ORIGINAL ARTICLE

UDC: 615.2:[615.451:633.846
DOI: https://doi.org/10.2298/VSP190212123P

Polyphenol rich horseradish root extracts and juice: *in vitro* antitumor activity and mechanism of action

Antitumorska aktivnost i mehanizam delovanja polifenolima bogatih ekstrakata i soka korena rena *in vitro*

Vidosava Petrović*†, Dragana Četojević-Simin‡§, Maja Milanović‖, Jelena Vulić¶, Nataša Milić‖

***University of Novi Sad, Faculty of Medicine, Novi Sad, Serbia; †Primary Health Center, Novi Sad, Serbia; ‡Oncology Institute of Vojvodina, Sremska Kamenica, Serbia; §University Singidunum, Belgrade, Serbia; ‖University of Novi Sad, Faculty of Medicine, Department of Pharmacy, Novi Sad, Serbia; ¶University of Novi Sad, Faculty of Technology, Novi Sad, Serbia**

Abstract

Background/Aim. Plant polyphenols are well known to show antimutagenic, anticarcinogenic, antiviral and antioxidative activity. The aim of this study was to investigate bioactive potential of *Armoracia rusticana* root juice and extracts: their polyphenol content, as well as *in vitro* antitumor activity and cell-death mechanism. **Methods.** Liquid-liquid extraction of polar and non-polar compounds was used and polyphenolic compounds were identified and quantified by high performance liquid chomatography (HPLC) analysis. Antiproliferative activity was examined *in vitro* on human cervix carcinoma (HeLa), breast adenocarcinoma (MCF7, MDA-MB-231), colon adenocarcinoma (HT-29), lung adenocarcinoma (A549), prostate adenocarcinoma (PC-3), melanocyte carcinoma (Hs 294T), hepatocyte carcinoma (Hep G2), as well as rat hepatocyte carcinoma (H-4-II-E), and normal human fetal lung (MRC-5) cell line using sulforhodamine B assay. The mechanism of cell-death in cell line was determined using Cell Death Detection ELISA^{PLUS} kit. **Results.** Dichloromethane extracts had the highest content of catechin, p-hydroxybenzoic, syringic and gallic acid (pulp, E1), and epicatechin (juice, E3). The results showed strong and non-selective antiproliferative activity of chloroform and dichloromethane extracts and root juice – highest being towards liver, breast and lung tissue cells. IC_{50} values of extracts and juice had low range of concentrations (IC_{50} = 3.49–26.5 μg/mL) and high range of dilutions (IC_{50} = 418–1,590). High and unfavorable potential of horseradish juice and chloroform juice extract (E4) to induce necrotic cell death was detected. **Conclusion.** Strong and non-selective *in vitro* antiproliferative activity of chloroform and dichloromethane extracts and root juice of horseradish was detected, with necrosis as a main mechanism of induced cell death. In order to utilize horseradish root bioactive potential further investigations that will pinpoint active components with more favourable apoptosis/necrosis inducing properties are needed.

Key words:
horseradish; phenols, antineoplastic agents; in vitro techniques; cell, death; apoptosis.

Apstrakt

Uvod/Cilj. Poznato je da polifenoli biljaka poseduju antimutagenu, antikancerogenu, antivirusnu i antioksidativnu aktivnost. Cilj ovog rada bio je ispitivanje bioaktivnog potencijala soka i ekstrakata korena *Armoracia rusticana*: određivanje polifenolnog sastava, *in vitro* antitumorske aktivnosti i mehanizma ćelijske smrti **Metode.** Primenom tečno-tečne ekstrakcije izolovane su i razdvojene polarne od nepolarnih komponenti, a HPLC metodom identifikovana su i kvantifikovana polifenolna jedinjenja. Antiproliferativna aktivnost ekstrakata i soka korena rena ispitana je *in vitro* na tumorskim ćelijskim linijama: karcinoma grlića materice (HeLa), adenokarcinoma dojke (MCF7 i MDA-MB-231), adenokarcinoma debelog creva (HT-29), adenokarcinoma pluća (A549), adenokarcinoma prostate (PC-3), karcinoma kože (Hs 294T), karcinoma jetre (Hep G2), kao i na ćelijskim linijama karcinoma jetre pacova (H-4-II-E) i normalnim fetalnim ćelijskim linijama pluća (MRC-5) upotrebom sulforodamin B testa. Mehanizam ćelijske smrti određen je detekcijom apoptoze i nekroze upotrebom Cell Death Detection ELISA^{PLUS} kompleta. **Rezultati.** Dihlormetanski ekstrakti korena rena imali su najveći sadržaj katehina, p-hidroksibenzoeve, siringinske i galne kiseline (pulpa, E1), i epikatehina (sok, E3). Utvrđena je

Correspondence to: Vidosava Petrović, Primary Health Center, Novi Sad, Bul. Cara Lazara 75, 21 000 Novi Sad, Serbia.
E-mail: vidapetrovicns@gmail.com

snažna i neselektivna antiproliferativna aktivnost hlonajsnažnijim delovanjem na ćelijske linije jetre, dojke i pluća. Dobijene IC_{50} vrednosti bile su u niskom rasponu koncentracija (IC_{50} = 3,49–26,5 μg/mL) i u visokim razblaženjima (IC_{50} = 418–1590). Sok i hloroformski ekstrakt soka rena (E4) pokazali su snažnu, nepoželjnu sposobnost indukcije nekroze. **Zaključak.** Hloroformski i dihlormetanski ekstrakti, kao i sok korena rena ispoljili su su snažnu i neselektivnu antiproliferativnu aktivnost *in vitro*, sa nekrozom kao dominantnim roformskih i dihlormetanskih ekstrakata i soka korena rena, sa mehanizmom ćelijske smrti. U cilju iskorišćenja bioaktivnog potencijala korena rena, neophodna su dalja ispitivanja i izolacija aktivnih komponenti sa povoljnijim odnosom indukcije apoptoze i nekroze.

Ključne reči:
ren; fenoli; antineoplastici; in vitro; ćelija, smrt; apoptoza.

Introduction

Horseradish (*Armoracia rusticana*, G. Gaertn, B. Mey. and Scherb.) is perennial plant from Brassicaceae family that is cultivated for its aromatic, fleshy root. Horseradish root possesses intense and opulent taste that produces the feeling of cooling during consumption due to the presence of sulfur compounds called glucosinolates (GSLs) [1–4]. Epidemiological and pharmacological studies have shown that GSLs and their degradation products – isothiocyanates (ITC), may reduce the risk of developing cancer in humans [5]. The most commonly found natural ITC is allyl isothiocyanate (AITC) i.e. "burning oil" [6] that is derived from sinigrin – GSL abundant in Brassicaceae family especially in mustard, horseradish and wasabi [7]. Agneta et al. [8] detected 16 different GSLs in horseradish juice.

Plant polyphenols are well known to show biological and pharmacological activity, such as antimutagenic, anticarcinogenic, antiviral and antioxidative [9]. Phenolic acids (chlorogenic, caffeic, and ferulic), flavonoids (quercetin, genistein, catechins, isoflavones), quinones, coumarins, stilbenes, curcuminoids and lignans possess potent antioxidant and also anticarcinogenic and antimutagenic activities [10–14]. Over 20 phenolic compounds have been identified in some varieties of the Brassicaceae family, which include kale, curly kale, white and black cabbage, cauliflower and tronchuda cabbage [15, 16]. The most important phenolic compounds in *Brassica* species are flavonoid glycosides, such as glycosides of kaempferol and quercetin, and their derivatives hydroxycinnamic and sinapic acid [17, 18]. Phenolic acids, among them gallic, protocatechuic, p-hydroxybenzoic, vanillic, syringic, salicylic, p-coumaric, caffeic, ferulic and sinapic acid were identified in the kale (*Brassica oleracea* var. *Acephala)* [19]. On the other hand, little is known about the polyphenolic composition of horseradish. Based on earlier research, *Armoracia rustucana* contains a small amount of flavonoids, mainly kaempferol and quercetin [20]. Cirimbei et al. [21] demonstrated great potential of the aqueous plant extract from *A. rusticana* and its main flavonoids, kaempferol and quercetin, to protect DNA from damage induced on human lymphocytes by the oxidative agent hydrogen peroxide.

In order to gain insight into its bioactive potential, present study focused on the evaluation of *Armoracia rusticana* root juice and extracts: (1) their polyphenol content, (2) *in vitro* antitumor activity and (3) cell-death mechanism using mammalian cell lines. To the best of our knowledge this is the first study evaluating sequential horseradish root extracts as well as horseradish root juice.

Methods

Chemicals and standards

Reference standards of ≥ 98% purity were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All used chemicals and solvents were of p.a. purity grade and were supplied by: Sigma-Aldrich (St. Louis, MO, USA), Acros Organics (New Jersey, USA), Lach-Ner s.r.o. (Neratovice, Czech Republic), Zorka Pharma, Šabac (Serbia), J.T. Baker (Deventer, Netherlands), AppliChem Panreac, (Darmstadt, Germany), Carlo Erba Reagenti (Milan, Italy), and Promochem (Wessel, Germany).

Preparation of horseradish root juice and extracts

Horseradish roots were obtained from Bački Petrovac, Serbia (45°21′5.32″N 19°37′21.65″E) in January 2016. Roots (2.64 kg) were peeled, chopped and grinded using Philips juicer (HR1858/55/BD, 650W). From 1.475 Kg of peeled horseradish 200 mL of juice and 1.25 kg of pulp was obtained. Juice and pulp were aliquoted in smaller portions and frozen (-20 °C) prior to extraction. Modified method of Kupchan [22] and Sarker et al. [23], based on the liquid-liquid extraction, was applied for the preparation of horseradish pulp and juice extracts. This method enables isolation and separation of polar and non-polar compounds. Mixtures of methanol and water (70:30, *v/v*), chloroform, dichloromethane or n-butanol were used as solvents for: 1) "crude extract" extraction (methanol), 2) extractions from methanol extract (chloroform, dichloromethane) and 3) further consecutive extractions (n-butanol and water) of horseradish root pulp and juice. The obtained extracts were gently evaporated to dryness using rota-evaporator. Dried extracts were sealed tight and kept at 4 °C until use.

Eight horseradish extracts from pulp, i.e. E1 (dichloromethane), E2 (chloroform), E7 (water) and E8 (n-butanol) and from juice E3 (dichloromethane), E4 (chloroform), E5 (water) and E6 (butanol) as well as horseradish juice J9 were used to examine chemical composition, *in vitro* antiproliferative activity and cell death activity.

Identification and quantification of phenolic acids and flavonoids by HPLC method

Horseradish root extracts and juice were analyzed using Shimadzu Prominence (Shimadzu, Kioto, Japan) high per-

formance liquid chromatography (HPLC) equipped with binary pump LC-20AT, thermostat CTO-20A and automatic dispenser SIL-20A connected to SPD-20AV UV/V detector. Chromatograms were recorded using different wavelengths for individual compounds: 280 nm for hydroxybenzoic acids, catechin and epicatechin, 320 nm for hydroxycinnamic acids, and 360 nm for flavonoids. Separation was performed on a Luna C-18 RP column, 5 mm, 250 x 4.6 mm with a C18 guard column, 4 × 30 mm (both from Phenomenex, Torrance, CA, USA). Two mobile phases, A (acetonitrile) and B (1% formic acid) were used at flow rates of 1 mL min^{-1} with the following gradient profile: 0–10 min from 10 to 25% B; 10–20 min linear rise up to 60% B, and from 20 min to 30 min linear rise up to 70% B, followed by 10 minutes reverse to initial 10% B with additional 5 min of equilibration time. Reference substances (flavonoids and phenolic acids) and samples were dissolved in 50% methanol.

In vitro antiproliferative activity

Cell lines

Human cervix epithelioid carcinoma (HeLa, ECACC 93021013), breast adenocarcinoma (MCF7, ECAACC 86012803; MDA-MB-231, ECAACC 92020424), colon adenocarcinoma (HT-29, ECAACC 91072201), lung adenocarcinoma (A549, ECACC 86012804), prostate adenocarcinoma (PC-3, ECACC 90112714), melanocyte carcinoma (Hs 294T, ATCC HTB-140), hepatocyte carcinoma (Hep G2, ECACC 85011430), as well as rat hepatocyte carcinoma (H-4-II-E, ATCC CRL-1548) and normal human fetal lung (MRC-5, ECACC 05090501) were used for the estimation of antiproliferative effects of horseradish root juice and extracts.

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^3 flasks (Corning, New York, USA) at 37 ºC in atmosphere of 5% CO_2, with high humidity, and subcultured twice a week. Single cell suspension was obtained using 0.1% trypsin (Serva, UK) with 0.04% EDTA.

Preparation of samples

Extracts were dissolved and further diluted in DMSO (to obtain five working concentration) and culture medium (1 μg/L of working concentration + 199 μg/L of culture medium; a = 200) to achieve required final concentrations. Ranges of final concentrations of extracts depended on extract yields and were in the range from 0.0625–1 mg/mL. Final concentration of DMSO in the samples was ≤ 0.05% (v/v).

Horseradish root juice was diluted in 0.9% NaCl to obtain four additional working dilutions and further in culture medium (1 μg/L of working concentration +199 μg/L of culture medium; a = 200) to achieve final dilutions in the range from 200–3200. The final concentrations of juice dilutions were in the range from 0.12–19.33 mg/mL, calculated on dry weight of juice (concentration of horseradish juice was 385.80 mg/mL).

Sulforhodamine B (SRB) assay

Cell lines were harvested and plated into 96-well microtiter plates (Sarstedt, Newton, NC, USA) at seeding density of 4–8 × 10^3 cells per well, in a volume of 199 μL, and pre-incubated in complete medium supplemented with 5% FCS, at 37 ºC for 24 h. Working concentrations of juice, extracts or solvents (1 μL) were added to the test and control wells. Microplates were then incubated at 37 ºC for an additional 48 h. Cell growth was evaluated by the colorimetric SRB assay, according to previously described procedure [24].

Absorbance was measured on a microplate reader (Multiscan Ascent, Labsystems) at 540/620 nm. Cell growth activity was expressed as a percent of the control and calculated as At/Ac × 100 (%), where At is the absorbance of the test sample and Ac is the absorbance of the control. The concentration-cell growth (dose effect) curves were drawn for each treatment and IC_{50} values (concentration that inhibit cell growth by 50%) were determined, using OriginPro 8 SRO (Origin-Lab Corporation, Northampton, USA).

In order to identify selectivity towards tumor cells compared to healthy tissue, non-tumor/tumor IC_{50} ratios were calculated as NT/T = $IC_{50}^{MRC-5}/IC_{50}^{repective\ tumor\ cell\ line}$ for extracts and as 1/NT/T for juice [25].

Cell death detection

Apoptosis and necrosis were detected using the Cell Death Detection ELISAPLUS kit (Roche, Version 11.0). Cell death detection was performed in HeLa, MCF7 and HT-29 cell lines using the most active extracts from cell growth experiments (E4 and J9), according to previously described procedure 25. Depending on the cell line used concentrations of extracts were 10 μg/mL (E4 in HeLa and MCF7) and 20 μg/mL (E4 in HT-29), while juice dilutions were a = 20 (J9 in HeLa and HT-29) for the 2 h exposition time.

Respective enrichment factors (ef) for apoptosis and necrosis (efA and efN) were calculated as ef = test/control using average absorbance-blank values for each sample and control [26, 27]. From these values, apoptosis/necrosis ratios were calculated indicating that apoptosis is a dominant mode of cell death if efA/efN > 1 or that necrosis is a dominant mode of cell death if efA/efN < 1.

Statistical analysis

Cell growth experiments were carried out in at least four repetitions (n = 4). Enrichment factors in cell death experiments were calculated using average absorbance-blank values (n = 2) from pooled quadruplicates (n = 4) for each sample and control. Results were expressed as mean ± standard deviation (SD). A comparison of the group means and the significance between the groups were verified by one-way ANOVA. Statistical significance was set at $p < 0.05$.

Results

Polyphenolic compounds in Armoracia rusticana root juice and extracts

Major polyphenolic compounds present in investigated horseradish root juice and extracts were identified and quantified by HPLC analysis (Figure 1). Phenolic acids (gallic, protocatechuic, caffeic, ferulic, isoferulic, chlorogenic, p-hydroxybenzoic, p-coumaric, syringic, and synapic acid) and flavonoids (catechin, epicatechin, quercetin, kaempferol, luteolin, apigenin and isorhamnetin) were identified by matching their retention times (RT) and on-line ultraviolet (UV) spectra with those of standards. The content of total and individual phenolic compounds, quantified at 280, 330 or 360 nm, depending on their maximal response, is listed in Table 1. Catechin (flavan-3-ol) was detected at relatively high levels in all samples (0.75–33.17 mg/g), where E1 had the highest catechin content. Besides that, E1 had the highest content of p-hydroxybenzoic (6.68 mg/g), syringic (3.65 mg/g) and gallic acid (3.34 mg/g), while E3 had the highest content of epicatechin (2.80 mg/g). The horseradish juice J9 contained mostly catechin and gallic acid.

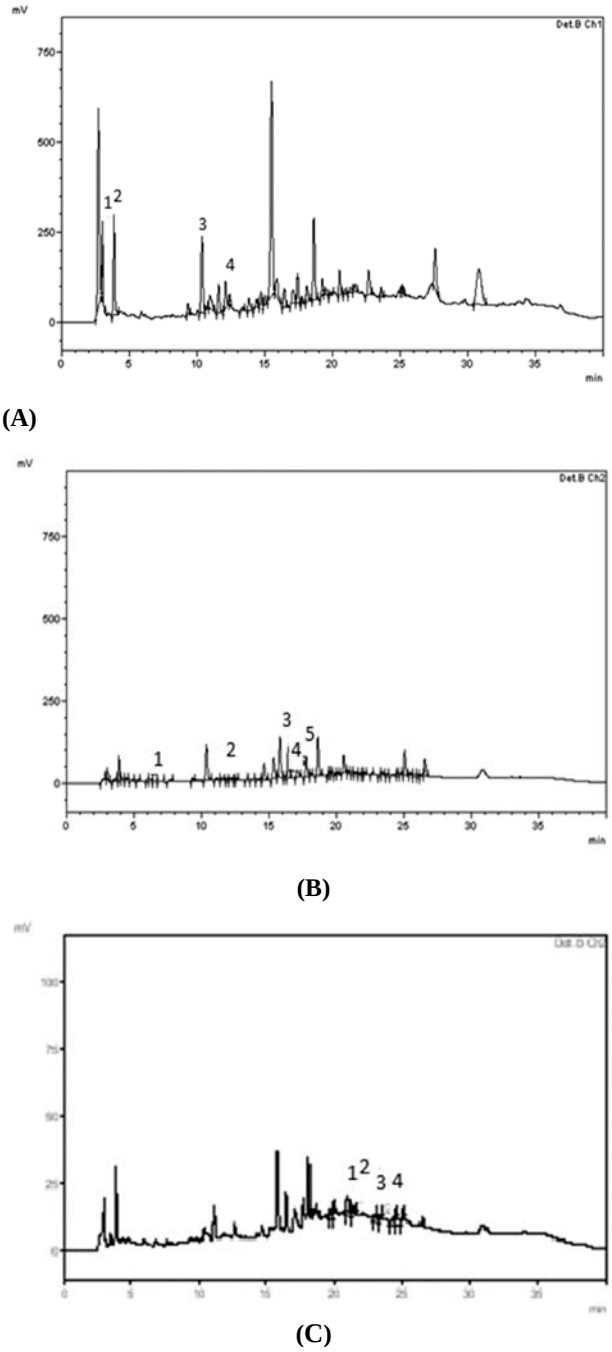

Fig. 1 – Phenolics in E1 extract. HPLC chromatograms on: A) 280 nm: 1-gallic acid; 2-catechin; 3-p-hydroxybenzoic acid; 4-syringic acid; B) 320 nm: 1-chlorogenic acid; 2-caffeic acid; 3-p-coumaric acid; 4-ferulic acid; 5-sinapic acid; C) 360 nm: 1-quercetin; 2-luteolin; 3-apigenin; 4-isoramnetin.

Horseradish extracts obtained from juice contained higher concentrations of epicatechin, ferulic and chlorogenic acid (dichloromethane); ferulic, isoferulic, p-coumaric acid and quercetin (chloroform); protocatechuic, syringic, chlorogenic, isoferulic acid and quercetin (n-butanol); and catechin, epicatechin, gallic and p-hydroxybenzoic acid (water) compared to extracts obtained from pulp.

In vitro antiproliferative activity

Obtained results revealed strong and non-selective antiproliferative activity of horseradish root extracts E4, E2, E3 and E1 and root juice J9 – highest being towards liver, breast and lung tissue cells (Table 2).

IC50 values of extracts and juice were obtained in low range of concentrations (IC50 = 3.49–26.5 μg/mL) and high range of dilutions (IC50 = 418–1590). Extract E4 and juice J9 possessed the highest cytotoxic activity towards rat hepatoma (IC50H-4-II-E = 3.49 μg/mL and IC50H-4-II-E = 1401.57) and human cervix carcinoma (IC50HeLa = 4.66 μg/mL and IC50HeLa = 1596.04), respectively (Table 2).

Higher antiproliferative activity of chloroform (E4), and n-butanol juice (E6) extracts was achieved compared to same extracts obtained from pulp (Table 2).

In order to identify selectivity towards tumor cells compared to healthy tissue, non-tumor/tumor ratios were calculated as NT/T for extracts and 1/NT/T for juice 25 (Table 3). Majority of extracts and juice demonstrated non-favourable (≤ 1) NT/T and 1/NT/T ratios indicating higher antiproliferative affinity towards healthy tissue. Most favourable (> 1) ratios were obtained in rat hepatoma cells using extracts E4 (NT/TH-4-II-E = 2.03) and E1 (NT/TH-4-II-E = 1.63), breast adenocarcinoma cells using E6 (NT/TMCF7 = 1.64) and cervix carcinoma cells using juice J9 (1/NT/THeLa = 1.18) indicating higher cell growth inhibition affinity towards tumor compared to healthy cells (Table 3).

In vitro cell death detection

In cervix carcinoma (HeLa) and colon adenocarcinoma cells (HT-29), horseradish juice J9 induced significant levels of apoptosis (efA = 2.59–4.73) compared to control, but also significant levels of necrosis (efN = 2.75–4.43) resulting in

Table 1

Polyphenolic compound contents (mg/g) in *Armoracia rusticana* root juice and extracts

Polyphenolic compounds	Pulp extracts (mg/g) mean ± SD				Juice extracts (mg/g) mean ± SD				Juice (mg/L) mean ± SD
	E1	E2	E7	E8	E3	E4	E5	E6	J9
Gallic acid	3.34 ± 0.17	1.87 ± 0.09	0.18 ± 0.01	0.60 ± 0.03	1.99 ± 0.09	0.21 ± 0.01	0.32 ± 0.01	0.53 ± 0.02	1.38 ± 0.06
Protocatechuic acid	0	0	0.36 ± 0.017	0	0	0	0.03 ± 0.001	0.03 ± 0.001	0.07 ± 0.002
Epicatechin	0	0	0.05 ± 0.002	0	2.80 ± 0.14	0	0.06 ± 0.003	0	0
Catechin	33.17 ± 1.66	8.36 ± 0.42	1.23 ± 0.06	1.07 ± 0.05	12.44 ± 0.62	1.24 ± 0.06	1.63 ± 0.08	0.75 ± 0.03	7.10 ± 0.35
Ferulic acid	0.01 ± 0.001	0	0	0.004 ± 0.001	0.45 ± 0.02	0.034 ± 0.001	0	0	0.01 ± 0.003
Caffeic acid	0.14 ± 0.006	0	0	0.003 ± 0.001	0.01 ± 0.001	0	0	0	0.01 ± 0.002
Syringic acid	3.65 ± 0.18	0.44 ± 0.02	0.02 ± 0.001	0.08 ± 0.003	0	0.10 ± 0.004	0.12 ± 0.004	0.06 ± 0.002	0.16 ± 0.01
Sinapic acid	0.65 ± 0.03	0	0	0.005 ± 0.001	0.11 ± 0.003	0	0	0	0.002 ± 0.001
p-coumaric acid	1.66 ± 0.08	0	0	0.01 ± 0.002	0.09 ± 0.004	0.01 ± 0.001	0	0	0.01 ± 0.002
Chlorogenic acid	0.07 ± 0.001	0	0.003 ± 0.001	0	0.20 ± 0.01	0	0	0.02 ± 0.001	0.02 ± 0.001
p-hydroxybenzoic acid	6.68 ± 0.33	0.26 ± 0.01	0.14 ± 0.01	0	0	0.11 ± 0.004	0.15 ± 0.01	0	0.64 ± 0.03
Isoferulic acid	0.02 ± 0.001	0	0	0.01 ± 0.001	0	0.04 ± 0.002	0	0.02 ± 0.002	0
Quercetin	0.23 ± 0.01	0.02 ± 0.001	0	0	0	0.04 ± 0.001	0	0.002 ± 0.001	0
Kaempferol	0	0.09 ± 0.003	0	0	0	0.02 ± 0.001	0	0	0
Luteolin	0.01 ± 0.001	0	0	0	0	0	0	0	0
Apigenin	0.02 ± 0.001	0	0	0	0	0	0	0	0
Isorhamnetin	0.02 ± 0.001	0	0	0	0	0.003 ± 0.001	0	0	0
Total	49.64	11.034	1.98	1.77	18.084	1.81	2.31	1.40	9.38

Pulp extracts: E1 – dichloromethane, E2 – chloroform, E7 – water, E8 – n-butanol; Juice extracts: E3 – dichloromethane, E4 – chloroform, E5 – water, E6 – n-butanol; Juice: J9. SD – standard deviation.

Table 2

Cell growth effects after 48 h exposure to horseradish extracts and juice (SRB test)

Extract, Juice/Cell line		HeLa	MCF7	HT-29	MRC-5	A549	H-4-II-E	MDA-MB-231	Hs294T	HepG2	PC-3
		IC_{50} (μg/mL), mean + standard deviation									
Pulp extracts	E1*	>31.25^{c}	>31.25^{c}	>31.25^{b}	>31.25^{c}	>31.25^{b}	19.19 ± 3.51^{b}	26.01 ± 2.45^{c}	>31.25^{c}	>31.25^{c}	>31.25^{b}
	E2*	21.31 ± 4.30^{b}	16.54 ± 1.20^{b}	28.46 ± 0.54^{b}	18.80 ± 3.88^{b}	28.11 ± 3.57^{b}	17.63 ± 0.88^{b}	19.44 ± 3.82^{b}	20.66 ± 3.00^{b}	15.42 ± 0.46^{b}	>31.25^{b}
	E7‡	>500^{f}	>500^{f}	>500^{e}	>500^{g}	>500^{e}	>500^{e}	>500^{f}	>500^{f}	>500^{f}	>500^{d}
	E8†	>125^{d}	>125^{d}	>125^{c}	>125^{d}	>125^{c}	>125^{c}	114.52 ± 0.28^{d}	>125^{d}	109.76 ± 9.67^{d}	>125^{c}
Juice extracts	E3*	>31.25^{c}	18.52 ± 2.19^{b}	>31.25^{b}	16.74 ± 0.33^{b}	>31.25^{b}	19.41 ± 3.45^{b}	26.50 ± 4.15^{c}	>31.25^{c}	>31.25^{c}	>31.25^{b}
	E4*	4.66 ⊥ 1.09^{a}	6.54 ± 0.66^{a}	12.17 ± 2.84^{a}	7.10 ± 1.87^{a}	11.43 ± 3.77^{a}	3.49 ± 1.24^{a}	5.52 ± 1.47^{a}	6.70 ± 1.81^{a}	5.68 ± 1.54^{a}	9.01 ± 1.89^{a}
	E5‡	>500^{f}	>500^{f}	>500^{e}	>500^{g}	>500^{e}	>500^{e}	>500^{f}	>500^{f}	>500^{f}	>500^{d}
	E6‡	239.86 ± 81.98^{e}	146.97 ± 4.29^{e}	460.97 ± 11.28^{e}	240.34 ± 40.20^{e}	354.66 ± 11.03^{d}	>500^{e}	>500^{f}	>500^{f}	>500^{f}	>500^{d}
Juice	J9§**	241.72^{e}	494.76^{f}	338.34^{d}	286.23^{f}	923.28^{f}	275.26^{d}	317.93^{e}	437.52^{e}	411.80^{e}	799.52^{e}
		IC_{50} (dilution), mean ± standard deviation									
	J9‖	1596.04 ± 386.77	779.77 ± 67.48	1140.26 ± 135.54	1347.86 ± 63.72	417.86 ± 3.61	1401.57 ± 240.37	1213.49 ± 97.68	881.79 ± 161.88	936.86 ± 79.22	482.54 ± 33.47

Investigated in 1.95–31.25*, 7.81–125† and 31.25–500‡ μg/mL range of concentrations. Investigated in 200–3200‖ range of dilutions or 0.12–19.33§ mg/mL range of concentrations (calculated based on dry weight of juice; concentration of horseradish juice was 385.80 mg/mL). Means within each column with different letter (a-g) differ significantly ($p < 0.05$). Calculated for mass concentrations of extracts and juice.**

Pulp extracts: E1 – dichloromethane, E2 – chloroform, E7 – water, E8 – n-butanol; Juice extracts: E3 – dichloromethane, E4 – chloroform, E5 – water, E6 – n-butanol; J9 – juice. SRB – sulforhodamine B; HeLa – human cervix epithelioid carcinoma; MCF7 – breast adenocarcinoma; HT-29 – colon adenocarcinoma; MRC-5 – normal human fetal lung; A549 – lung adenocarcinoma ; H-4-II-E – rat hepatocyte carcinoma ; MDA-MB-23 – breast adenocarcinoma; Hs294T – melanocyte carcinoma; HepG2 – hepatocyte carcinoma; PC-3 – prostate adenocarcinoma.

low efA/efN ratios (efA/efN = 0.94–1.07; Table 4). In cervix carcinoma (HeLa), breast (MCF7) and colon (HT-29) adenocarcinoma cells, extract E4 induced low levels of apoptosis (efA = 0.74–1) compared to control, but significant levels of necrosis (efN = 1–4.5) resulting in low efA/efN ratios (efA/efN = 0.22–0.74; Table 4).

Higher induction of necrosis was observed using juice extract E4 compared to horseradish juice J9 (Table 4).

Table 3

Non-tumor/tumor (NT/T) ratio of extracts and juice in different cell lines

Extract, Juice/ Cell line		HeLa	MCF7	HT-29	A549	H-4-II-E	MDA-MB-231	Hs294T	HepG2	PC-3
		NT/T								
Pulp extracts	E1	1	1	1	1	1.63	1.2	1	1	1
	E2	0.88	1.14	0.66	0.67	1.07	0.97	0.91	1.22	< 0.6
	E7	1	1	1	1	1	1	1	1	1
	E8	1	1	1	1	1	> 1.09	1	> 1.14	1
Juice extracts	E3	< 0.54	0.9	< 0.54	< 0.54	0.86	0.63	< 0.54	< 0.54	< 0.54
	E4	0.21	1.08	0.58	0.62	2.03	1.29	1.06	1.25	0.79
	E5	1	1	1	1	1	1	1	1	1
	E6	1	1.64	0.52	0.68	< 0.48	< 0.48	< 0.48	< 0.48	< 0.48
		1/NT/T*								
Juice	J9	1.18	0.58	0.85	0.31	1.04	0.90	0.65	0.69	0.36

***calculated for juice dilutions.**
Favourable (>1) ratios indicate higher cell growth inhibition of the extract/juice towards tumor compared to healthy cells
Pulp extracts: E1 – dichloromethane, E2 – chloroform, E7 – water, E8 – n-butanol; Juice extracts: E3 – dichloromethane, E4 – chloroform, E5 – water, E6 – n-butanol; J9 – juice.
HeLa – human cervix epithelioid carcinoma; MCF7 – breast adenocarcinoma; HT-29 – colon adenocarcinoma;
MRC-5 – normal human fetal lung; A549 – lung adenocarcinoma; H-4-II-E – rat hepatocyte carcinoma;
MDA-MB-23 – breast adenocarcinoma; Hs294T – melanocyte carcinoma; HepG2 – hepatocyte carcinoma;
PC-3 – prostate adenocarcinoma.

Table 4

Apoptosis and necrosis induced by horseradish root juice J9 and extract E4 in HeLa, HT-29 and MCF7 cell line

Cell line	Sample	Apoptosis		Necrosis		efA/efN
		absorbance	efA	absorbance	efN	
HeLa	J9	1.446	4.72	0.031	4.43	1.07
	Control*	0.306		0.007		
	E4	0.304	0.74	0.001	1.00	0.74
	Control**	0.411		0.001		
HT-29	J9	1.750	2.58	0.022	2.75	0.94
	Control*	0.677		0.008		
	E4	0.750	0.99	0.009	4.50	0.22
	Control**	0.761		0.002		
MCF7	E4	0.294	1.00	0.005	5.00	0.20
	Control**	0.295		0.001		

E4 – chloroform juice extract; J9 – juice; HeLa – human cervix epithelioid carcinoma; HT-29 – colon adenocarcinoma; MCF7 – breast adenocarcinoma; *0.9% NaCl; **dimethyl sulfoxide (DMSO); efA – enhancement factor for apoptosis; efN – enhancement factor for necrosis.

Discussion

Earlier reports demonstrated that the methanol-aqueous extract of horseradish root was rich in polyphenols: malic acid, gallic acid, ferulic acid and epigallocatechin-3-gallate. The most abundant compound of the extract was allyl isothiocyanate [26]. Herz et al. [27] showed that prominent compounds in the aqueous extract from horseradish root were the amino acids arginine and proline, citric acid, phenolic compounds (caffeic acid and kaempferol derivatives), the main glucosinolates, 2-propenyl-GLS and 3-methylsulfinyl-propyl-GLS, and fatty acid derivatives. The study of Marzocco et al. [28] reported the highest content of sinigrin in the methanol extract from horseradish root.

The obtained HPLC results pointed out rich content of total polyphenolics in dichloromethane extracts from pulp E1 (49.64 mg/g) and juice E3 (18.084 mg/g).

In the study of Calabrone et al. [29] total polyphenolic contents of methanol, methanol/water (70/30, v/v) and methanol/water (50/50, v/v) horseradish root extracts that were

determined spectrophotometrically were: 1.80 ± 0.17, 2.36 ± 0.14 and 2.61 ± 0.20 mg/g DW, respectively. Similar results were presented in Tomsone et al. [30] study, where total phenolics in horseradish roots extracts obtained by convectional and Soxhlet extraction ranged from 334.29 mg GAE/100 g DW to 985.87 mg GAE/100 g DW depending on solvents used. The differences in total polyphenolics comparing HPLC and Folin–Ciocalteau method could be explained by the fact that spectrophotometric method is not an absolute measurement of the amount of phenolics, because some other substances such as organic acids, residual sugars, amino acids, proteins and other hydrophilic compounds interfere with this assay. Also, different classes of phenolic contribute to different extents to the absorbance produced by reaction with the Folin–Ciocalteau reagent when compared to gallic acid [31].

Antioxidative activity of phenolic acids is based on scavenging of reactive electrophiles and oxygen radicals [32]. The proposed mechanism by which phenolic acids induce apoptosis in tumor cells is by activation of proapoptotic factors [33]. Anticarcinogenic activity of flavonols is partially due to their antioxidative activity, since it has been shown that quercetin and kaempferol increase intracellular levels of natural antioxidant glutathione[34].

In this study, antiproliferative activity of extracts and juice horseradish root in cancer cell lines were evaluated. All extracts and juice contained catechin and gallic acid.

As confirmed by previous research (based on IC_{50} values) among twenty-four evaluated flavonoids and phenolic acids kaempferol > gallic acid > quercetin > caffeic acid (in this order of activity) had most prominent antiproliferative activity [35]. Numerous studies have shown that gallic acid exhibit antitumor and antiproliferative activity toward many types of human cancer cells [36], such as human cervix carcinoma [37], prostate cancer, and lung cancer [38]. On the other hand, catechin, epicatechin, p-coumaric acid, p-hydroxybenzoic, protocatechuic, syringic, sinapic, chlorogenic and ferulic acids had no significant cell growth activity [35].

Extracts E4 (chloroform from juice) and E2 (chloroform from pulp) that showed highest antiproliferative activity contained similar classes of polyphenols but in lower concentrations compared to E1 (dichloromethane from pulp) that had widest range and highest concentrations of polyphenols. Higher activity of extracts E4 and E2 compared to E1 can be attributed to kaempferol, one of the flavonoids with most potent antiproliferative activity [35], while higher activity of extract E4 compared to E2 can be explained by additional presence of isorhamnetin with proven powerful antiproliferative activity – even higher than activity of kaempferol, quercetin or gallic acid (based on IC_{50} values) [39]. Kaempferol exerted a dose-dependent reduction in cell viability and DNA synthesis on human lung cancer cell line A549 at different doses from 0–70 μM [40]. Hung [41] has shown that kaempferol at concentration of 35 μM significantly reduced the number of viable estrogen receptor-positive MCF7 breast cancer cells. Isorhamnetin suppressed cell proliferation of the human colon adenocarcinoma cell line HT-29, and induced arrest of cell growth in G2/M phase [42].

Higher concentrations of phenolics in extracts obtained from juice compared to same extracts obtained from pulp resulted in higher antiproliferative activity of chloroform (E4), and n-butanol juice (E6) extracts due to presence of quercetin. Higher concentration of cell growth active gallic acid in water extract from juice (E5) did not result in its higher antiproliferative activity compared to same extract from pulp, most likely due to its low concentration.

Numerous studies have shown that ITC exhibit antitumor activity, inhibiting *in vitro* growth of many types of human cancer cells, such as leukemia [43], prostate cancer [44], breast cancer [45], lung cancer [46], carcinoma of the cervix [47] and colorectal carcinoma [48]. A ITC inhibits *in vitro* proliferation of various types of human cancer cells in low micromolar range of concentrations [49–52, 43] while being less toxic to normal cells [51]. In recent study, antiproliferative activity of three isothiocyanates: sulforaphane, benzyl isothiocyanate (BITC) and phenylethyl isothiocyanate, on human cervix carcinoma cell line (HeLa), melanoma cell line (Fem-x), colon cancer cell line (LS 174), and peripheral blood mononuclear cells (PBMC) was evaluated and selectivity of BITC towards malignant cells was confirmed [53]. Due to their nonpolar nature (chloroform) it is highly unlikely that extracts E4 and E2 contained or that their activity can be attributed to isothiocyanates or sinigrin. Due to their more polar nature (dichloromethane), activity of extracts E3 and E1 can be attributed to activities of detected polyphenols as well as isothiocyanates and sinigrin.

Both water extracts, E5 (from juice) and E7 (from pulp) showed lowest antiproliferative activity, confirming that extraction of active components occurred in earlier steps of extraction (in chloroform, dichloromethane and n-butanol extracts).

High and non-selective cell growth activity of horseradish root juice towards all evaluated cell lines was achieved due to high mass concentration of juice (385.80 mg/mL; based on dry weight of the juice). Evaluated ranges of concentrations of extracts depended on extract yields and were from 0.0625–1 mg/mL. On the other hand, final concentration of juice was in the range from 0.12–19.33 mg/mL. This was almost 20 fold higher compared to extracts. When activity of extracts and juice was compared using IC_{50} values, it was clear that highest activity of juice (IC_{50}^{HeLa} = 241.72 μg/mL), that could only be attributed to gallic acid was from 8–70 fold lower compared to the most potent extracts (E1–E4).

Results in this study indicate unfavorable, high horseradish juice J9 and extract E4 potential to induce necrotic cell death of tumor cells with a possible consequence of simultaneous damage to adjacent healthy tissue (that follows necrosis). Further investigation of active components should pinpoint ones with more favourable apoptosis/necrosis inducing activities.

Conclusion

The highest and non-selective *in vitro* antiproliferative activity of chloroform and dichloromethane extracts and juice of horseradish root was detected, with necrosis as a

main mechanism of induced cell death. Chloroform extracts showed high antiproliferative activity most likely due to the presence of kaempferol, while the highest activity of chloroform extract from juice can be explained by additional presence of isorhamnetin. Due to more polar nature of dichloromethane, the activity of these extracts that was lower compared to the activity of non-polar extracts could be attributed to detected polyphenols as well as to isothiocyanates and/or sinigrin. In order to utilize horseradish root bioactive potential further investigations that will pinpoint active components with more favourable apoptosis/necrosis inducing properties are needed.

Acknowledgement

This research was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (Grant No. 172058).

R E F E R E N C E S

1. *Weber WW*. Seed production in horseradish. J Hered 1949; 40(8): 223–7.
2. *Balasinska B, Nicolle C, Gueux E, Majewska A, Demigne C, Mazur A*. Dietary horseradish reduces plasma cholesterol in mice. Nutr Res 2005; 25(10): 937–45.
3. *Shehata A, Mulwa RMS, Babadoost M, Uchanski M, Norton MA, Skirvin R*, et al. Horseradish: botany, horticulture and breeding. In: *Janick J*, editor. Horticultural reviews. Hoboken: Wiley-Blackwell; 2009. p. 222–61.
4. *Walters SA, Wahle EA*. Horseradish production in Illinois. Hortic Technol 2010; 20(2): 267–76.
5. *Dinkova-Kostova AT, Kostov RV*. Glucosinolates and isothiocyanates in health and disease. Trends Mol Med. 2012; 18(6): 337–47.
6. *Fahey JW, Zalcmann AT, Talalay P*. The chemical diversity and distribution of glucosinolates and isothiocyanates among plants. Phytochemistry 2001; 56(1): 5–51.
7. *Sultana T, Savage GP, McNeil DL, Porter NG, Martin RJ, Deo B*. Effects of fertilisation on the allyl isothiocyanate profile of above-ground tissue of New Zeland-grown wasabi. J Sci Food Agr 2002; 82(13): 1477–82.
8. *Agneta R, Rivelli AR, Ventrella E, Lelario F, Sarli G, Bufo SA*. Investigation of glucosinolate profile and qualitative aspects in sprouts and roots of horseradish (Armoracia rusticana) using LC-ESI-hybrid linear ion trap with Fourier transform ion cyclotron resonance mass spectrometry and infrared multiphoton dissociation. J Agric Food Chem 2012; 60(30): 7474–82.
9. *Okuda T, Yoshida T, Htano T*. Economical and medicinal plant research. In: *Wagner H, Hikino H, Farnsworth N*, editors. Economical and medicinal plant research. New York: Academic press; 1991. p. 129.
10. *Ho CT, Osawa T, Huang MT, Rosen RT*. Food phytochemicals for cancer prevention. Washington, DC: American Chemical Society; 1994.
11. *Gao X, Bjork L, Trajkovski V, Uggla M*. Evaluation of antioxidant activities of rosehip ethanol extracts in different test systems. J Sci Food Agr 2000; 80(14): 2021–7.
12. *Owen RW, Giacosa A, Hull WE, Haubner R, Spiegelhalder B, Bartsch H*. The antioxidant/anticancer potential of phenolic compounds isolated from olive oil. Eur J Cancer 2000; 36(10): 1235–47.
13. *Yang CS, Landau JM, Huang MT, Newmark HL*. Inhibition of carcinogenesis by dietary polyphenolic compounds. Annu Rev Nutr 2001; 21(1): 381–406.
14. *Tapiero H, Tew KD, Ba GN, Mathé G*. Polyphenols: do they play a role in the prevention of human pathologies? Biomed Pharmacother 2002; 56(4): 200–7.
15. *Cartea ME, Francisco M, Soengas P. Velasco P*. Phenolic compounds in Brassica vegetables. Molecules 2011; 16(1): 251–80.
16. *Podsedek A*. Natural antioxidants and antioxidant capacity of Brassica vegetables: a review. LWT – Food Sci Technol 2007; 40(1): 1–11.
17. *Vallejo F, Tomas-Barberan FA, Garcia-Viguera C*. Potential bioactive compounds in health promotion from broccoli cultivars grown in Spain. J Sci Food Agr 2002; 82(11): 1293–7.
18. *Martínez-Sánchez A, Gil-Izquierdo A, Gil MI, Ferreres F*. A comparative study of flavonoid compounds, vitamin C, and antioxidant properties of baby leaf Brassicaceae species. J Agric Food Chem 2008; 56(7): 2330–40.
19. *Nićiforović N, Abramovič H*. Sinapic acid and its derivatives: natural sources and bioactivity. Compr Rev Food Sci Food Saf 2014; 13(1): 34–51.
20. *Fursa NS, Litvinenko VI, Krivenchuk PE*. Flavonoids of Armoracia rusticana and Barbarea arcuata. Chem Nat Compd 1969; 5(4): 270–1.
21. *Cirimbei MR, Dinica R, Gitin L, Vizireanu C*. Study on herbal actions of horseradish (Armoracia rusticana). J Agroaliment Proc Technol 2013; 19(1): 111–5.
22. *Kupchan SM*. Recent advances in the chemistry of terpenoid tumor inhibitors. In: *Kupcan SM*, editor. Tumor inhibitors. Virginia: University of Virginia; 1969. p. 227–46.
23. *Sarker SD, Latif Z, Gray AI*. Natural products isolation. New Jersey: Humana Press Inc; 2006.
24. *Četojević-Simin DD, Velićanski AS, Cvetković DD, Markov SL, Mrđanović JŽ, Bogdanović VV*, et al. Bioactivity of lemon balm Kombucha. Food Bioprocess Tech 2012; 5(5): 1756–65.
25. *Četojević-Simin DD, Velićanski AS, Cvetković DD, Markov SL, Cetković GS, Tumbas Šaponjac VT*, et al. Bioactivity of Meeker and Willamette raspberry (Rubus idaeus L.) pomace extracts. Food Chem 2015; 166: 407–13.
26. *Aissani N, Tedeschi P, Maietti A, Brandolini V, Garau VL, Caboni P*. Nematicidal activity of allyl isothiocyanate from horseradish (Armoracia rusticana) roots against Meloidogyne incignita. J Agric Food Chem 2013; 61(20): 4723–7.
27. *Herz C, Tran HTT, Márton MR, Maul R, Baldermann S, Schreiner M*, et al. Evaluation of an aqueous extract from horseradish root (Armoracia rusticana Radix) against lipopolysaccharide-induced cellular inflammation reaction. Evid Based Complement Alternat Med 2017; 2017: 1950692.
28. *Marzocco S, Calabrone L, Adesso S, Larocca M, Franceschelli S, Autore G*, et al. Anti-inflamatory activity of horseradish (Armoracia rusticana) root extracts in LPS-stimulated macrophages. Food Funct 2015; 6(12): 3778–88.
29. *Calabrone L, Larocca M, Marzocco S, Martelli G, Rossano R*. Total phenols and flavonoids content, antioxidant capacity and lipase inhibition of root and leaf horseradish (Armoracia rusticana) extracts. Food Nutr Sci 2015; 6(1): 64–74.
30. *Tomsone L, Kruma Z*. Comparison of different solvents for isolation of phenolic compounds from horseradish (Armoracia Rusticana L.) leaves. Res Rural Dev 2013; (19): 104–10.
31. *Singleton VL, Orthofer R, Lamuela-Raventos RM*. Methods in enzymology, oxidant and antioxidants (Part A). In: Packer L, editor. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. San Diego: Academic Press; 1999. p. 152–78.

32. *Teel RW, Huynh H.* Modulation by phytochemicals of cytochrome P450-linked enzyme activity. Cancer Lett 1998; 133(2): 135–41.
33. *Watabe M., Hishikawa K, Takayanagi A, Shimizu N, Nakaki T.* Caffeic acid phenethyl ester induces apoptosis by inhibition of NF-kappa B and activation of Fas in human breast cancer MCF-7 cells. J Biol Chem 2004; 279(7): 6017–26.
34. *Myhrsta MC, Carlsen H, Nordström O, Blomhoff R, Moskaug JØ.* Flavonoids increase the intracellular glutathione level by transactivation of the gamma-glutamylcysteine synthetase catalytical subunit promoter. Free Radic Biol Med 2002; 32(5): 386–93.
35. *Četojević-Simin D.* Tumor cell growth activity of fruit and pomace extracts. In: *Oven JP,* editor. Fruit and pomace extracts: biological activity, potential applications and beneficial health effects. New York: Nova Science Publishers; 2015. p. 241–53.
36. *Simin N, Orcic D, Cetojevic-Simin D, Mimica-Dukic N, Anackov G, Beara I*, et al. Phenolic profile, antioxidant, anti-inflammatory and cytotoxic activities of small yellow onion (Allium flavum L. subsp. flavum, Alliaceae). LWT – Food Sci Technol 2013; 54(1): 139–46.
37. *Zhao B, Hu M.* Gallic acid reduces cell viability, proliferation, invasion and angiogenesis in human cervical cancer cells. Oncol Lett 2013; 6(6): 1749–55.
38. *Verma S, Singh A, Mishra A.* Gallic acid: Molecular rival of cancer. Environ Toxicol Pharmacol 2013; 35(3): 473–85.
39. *Guo D, Pan H, Li X, Guo D.* Metabolic engineering of Escherichia coli for production of biodiesel from fatty alcohols and acetyl-CoA. Appl Microbiol Biotechnol 2015; 99(18): 7805–12.
40. *Nguyen TT, Tran E, Ong CK.* Kaempferol-induced growth inhibition and apoptosis in A549 lung cancer cells is mediated by activation of MEK-MAPK. J Cell Physiol 2003; 197(1): 110–21.
41. *Hung H.* Inhibition of estrogen receptor alpha expression and function in MCF7 cells by kaempferol. J Cell Physiol 2004; 198(2): 197–208.
42. *Li C, Yang X, Chen C, Cai S, Hu J.* Isorhamnetin suppresses colon cancer cell growth through the PI3K-Akt-mTOR patway. Mol Med Rep 2014; 9(3): 935–40.
43. *Xu K, Thornalley PJ.* Involvement of glutathione metabolism in the cytotoxicity of the phenethyl isothiocyanate and its cysteine conjugate to human leukaemia cells in vitro. Biochem Pharmacol 2001; 61(2): 165–77.
44. *Gong A, He M, Krishna Vanaja D, Yin P, Karnes RJ, Young CY.* Phenethyl isothiocyanate inhibits STAT3 activation in prostate cancer cells. Mol Nutr Food Res 2009; 53(7): 878–86.
45. *Kang L, Ding L, Wang ZY.* Isothiocyanates repress estrogen receptor alpha expression in breast cancer cells. Oncol Rep 2009; 21(1): 185–92.
46. *Mi L, Gan N, Cheema A, Dakshanamurthy S, Wang X, Yang DC*, et al. Cancer preventive isothiocyanates induce selective degradation of cellular alpha- and beta-tubulins by proteasomes. J Biol Chem 2009; 284(25): 17039–51.
47. *Mukherjee S, Dey S, Bhattacharya RK, Roy M.* Isothiocyanates sensitize the effect of chemotherapeutic drugs via modulation of protein kinase C and telomerase in cervical cancer cells. Mol Cell Biochem 2009; 330(1–2): 9–22.
48. *Prawan A, Saw CL, Khor TO, Keum YS, Yu S, Hu L*, et al. Anti-NF-kappaB and anti-inflammatory activities of synthetic isothiocyanates: effect of chemical structures and cellular signaling. Chem Biol Interact 2009; 179(2–3): 202–11.
49. *Zhang Y, Tang L, Gonyalez V.* Selected isothiocyanates rapidly induce growth inhibition of cancer cells. Mol Cancer Ther 2003; 2(10): 1045–52.
50. *Xiao D, Srivastava SK, Lew KL, Zeng Y, Hershberger P, Johnson CS*, et al. Allyl isothiocyanate, a constituent of cruciferous vegetables, inhibits proliferation of human prostate cancer cells by causing G_2/M arrest and inducing apoptosis. Carcinogenesis 2003; 24(5): 891–7.
51. *Musk SR, Johnson IT.* Allyl isothiocyanate is selectively toxic to transformed cells of the human colorectal tumor line HT29. Carcinogenesis. 1993; 14(10): 2079–83.
52. *Tang L, Zhang Y.* Dietary isothiocyanates inhibit the growth of human bladder carcinoma cells. J Nutr. 2004; 134(8): 2004–10.
53. *Konić-Ristić A, Stanojković T, Srdić-Rajić T, Dilber S, Đorđević B, Stanković I,* et al. In vitro assessment of antiproliferative action selectivity of dietary isothiocyanates for tumor versus normal human cells. Vojnosanit Pregl 2016; 73(7): 636–42.

Received on February 12, 2019
Revised on October 29, 2019
Accepted November 6, 2019
Online First November, 2019