\%] \quad (2)$$

where h_0 and h_x are the height of the second peak in the ESR spectrum of DMPO/$O_2^{\cdot-}$ spin adduct of the sample without and with oleoresin, respectively.

2.5. Growth and culture of the cell lines

For the estimation of cell growth effects, human tumor cell lines HeLa (cervix epitheloid carcinoma), MCF7 (breast adenocarcinoma) and HT-29 (colon adenocarcinoma) were used. Cell lines were grown in Dulbecco's modified Eagle's medium (DMEM; PAA Laboratories GmbH, Pashing, Austria) with 4.5% glucose, supplemented with 10% heat inactivated fetal calf serum (FCS, PAA Laboratories GmbH, Pashing, Austria), 100 IU mL^{-1} of penicillin and 100 µg mL^{-1} of streptomycin (Galenika, Belgrade, Serbia). They were cultured in 25 cm^2 flasks (Corning, New York, NY, USA) at 37°C in atmosphere of 5% CO_2 and high humidity and sub-cultured twice a week. Single cell suspension was obtained using 0.1% trypsin (Serva, Heidelberg, Germany) with 0.04% EDTA (Ethylenediaminetetraacetic acid; Sigma Chemical Co., St. Louis, MO, USA).

2.6. Sulforhodamine B (SRB) assay

For the analysis of cell growth effects, serial dilutions in 0.9% NaCl were used. Samples were filtered through a 0.22 µm microfilters to obtain sterility. Final concentrations were in the range of 0.176-90 mg mL^{-1}. Doxorubicin (Doxorubicin-Teva®) was purchased from Pharmachemie B.V., Haarlem, Netherlands, Gemcitabine (Gemzar®) from Lilly France S.A., Fegersheim, France and β-carotene from Sigma Chemical Co., St. Louis, MO, USA.

Cell lines were harvested and plated into 96-well microtiter plates (Sarstedt, Newton, NC, USA) at seeding density of 4×10^3 cells per well [23], in a volume of 180 µL, and preincubated in complete medium supplemented with 5% FCS, at 37°C for 24 h. Serial dilutions of extracts and 0.9% NaCl were added (20 µL/well) to achieve required final concentrations and control. After adding the treatments, microplates were vortexed and incubated at 37°C for an additional 48 h. Cell growth was evaluated by the colorimetric SRB assay [24]. Cells were fixed with 50% trichloractic acid (TCA; 1 h, +4°C), washed with distilled water (Wellwash 4, Labsystems; Helsinki, Finland) and stained with 0.4% SRB (30 min; room temperature). The plates were then washed with 1% acetic acid to remove unbound dye. Protein-bound dye was extracted with 10 mM TRIS (tris(hydroxymethyl)aminomethane) base. Absorbance was measured on a microplate reader (Multiscan

Table 1. α-Tocopherol content, extractable colour and superoxide anion radical scavenging activity of paprika oleoresins.

Paprika oleoresin	α-Tocopherol content (mg/100 g)	Extractable colour (ASTA)	EC_{50} (mg mL^{-1})
SX	334.7±9.8[a]	886.0±26.3[a]	4.42±0.18[a,b]
SF20	384.7±12.4[b]	133.5±3.9[b]	4.14±0.11[b]
SF30	308.8±8.6[a]	369±17[c]	3.66±0.15[c]
SF40	326.3±10.9[a]	417.2±14.4[c]	5.04±0.19[a]

Values are mean ± SD of three replicates. Different letters in the same column indicate significantly different values ($p<0.01$) among the oleoresin samples

Ascent, Labsystems, Helsinki, Finland) at 540/620 nm. Effect on cell growth was expressed as a percent of the control, and calculated by Eq. 3:

$$\% \text{ Control} = (At/Ac) \times 100 \ [\%] \quad (3)$$

where At is the absorbance of the test sample and Ac is the absorbance of the control. b-carotene and cytotoxic drugs Doxorubicin and Gemcitabine were tested in the same manner, as reference substances.

2.7. Statistical analyses

All analysis were expressed as mean ± standard deviation (SD), and were run in triplicate except results of cell growth activity, which were carried out as two independent experiments performed in quadruplicate. Statistical analysis and EC_{50} values (half-maximal effective concentration, *i.e.*, concentration of oleoresin at which 50% of its maximal effect on superoxide anion radicals is observed) were done using Origin 6.1 software package (OriginLab Corporation, Northampton, MA, USA) and Microsoft Office Excel 2003 software. Significant differences were calculated by ANOVA test and then least significant difference (LSD) test ($p<0.05$), unless noted otherwise. IC_{50} values (half-maximal inhibitory concentration, *i.e.*, the concentration of oleoresin/refernce substance needed to inhibit cell growth by 50%) were calculated using Calcusyn for Windows (Version 1.1.0.0.; Biosoft).

3. Results and discussion

3.1. Characterization of paprika oleoresins

The colour of paprika mainly originates from carotenoids formed in the fruit during ripening [25]. Ketocarotenoids, capsanthin and capsorubin [26], are responsible for the intense red colour of the paprika. Paprika contains also yellow pigments, mainly zeaxanthin, violaxanthin, antheraxanthin, β-cryptoxanthin, β-carotene and capsolutein [11]. Colour and quality of paprika are closely connected terms;the quality of paprika, and therefore, its commercial value, is evaluated by its "colouring capacity" [27]. Paprika is normally classified by its extractable colour - ASTA (American Spice Trade Association) colour. Industrial oleoresins overall quality is expressed in terms of colour units: ASTA, SCU and Tint value parameters [28]. ASTA color is used for evaluating the quality of ground paprika by international standard ISO 7540, as a parameter directly correlated to pigment (carotenoid) content. This method was used for testing oleoresin, too, to evaluate the quality of oleoresin in terms of pigment content, as this is a simple and rapid method. Oleoresin is commercially used for colouring various food products, and therefore, ASTA value directly reflects its quality. Commercial oleoresins are available in strengths ranging from 300 to 2500 ASTA colour units [29].

Table 1 reports total content of α-tocopherol, extractable colour and superoxide anion radical scavenging activity of paprika oleoresin as a function of extraction procedure.

The colour intensity in oleoresins obtained by supercritical fluid extraction was increased 3 times (from 133.48 ASTA to 417.24 ASTA) as a result of increase in extraction pressure from 20 to 40 MPa and mimics the increase in the solubility of pigments in supercritical CO_2 with pressure. In accordance with this, it was reported in our previous study that supercritical CO_2 extracted the least carotenoids under lowest extraction pressure (20 MPa), whereas the extraction of carotenoids was increased with the increase in pressure [19]. Other investigators also confirmed the improvement of carotenoid pigment extraction from paprika with increasing process pressure: 3-fold increase [16] with increase in pressure from 137.8 to 413.4 bar, significant increase by Gnayfeed *et al.* [30] in the experimental range from 100-400 bar at 40°C, 5-fold increase by Daood *et al.* [11] detected for the same pressure range, at the temperatures from 35-55°C and improvement of extraction kinetics by Ambrogi *et al.* [31] with an increase in process

pressure (300–500 bar) at 60°C. Furthermore, Lock and Simándi [32] reported that the optimal pressure for paprika oleoresin production should be in the range of 35–50 MPa. Duarte *et al.* have examined the effects of pressure on the extraction of red pepper and found that high extraction yields in oleoresins and capsaicinoids were obtained at pressures around 20–22 MPa [33]. Perva-Uzunalic *et al*. have shown that the total extraction yield and extraction efficiency of capsaicinoids increased with increasing pressure (100–400 bar) at constant temperature [34]. Valle *et al*. observed that a pressure above 290 bar had no effect on extraction yield of pelletized Jalapeno peppers at 45°C; while at 360 bar, the extraction rate increased with the process temperature [35]. Fernández-Ronco *et al*. showed that 300 bar and 333 K were selected as the best operational conditions to get the fractionation of oleoresin into the fractionation of pigments present in oleoresin (carotenoids and capsaicinoids) and producing extracts and raffinates enriched in those compounds. At these conditions in the raffinate, the concentration of carotenoids and capsaicinoids is the highest and lowest, respectively. In the extract, the concentration of capsaicinoids is quite high and enough to fulfill trader's needs, and the extraction yield of the SFE process is good enough to make this technique economically interesting [28].

Colour grade (886.02 ASTA) in conventional oleoresin sample, in accordance with total carotenoid concentration reported in the previous work [19], was twice as high than in SF40 oleoresin (417.24 ASTA), suggesting that hexane shows higher selectivity for the extraction of carotenoids and overall pigments than CO_2. Various literature data state that colour of commercial paprika oleoresins should be in the range from 300 to 3600 ASTA [29,36]. Therefore, paprika oleoresin SF20 obtained in this study is not commercially acceptable from the colour criterion point of view.

Paprika distributes considerable amounts of fat soluble antioxidants such as tocopherols, (mainly α-tocopherol), which are very important factor for colour stability in paprika, and their content depends on the variety and maturity of paprika [5]. The lowest pressure applied for supercritical extraction (20 MPa) resulted in highest content of α-tocopherol (384.7 mg per 100 g) in SF20 oleoresin (Table 1). These results are in agreement with findings of Daood *et al.* [11] who also suggests that higher solubility in supercritical carbon dioxide, and therefore, higher yields of tocopherol, could be achieved by extraction at subcritical temperatures (<25°C). In our study, the amount of α-tocopherol in oleoresin obtained by conventional extraction (334.7 mg per 100 g) was comparable to that obtained by supercritical extraction

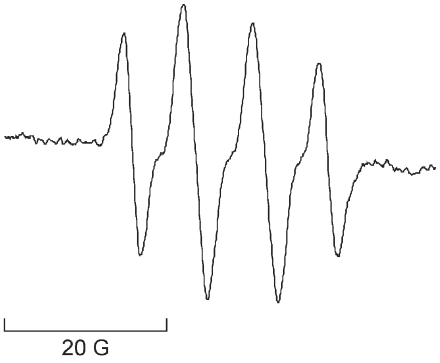

Figure 1. ESR spectrum of the DMPO/$O_2^{\bullet-}$ spin adduct (blank).

at the highest pressure (326.3 mg per 100 g) (Table 1). Our results are comparable to the results obtained by Daood *et al*. [5] and Gnayfeed *et al*. [30], but higher tocopherol contents in hexane and supercritical carbon dioxide paprika extracts (4-10 mg g^{-1}) were reported in the studies of Vesper and Nitz [37,38]. Daood *et al*. [11] reported that after saponification of the extract of paprika sample tocopherols fraction was found to compose of α-, β-, and γ-tocopherols (70%, 2% and 28%, respectively).

3.2. Antioxidant activity of paprika oleoresins

Some of the applications of paprika relate to its varied flavours while others are related to colour. Paprika is used in pharmaceutical industry also, due to numerous biological activities of its ingredients. With respect to humans, carotenoids have important health promoting properties; for example, α, β, γ-carotene and β-cryptoxanthin are known as pro-vitamin A. It has been suggested by Seppanen and Csallany [39] that dietary paprika carotenoids and β-carotene, when fed at high levels, exhibit similar antioxidant effects on inhibiting *in vivo* lipid peroxidation but do not compensate for the role of physiological levels of vitamin E in normal growth and weight gain.

Free radical scavenging ability of paprika on 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical [40] and ferric reducing antioxidant power (FRAP) [41] were already established. Molnár *et al.* [40] reported that paprika extract did not scavenge the $O_2^{\bullet-}$ produced by the hypoxanthine and xanthine oxidase reaction. In this study, radical scavenging activity of paprika oleoresins was tested on chemically generated superoxide anion radicals using KO_2 solubilized in crown ether as a source of superoxide. The advantage of this system for determination of superoxide anion radical scavenging activity compared to enyzmatic is the simplicity in interpretation of results, since possible inhibition of the xanthine oxidase enzyme by antioxidants is not a

problem. The unstable superoxide anion radicals were trapped with 5,5-dimethyl-l-pyrroline-N-oxide (DMPO) as a spin trap and the corresponding spin adduct has been detected by electron spin resoance (ESR) spectroscopy. The ESR spectra of the DMPO/$O_2^{\cdot-}$ spin adduct obtained in this study is presented in Fig. 1. The ESR hyperfine splitting constants of the DMPO/$O_2^{\cdot-}$ spin adduct were: a_N = 12.65 G; $a_{H\beta}$ = 10.4 G; $a_{Hγ}$ = 1.3 G. The concentration of paprika oleoresin providing 50% of superoxide anion radical scavenging (EC_{50}) was calculated from the graph of $SAo_2^{\bullet-}$ (%) against oleoresin concentration, and the results are presented in Table 1. As presented, all four oleoresin samples were able to scavenge superoxide anion radicals; SF30 being the best superoxide anion radical scavenger. Highest EC_{50} value, therefore, the worst superoxide anion radical scavenging activity, was expressed by SF40 which was not significantly different ($p<0.01$) from SX oleoresin.

Among the various defense strategies, carotenoids are most likely involved in the scavenging of two of the reactive oxygen species, singlet molecular oxygen ($^1O_2$), and peroxyl radicals. Further, they are effective deactivators of electronically excited sensitizer molecules which are involved in the generation of radicals and singlet oxygen [42]. In addition, there is experimental evidence indicating the effectiveness of carotenoids in inhibiting lipid peroxidation induced by xenobiotics well-known to be implicated in the production of oxy radicals [43].

α-Tocopherol is an excellent quencher of singlet oxygen, and has been shown to react very rapidly with peroxyl radicals, by providing hydrogen atoms [44]. Several studies suggest that use of vitamin E may contribute to help lower the risks of specific chronic and degenerative diseases such as Alzheimer's disease, age-related macular degeneration, some types of cancer, cataracts and ischemic heart disease [45].

Many authors suggest that β-carotene and α-tocopherol can act synergistically [46] by electron transfer from carotenoids (CAR) to α–tocopheroxyl radical ($TO^{\bullet}$):

$$TO^{\bullet} + CAR \rightarrow TOH + CAR^{\bullet+}$$

However, Böhm *et al.* [47] proposed that the reverse reaction does not occur, e.g. α-tocopherol does not protect β-carotene by a repair mechanism. The same authors, as well as others [48], observed a synergistic effect in cell protection by β-carotene and vitamins E and C, suggesting that it may be related to the fact that β-carotene is not only quenching oxy-radicals but is also repairing the α-tocopheroxyl radical, which is produced when α-tocopherol scavenges an oxy-radical. The conclusion is that these antioxidants have different cellular locations, and that carotenoids could substantially enhance vitamin E/C protection. β–Carotene is located in the interior of the membrane, between lipophilic α-tocopherol and aqueous phase antioxidants like vitamin C.

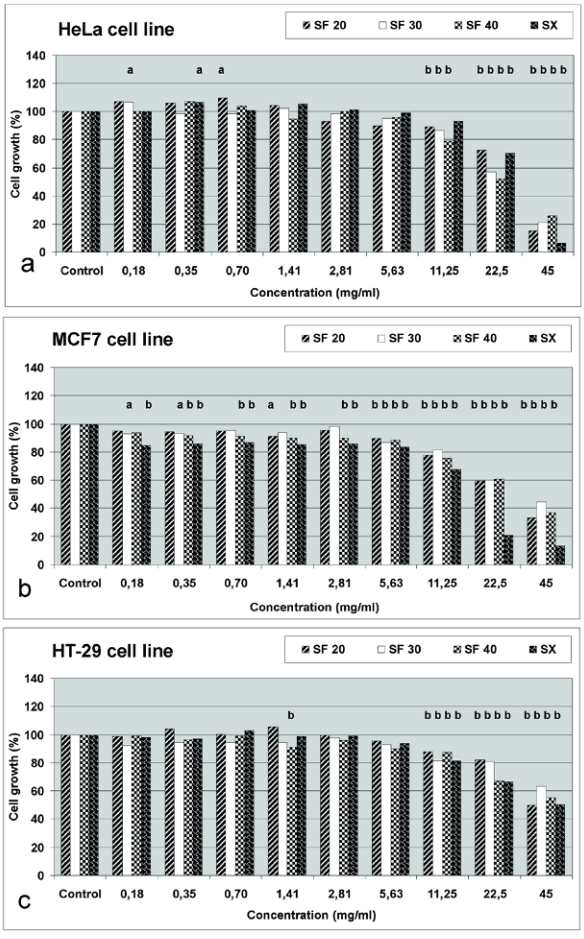

Figure 2. Cell growth activity of SF20, SF30, SF40 and SX paprika oleoresins in A) HeLa, B) MCF7 and C) HT-29 cell lines. (One way analysis of variance, compared to the control; a: $p<0.05$, b: $p<0.01$). Results are expressed as mean ± SD of two experiments, each performed in quadruplicate (n = 8).

3.3. Antiproliferative activity of paprika oleoresins

The cell growth activity of paprika oleoresins was investigated *in vitro* in a panel of three human tumor cell lines: HeLa (cervix epitheloid carcinoma), MCF7 (breast adenocarcinoma) and HT-29 (colon adenocarinoma) at 0.176 - 45 mg mL^{-1} range of mass concentrations (Fig. 2). Multi-endpoint bioassays based on whole cell response in human cell lines are powerful indicators of metabolic, biochemical and genetic alterations that arise under the influence of evaluated compounds.

Table 2. IC_{50} values of SX, SF20, SF30, SF40 paprika oleoresins and reference substances in human tumor cell lines.

Paprika oleoresin/reference substance	IC_{50}^{HeLa} (mg mL^{-1})	IC_{50}^{MCF7} (mg mL^{-1})	IC_{50}^{HT-29} (mg mL^{-1})
SX[a]	28.07±3.14	14.28±2.60	25.90±3.64
SF20[a]	28.75±6.13	30.01±5.95	33.10±6.56
SF30[a]	25.64±3.44	29.29±7.95	>45.00
SF40[a]	23.54±3.86	30.03±6.43	27.47±6.17
b-carotene[b]	13.83±10.87	10.65±1.71	21.08±5.24
Doxorubicin[c]	0.254±0.082	0.261±0.018	0.378±0.045
Gemcitabine[d]	0.127±0.099	0.038±0.004	>31.30

[a] *In 0.176 - 45.00 mg mL^{-1} concentration range;* [b] *In 1.95 - 31.25 mg mL^{-1} concentration range*
[c] *In 0.006 - 58.10 µg mL^{-1} concentration range;* [d] *In 0.003 - 31.30 µg mL^{-1} concentration range*

At concentrations higher than 6 mg mL^{-1}, paprika oleoresins inhibited the growth of all examined cell lines. The most sensitive cell line was MCF7 where the growth was significantly ($p<0.01$) inhibited in the whole concentration range (Fig. 2b). The highest antiproliferative activity was exhibited by SX extract in MCF7 cell line reaching IC_{50}=14.28 mg mL^{-1} (Table 2), while the activity of SF20, SF30 and SF40 extracts were similar, reaching IC_{50} values of 30 mg mL^{-1}. In HeLa cell line, the effects of paprika oleoresins on growth based on IC_{50} values were fairly similar among the extracts (IC_{50} values were in the range from 23.54-28.75 mg mL^{-1}) with SF40 extract being the most active. IC_{50} values in HT-29 cell line were in the 25.90-33.10 mg mL^{-1} range. SF30 extract was the only one that did not reach the 50% inhibition of cell growth in the investigated concentration range. In HeLa and HT-29 cell line, significant ($p<0.05$) cell growth stimulation was also observed in the lowest range of concentrations (Figs. 2a,2c).

In agreement with our results, are the studies of Molnar *et al.* [48] and Dou *et al.* [49] that showed inhibition of the growth of human breast cell lines *in vitro* using different pepper extracts by slowing-down the cell cycle progression through phases G1-S *via* the reduction of cyclinD1. Dou *et al.* [49] correlated significant growth arrest and apoptosis with the capsaicin content and its accepted mechanism of anticancer activity through the generation of reactive oxygen species (ROS) especially hydroxyl radicals [50,51] and suggested the involvement of free radicals in mediating cell growth inhibition and caspase-3/7 activity produced by the pepper extracts [49]. The redox alterations play a significant role in a signal transduction pathway important for cell growth regulation, and a low level of free oxygen species is necessary for the promotion of cell proliferation [49]. But, it is not uncommon that extracts of natural products possessing high antioxidative activity do not exhibit high antiproliferative activity and *vice versa* [52,53]. In our work, it was shown that strong antioxidative and strong antiproliferative activity was sharply separated between the extracts.

Activity of b-carotene and cytotoxic drugs Doxorubicin and Gemcitabine was investigated in the same panel of cell lines and IC_{50} values are presented in Table 2.

Although the activities of b-carotene were roughly 50 fold less potent than of two well-known cytostatic drugs Doxorubicine and Gemcitabine (Table 2), they correlated well with the observed highest antiproliferative activity of SX extract (with highest amount of pigments) in MCF7 and HT-29 cell line. This is in agreement with the results of Zhang *et al.* [54] that reported dose- and time-dependent inhibition of the proliferation, decrease of the viability, induced apoptosis and interference with cell cycle progression of leukemia K562 cells by the activity of carotenoids b-carotene, astaxantin, capsantin and bixin. Extract SF40, which contained high concentrations of α-tocopherol, produced significant and selective antiproliferative action towards HeLa cell line indicating its possible role in the observed effect.

4. Conclusion

Four different paprika oleoresins exhibited good superoxide anion radical scavenging activity with SF30 being the best superoxide anion radical scavenger. The highest antiproliferative activity was exhibited by SX extract in the MCF7 cell line. Extract SF40 produced significant and selective antiproliferative action towards HeLa cell line. Activities of b-carotene correlated well with the observed highest antiproliferative activity of SX extract in MCF7 and HT-29 cell line. Taken together, our results suggest that extracts from

paprika products can be regarded as functional food ingredients. Such concentrates, encapsulated or not, could be considered as food supplements for direct use or for the fortification of meals prepared in a kitchen. However, further toxicological studies are necessary, especially in respect to residues of organic solvents. Supercritical extracts are solvent free, and CO_2 is non-toxic and is considered a GRAS solvent, which means that it is acceptable for use in food. Conversely, extracts obtained by conventional extraction can contain some residues of solvents. In order to put this product on the market, it must satisfy the valid regulations requirements concerning the maximum level of residues.

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