Determination of the Total Phenolic Content, Antioxidant Activity and Cytotoxicity of Selected Aromatic Herbs

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Summary

Aromatic plants used as culinary herbs contain phytochemicals with distinct properties affecting the population that utilizes them, yet there is still insufficient data on their bioactive profile. The present study investigated the antioxidant and cytotoxic properties of five aromatic herbs: *Allium schoenoprasum* L. (ASPR), *Allium ursinum* L. (AUR), *Anthriscus cerefolium* L. Hoffm. (ACH), *Capsicum annuum* L. *var. annuum* (CAF) and *Foeniculum vulgare* Mill (FVH).

Total phenolic content (TPC) and total flavonoid content (TFC) were determined by Folin-Ciocalteu method and $AlCl_3$ method. Antioxidant activity of the extracts was examined by 2,2'-diphenyl-1-pycrylhydrazyl (DPPH), Ferric reducing antioxidant power (FRAP), Non-site-specific-degradation (NSSOH) and Site-specific-deoxyribose-degradation (SSOH) assays. The cytotoxicity of the extracts was evaluated by Brine shrimp lethality assay (BSLA). Considerable variations were observed for TPC values from 65.03 to 253.74 mg GAE/g crude extract and TFC values from 8.02 to 49.58 mg QE/g crude extract. Highest quantity of total polyphenols and flavonoids was measured in CAF, which also demonstrated strong radical scavenging ability, reducing power and chelating activity. ACH showed lower amount of polyphenols and weak antioxidant activity. Obtained LC_{50} values by BSLA revealed strong cytotoxicity for CAF, moderate for FVH and ASPR, weak cytotoxicity for AUR, while ACH caused no toxic effects against the shrimps.

Obtained data indicate that certain extracts have notable antioxidant and cytotoxic properties. Therefore, they present promising dietary sources in prevention of pathological conditions associated with accumulation of free radicals.

Key words

dietary antioxidants, antioxidant capacity, cytotoxic potential, brine shrimp lethality assay, LC_{50}

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Introduction

Epidemiological studies have shown that a diet rich in plant-derived foods is associated with a reduced risk of developing chronic diseases and lower risks of cancer. Only 5–10% of all cancer cases can be attributed to genetic defects, whereas the environment and lifestyle account for 90–95% of most chronic illnesses (Anand et al., 2008). Higher consumption of plant-derived foods increases the availability of antioxidants in biological systems, which may help to maintain the balance between generated free radicals and the endogenous antioxidant defense. Oxidative stress occurs when the production of reactive oxygen species (ROS) exceeds the limited capacity of the cellular antioxidant system (Treml and Šmejkal, 2016), resulting in their accumulation and further involvement in tumor promotion and cancer development (Shimoi et al., 1996). Therefore, an increasing interest has been recently projected in utilizing the biologically active phytochemicals in the prevention of oxidative damage in biological systems. Extensive research on the biochemistry of plant-derived compounds indicated that secondary metabolites, and especially phenolics, are highly potent class of compounds with various protective roles in plants. In the group of total polyphenols, flavonoids deserve special attention because of their structural diversity and high reactivity with free radicals, mainly as a result of their radical scavenging properties (López-Lázaro, 2009).

Many natural bioactive compounds have been identified as potential anti-cancer agents, such as catechins from green tea, curcuminoids from turmeric, isoflavones from soybean, capsaicin from hot chili peppers (Pramanik and Srivastava, 2013). *Capsicum annuum* L. var. *annuum* fruits have a long history of use as spice because of its high pungency, but also as a remedy in traditional medicine for the treatment of arthritis, rheumatism, stomach ache, and skin rashes (Rigon Zimmer et al., 2012). An ethnopharmacological survey on herbal remedies used for treatment of various types of cancer reported that *C. annuum* water decoction is commonly used to treat skin and bladder cancers in the West Bank, Palestine (Jaradat et al., 2016). Due to its pleasant spicy aroma, *Foeniculum vulgare* Mill. fruits are widely used for flavoring foods, as well as for the treatment of menstrual disorders, dyspepsia, flatulence, cough, or as a galactagogue in lactating mothers. Aqueous preparations of the leaves and flowers for oral use are also traditionally used to treat gastritis, conjunctivitis and cancer (Tene et al., 2007). The genus *Allium* is characterized by the presence of diallyl sulfides, the main responsible compounds for the antimicrobial potency (Casella et al., 2013; Lu et al., 2011). Fresh bulbs are traditionally used as antiseptic agents and remedies for lung congestion and flu. In traditional folk medicine *A. schoenoprasum* L. is recognized as agent that lowers blood pressure, aids digestion and enhances the immune system (Esmail Al-Snafi, 2013), while *A. ursinum* L. is frequently used in European traditional medicine as digestive stimulant, detoxifying agent and as treatment of bronchitis (Sobolewska et al., 2015). Regular consumption of *Allium* bulbs is associated with decreased risk of cancer, particularly cancers of the gastrointestinal tract. *Anthriscus cerefolium* L. Hoffm. leaves are known for their expectorant, stimulant and diuretic properties. Despite its common intake as aromatic herb in the diet, little is known of the bioactive properties. Records on the traditional use of this herb report the utilization of whole aerial parts to treat eczema, high blood pressure, gout and kidney stones (Charles, 2013). No information on its traditional use for the treatment and/or prevention of cancers was reported in literature, although in the taxonomically closer member *Anthriscus sylvestris* (L.) Hoffm. the presence of deoxypodophyllotoxin was detected, a compound with antitumor and anti-proliferative effects (Olaru et al., 2015).

The current study examined antioxidant and cytotoxic properties of several plant species commonly utilized as natural flavouring agents and dietary sources of antioxidants, to justify their utilization in preventive purposes and treatment of pathological conditions. According to the obtained results in the current study, further examination of certain plant species has been suggested for a detailed analysis on the possible mechanisms of their cytotoxicity *in vivo*.

Materials and methods

Plant samples

Five commercial aromatic herbs and spices were investigated for their total phenolic content, total flavonoid content, *in vitro* antioxidant activity and *in vivo* cytotoxicity. The commercial samples were purchased from a local herbal products sector of different plant manufacturers in Macedonia: fruits from *Capsicum annuum* L. *var. annuum* (cayenne pepper), aerial parts from *Foeniculum vulgare* Mill. (fennel) and *Anthriscus cerefolium* (L.) Hoffm. (chervil), and bulbs from *Allium schoenoprasum* L. (chives) and *Allium ursinum* L. (wild garlic).

Preparation of extracts

Dry plant material was milled in electric grinder and 625 mg of the fine powder were extracted with 25 mL 96% (v/v) ethanol in ultrasonic bath (50/60 Hz, 720W) for 30 minutes at room temperature. After filtration, the volume of the extracts was adjusted to 25 mL with ethanol to final concentration 25 mg/mL and stored in dark and cold place. For the cytotoxicity study, extracts were prepared as water:ethanol (1:1, v/v) mixtures by ultrasonification for 60 minutes at 40°C, filtered and evaporated until dry on vacuum rotary evaporator, followed by lyophilization. Obtained samples were stored in dark airtight containers at -18°C until use.

Total polyphenolic content and Total flavonoid content

The total polyphenolic content was measured using Folin-Ciocalteu method as described by Singleton et al. (1999) with slight modifications. In the test tubes 0.1 mL of each extract were pipetted and mixed with 2.5 mL Folin-Ciocalteu reagent (previously diluted in 1:10 with deionized water). The mixtures were incubated at room temperature with periodical mixing and allowed to stand for five minutes. After incubation, 3 mL Na_2CO_3 (7%, w/v) were added, followed by rigorous mixing. The volume of the mixtures was adjusted to 10 mL with deionized water and incubated for 60 minutes in dark place. Absorbance of the blue-coloured solutions were measured at 765 nm and final results expressed as gallic acid equivallents/g crude extract based on the equation from gallic acid standard curve (20 – 200 µg/mL).

To determine the total flavonoid content, the Aluminum chloride method was conducted according the described procedure by Lallianrawna et al. (2013). The reaction mixture was

prepared by mixing 0.1 mL of extract and 0.1 mL $NaNO_2$ (5%) and allowed to stand at room temperature for five minutes, followed by addition of 0.15 mL $AlCl_3$ (10%) and incubation for six more minutes. After adding 0.5 mL NaOH (1M) and vigorously shaking, the reaction solution was adjusted to final volume 2.5 mL with deionized water, followed by immediate measurement of the absorbance at 510 nm. Results were expressed as quercetin equivallents/g crude extract using the equation from quercetin standard calibration curve (10 – 120 μg/mL).

In vitro antioxidant activity

The antioxidant properties of the selected spices were evaluated spectrophotometrically by in vitro antioxidant methods: DPPH (1,1-diphenyl-2-picrylhydrazyl) assay, FRAP (Ferric reducing antioxidant power) assay, NSSOH (Nonsite-specific hydroxyl radical-mediated 2-deoxyribose degradation) assay and SSOH (Site-specific hydroxyl radical-mediated 2-deoxyribose degradation) assay.

DPPH assay: The radical scavening ability of the extracts was measured according the described procedure by Brand-Williams et al. (1995). Reaction solutions were prepared by dilution of the basic extract in three concentrations: 2, 5 and 10 mg/mL. Aliquots of each concentration were added to 4 mL DPPH ethanol solution (100 μM) and the mixtures were incubated for 10 minutes in the dark. Absorption of the test solutions was measured at 517 nm and the obtained results were compared against quercetin, BHA and ascorbic acid as standards. Based on the measured absorbance, a percentage of inhibition (%) was calculated for each concentration according the following equation:

$$I_{\%} = [(A_{blank} - A_{sample})/A_{blank}] \times 100\%$$

The radical scavenging capacity of the samples was calculated as IC_{50} values (inhibitory concentration of extract reducing the absorbance of DPPH solution by 50%) by regression analysis:

$$IC_{50}\ (mg/mL) = (50 - b)/a^*$$

(*a – slope; b – intercept)

Final results were presented as AAI (antioxidant activity index) and classified according the Scherer and Godoy's scale (2009):

$$AAI = \text{final concentration of DPPH } (\mu g/mL) / IC_{50}\ (\mu g/mL)$$

FRAP assay: Reducing power of the extracts was determined as percentage of ferric reducing capacity (FRAP) according the method of Oyaizu (1986) with minor modifications. Different concentrations of the extract (0.25 mL) were added to 2.5 mL phosphate buffer (0.2 M, pH 6.6) and 2.5 mL $K_3Fe(CN)_6$ (1%), and the mixture was incubated for 20 minutes at 50°C. The reaction was stopped by introducing 2.5 mL CCl_3COOH (10%) in the test tube and allowed to cool down. An aliquot (2.5 mL) was transferred into another tube containing 2.5 mL distilled water and 0.5 mL $FeCl_3$ (1%) and vigorously shaken. Absorbance of the reaction mixture was determined after 30 minutes at 700 nm and final results were calculated based on the $FeSO_4 \times 7H_2O$ (0.5 – 15 mmol/mL) calibration curve. FRAP values for the extracts were calculated based on the obtained regression equation and later compared against quercetin, BHA and ascorbic acid as standards.

NSSOH and SSOH assays: The hydroxyl radical scavenging ability of herbal extracts was evaluated according the procedure of Halliwell et al. (1987). Aliquot (100 μL) of different concentrations of extract were mixed with 500 μL 2-deoxy-D-ribose (5.6 mM, in KH_2PO_4-NaOH buffer, pH 7.4), 200 μL premixed (1:1 v/v) $FeCl_3$ (100 μM) and phosphate buffer/EDTA (104 μM), 100 μL H_2O_2 (1 mM) and 100 μL ascorbic acid (1 mM). Reaction mixtures were vortexed and incubated at 50°C. After 30 minutes of incubation the reaction was interrupted by adding 1 mL CCl_3COOH (2.8%), followed by the addition of 1 mL thiobarbituric acid (1%). The contents were vortexed and heated at 50°C for 30 minutes and the extent of 2-deoxyribose degradation was measured at 532 nm. Final results were calculated using regression analysis and expressed as IC_{50} values (inhibitory concentration of extract that reduces the absorbance of thiobarbituric acid reactive substances (TBARS) by 50%). The chelating ability was evaluated by comparing IC_{50} values obtained without chelator EDTA (SSOH assay) and IC_{50} values obtained in the presence of EDTA (NSSOH assay). Herbal extracts with lower IC_{50} values in the absence of EDTA were identified as samples with potent chelating activity.

In vivo cytotoxic potential

Herbal extracts were examined for their cytotoxicity according the Brine shrimp lethality assay described by Meyer et al. (1982) and McLaughlin et al. (1998). Extracts were prepared by reconstitution of the lyophilizates with DMSO (0.05%) to final concentration 10 mg/mL and refrigirated until use.

Preparation of the medium and hatching of Artemia larvae: The medium for hatching *Artemia* larvae is prepared as NaCl water solution in concentration c = 38 mg/mL and pH 9.00. Brine cysts are applied in the artificial sea water and hatched to adult larvae under constant aeration, light exposure and a temperature range between 25 and 28°C. After 48 hours hatching, *Artemia* nauplii reach adult form of instar stage III, the most optimal stage for the Brine shrimp assay (Vanhaecke et al., 1981; Sorgeloos et al., 1978).

Setting the Brine shrimp lethality assay: The mortality of the *Artemia* larvae was observed in a concentration range 0.01 to 10 mg/mL for each extract. From the stock solution (10 mg/mL), six concentrations were prepared (5, 3, 1, 0.5, 0.1 and 0.01 mg/mL) and ten shrimps were applied in each concentration of extract. Number of dead brine shrimps was recorded after 6, 10, 24, 30, 36, 48, 54 and 60 hours of exposure. Mortality of the exposed larvae was established if no movement was detected after 10 seconds of observation. Final results were calculated by probit regression analysis and expressed as LC_{50} values (lethal concentration that kills 50% of the exposed population of shrimps) after 24-hour exposure of *A. salina*.

Statistical and correlation analysis

All tests were conducted in triplicate and final results were expressed as mean ± standard deviation. The correlation between total polyphenols, antioxidant activity and the cytotoxic potential was examined by regression analysis. Statistical significance for the cytotoxicity studies was assigned at $p < 0.05$ and the probit regression analysis was conducted using IBM SPSS 20.0 statistical software based on Finney computation method (Finney, 1949).

Results and discussion

Total polyphenolic content and total flavonoid content

Phenolic compounds are an important group of secondary metabolites produced as adaptive response to biotic and abiotic stress conditions (Oboh and Rocha, 2007). Knowledge on the effects of dietary polyphenols on human health is constantly growing and it strongly supports their preventive role from degenerative diseases (Scalbert et al., 2005). More than 8,000 polyphenolic compounds have been identified, of which flavonoids comprise the largest and most studied group of polyphenols. Individual differences within each group of flavonoids arise from the variation in the number and arrangement of hydroxyl groups and their extent of alkylation and/or glycosylation (Pandey and Rizvi, 2009).

Total polyphenols in the examined samples ranged from 65.03 to 253.74 mg GAE/g crude extract, while total flavonoid content was detected in the range from 8.02 to 49.58 mg GAE/g crude extract (Table 1). *Capsicum annuum* was characterized as a sample with the highest total polyphenolic content and total flavonoid content.

A slightly different descending trend was observed for the TFC values in comparison to the total polyphenols. This is mostly evident from the percentage fraction of flavonoids calculated for the samples: highest fraction of flavonoids in the total polyphenolic content was observed for *Anthriscus cerefolium* (21.39%), followed by *Capsicum annuum* (19.54%) and *Allium ursinum* (14.59%).

Table 1. Total phenolics and total flavonoids of selected aromatic herbs

Plant species	TPC (mg GAE/g)	TFC (mg QE/g)	(TFC/TPC) x 100%
Allium schoenoprasum L.	112.67 ± 5.08	8.02 ± 0.51	7.12
Allium ursinum L.	111.69 ± 2.78	16.30 ± 2.37	14.59
Anthriscus cerefolium L.	65.03 ± 1.56	13.91 ± 0.33	21.39
Capsicum annuum L.	253.74 ± 12.03	49.58 ± 3.18	19.54
Foeniculum vulgare Mill.	136.82 ± 6.15	10.41 ± 0.33	7.61

Antioxidant activity

The mechanism of DPPH assay is based on the reduction of the stable DPPH radical in the presence of hydrogen-donating antioxidants. DPPH radical is stabilized as a result of delocalisation of the spare electron giving rise to a deep violet color that is lost upon receiving hydrogen forming yellow-coloured 2,2-diphenyl-1-pycrylhydrazine (Kedare and Singh, 2011). The physicochemical properties of DPPH radical highly resemble the free radicals *in vivo* formed as a result of lipid autooxidation. Therefore, this test is intended to represent the formation of these radicals and their supression *in vivo* by hydrogen donating substances such as vitamins C and E (Njus and Kelley, 1991). Obtained results for the antioxidant properties using DPPH assay demonstrate that all examined spices possess radical scavenging ability to a different extent (Table 2).

Table 2. Antioxidant activity of selected aromatic herbs according DPPH and FRAP assays

Plant species	Abbr.	$I_{\%}$	DPPH IC_{50} (µg/mL)	AAI	FRAP (%)
Allium schoenoprasum L.	ASPR	18.85	3700.99	0.011	13.21
Allium ursinum L.	AUR	11.96	4936.60	0.007	14.49
Anthriscus cerefolium L.	ACH	9.98	7430.54	0.005	11.65
Capsicum annuum L.	CAF	90.29	22.30	1.791	20.93
Foeniculum vulgare Mill.	FVH	83.86	51.52	0.758	14.02
Quercetin*		92.30	2300.63	17.126	31.71
ascorbic acid*		95.84	5844.74	6.741	16.85
BHA*		51.85	11914.92	3.307	21.38

*initial concentration of standards is 250 µg/mL

The highest percentage of DPPH inhibition was observed for CAF (90.29%) and FVH (83.86%). According to Scherer and Godoy's scale, CAF is classified as sample with strong antioxidant potency (AAI > 1), while *Foeniculum vulgare* is classified as sample with moderate antioxidant ability (AAI > 0.5). Additionally, *Capsicum annuum* and *Foeniculum vulgare* showed very close values for the radical scavenging capacity in comparison with standards quercetin (92.30%) and ascorbic acid (95.84%), and a higher activity compared to the synthetic antioxidant BHA (51.85%).

FRAP assay is based on the reducing capability of antioxidants in the presence of Fe^{3+} ions as oxidants in the reaction system. The mechanism of action follows single electron transfer from the antioxidant molecule to the oxidant (Ou et al., 2002), resulting in the formation of blue-coloured Fe^{2+}- complexes (Perl's Prussian). The selected herbal extracts demonstrated proximate values for the reducing power, with CAF possessing most prominent ferric reducing activity (20.93%) (Table 2). FRAP values are descending in the following order: Quercetin > BHA > CAF > ascorbic acid > AUR > FVH > ASPR > ACH.

Comparison of the measured IC_{50} values by NSSOH and SSOH assays revealed that all samples have lower IC_{50} values in the presence of EDTA and was identified as a good source of antioxidant compounds with better hydroxyl radical scavenging properties than chelating ability (Fig. 1). The mechanism of reaction consists of three steps: 1) hydroxyl radical generation as a result of Fenton reaction between H2O2 and ferrous ions, 2) hydroxyl-induced degradation of 2-deoxyribose and 3) formation of thiobarbituric acid reactive substances (TBARS) in a reaction between 2-deoxyribose radicals and thiobarbituric acid.

Strong hydroxyl radical scavenging activity was observed for *C. annuum*, whereas *A. cerefolium* demonstrated moderate scavenging capacity. Notable chelating ability was only observable for *C. annuum* (IC_{50} 0.45 mg/mL).

Previous studies on the chemical composition of *Capsicum annuum* reported that capsaicin and its derivatives are the main bioactive compounds of *Capsicum* species and the antioxidant properties are mainly due to the presence of these secondary

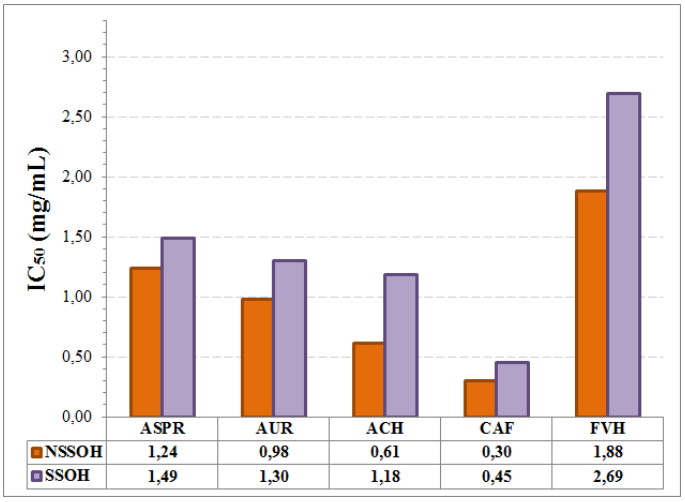

Figure 1. Comparison of IC_{50} values obtained with EDTA (NSSOH) and without EDTA (SSOH) in the reaction mixture

metabolites. High concentration of phenolics and a strong antioxidant activity was also observed for red varieties of *C. annuum* compared to green and yellow cultivars (Aniel Kumar et al., 2010; Castro-Concha et al., 2014; Chávez-Mendoza et al., 2015). Higher amount of total polyphenols in red varieties compared to green varieties was also demonstrated in the study of Ciulu-Costinescu et al. (2015) by employing FT-IR analysis of *C. annuum* extracts. On the other hand, Alvarez-Parrilla et al. (2011) reported the presence of chlorophyl in green peppers and its significant role in the radical scavenging activity observed by DPPH assay, suggesting of the complexity and synergistic mechanisms of several classes secondary metabolites (alkaloids, phenolics) and involvement of primary metabolites (such as chlorophyl) in the total antioxidant capacity of *Capsicum* species.

Extensive phytochemical analysis of *Foeniculum vulgare* conducted by previous authors revealed *trans*-anethole, estragole, limonene and fenchone as the principal compounds of the essential oil (Martins et al., 2012; Ruberto et al., 2000; Telci et al., 2009), whereas the crude extract contains phenolic acids (chlorogenic acid, caffeic acid, *p*-coumaric acid, rosmarinic acid), coumarins (6,7-dihydroxycoumarin), flavonoids (quercetin, kaempferol) and their glucosides (ferulic acid-7-o-glucoside, quercetin-7-o-glucoside) (Badgujar et al., 2014; Cai et al., 2004; Parejo et al., 2004). Shahat et al. (2011) postulated that the prominent antioxidant activity of *Foeniculum vulgare* is mostly attributed to *trans*-anethole identified as the main component of essential oils from two varieties: *azoricum* and *dulce*. Moreover, they described the variety *vulgare* as sample with significantly lower amount of *trans*-anethole and weak antioxidant activity compared to other varieties. Despite the high levels of *trans*-anethole in the cultivated varieties (Bernáth et al., 1996), wild fennel was found to exhibit stronger radical scavenging activity because of the higher phenolic and flavonoid content than the cultivated medicinal and edible fennel (Faudale et al., 2008), suggesting of possible synergistic effect of highly potent compounds detected in *F. vulgare*.

Cytotoxic potential

Previous research on the cytotoxicity of herbal extracts demonstrated positive correlation between the Brine shrimp lethality assay and animal models (Logarto Parra, 2001; Naidu et al., 2014; Obembe et al. 2014; Sahgal et al., 2010; Shafii et al., 2011; Sharma et al., 2013; Syahmi et al., 2010), as well as with *in vitro* cell cultures (Anderson et al., 1991; Rajabi et al., 2015). Therefore, Brine shrimp lethality assay is considered an excellent approximation of the presence of potential cytotoxic agents from plant origin. Obtained results for the toxicity of selected plant species against *A. salina* are presented in Figure 2 as percentage of dead larvae after 24-hour exposure to different concentrations

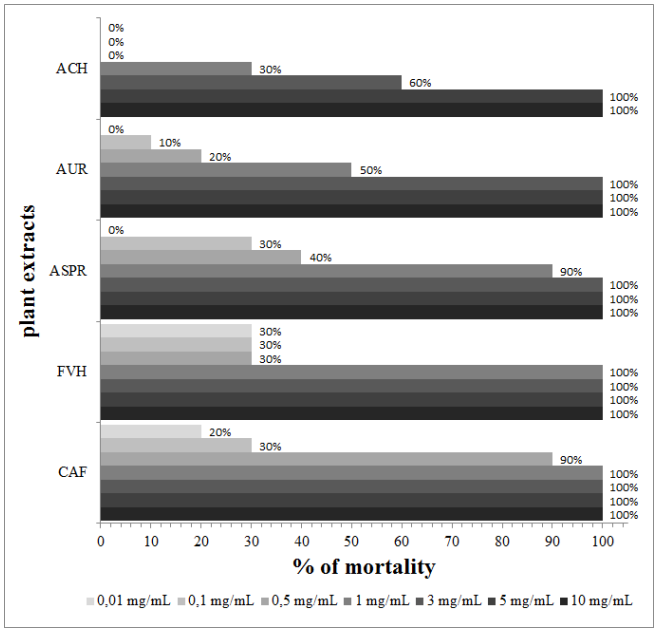

Figure 2. Mortality rate for *A. salina* after 24-hour exposure against different concentrations of extracts

of the extracts. Maximal mortality rate of 100% was observed in the high concentration range (3, 5 and 10 mg/mL) for CAF, FVH, ASPR and AUR. Divergent mortality for the larvae was observed at 1 mg/mL with CAF and FVH causing 100% mortality, whereas ASPR, AUR and ACH caused mortality of 90%, 50% and 30%, respectively. Toxic effects at the lowest concentration of extract (0.01 mg/mL) were only detected for CAF (20%) and FVH (30%).

Classification of the herbal extracts was achieved according Meyer's scale (Meyer et al., 1982) and Clarkson's scale of toxicity (Clarkson et al., 2004) based on their calculated LC_{50} values. Plant samples were divided into two main groups: extracts with cytotoxic properties (< 1000 µg/mL) and extracts with no cytotoxicity against *A. salina* (> 1000 µg/mL). Extracts were also classified into three sub-categories according Clarkson's scale: strong (0 – 100 µg/mL), moderate (100 – 500 µg/mL) and weak (500 – 1000 µg/mL) cytotoxicity.

Two samples demonstrated insignificant toxic effects against *Artemia* larvae: *A. ursinum* was classified as extract with weak cytotoxicity, while *A. cerefolium* was described as sample with no cytotoxic properties (LC_{50} 743 and 1822 µg/mL, respectively).

F. vulgare and *A. schoenoprasum* were identified as extracts with moderate cytotoxic properties (LC_{50} 129 and 302 µg/mL, respectively) whereas *C. annuum* was classified as extract with strong cytotoxic potential (LC_{50} 80 µg/mL).

The moderate cytotoxicity of *F. vulgare* obtained in the current study is in accordance with results from previous *in vitro* cytotoxicity testing on several cell lines. Sharopov et al. (2017) demonstrated the cytotoxicity of essential oil from *F. vulgare* against HeLa (human cervical cancer), Caco-2 (human colorectal adenocarcinoma), MCF-7 (human breast adenocarcinoma), CCRF-CEM (human T lymphoblast leukaemia) and CEM/ADR5000 (adriamycin resistant leukaemia) cell lines with IC_{50} values between 30 – 210 µg/mL and the activity was attributed to the presence of *trans*-anethole and *p*-anisealdehyde. Essential oils rich in aldehydes often exhibit cytotoxic activity via formation of Shiff's bases with free amino groups (Bassi et al., 1997), such as amino acid residues in proteins and nucleic acids.

On the other hand, a considerable amount of evidence in literature suggests that capsaicin, the main compound in *Capsicum* species, has anti-inflammatory, antitumor and chemopreventive effects (Campos et al., 2013) and the proposed mechanisms for the anti-cancer effects include cytochrome P-450 in targetting the excessive production of ROS in the mitochondrial respiratory chain, suppression of transcription factors NF-kB and STAT-3, inhibition of cell survival pathways and intrinsic mitochondrial cell death pathway (Pramanik and Srivastava, 2013). *C. annuum* may also contain potential chemopreventive compounds such as phenols and capsaicinoids that interact in synergistic and additive mode resulting in antimutagenic activity (El Hamss et al., 2003). The strong cytotoxic potential of *C. annuum* water: ethanol extracts observed in the current study (80 µg/mL) is in accordance with previously reported data on other members from the *Capsicum* genus using Brine shrimp assay. Similar results for the cytotoxicity were obtained for ethanol extracts of *C. frutescens* with LC_{50} value 83.33 µg/mL in the study of Anwar et al. (2013). Strong cytotoxicity for *C. annuum* ethanol extracts was also demonstrated by Bertão et al. (2016) (78,14 µg/mL) and surprisingly, the chemical analysis showed low levels of capsaicin and dihydrocapsaicin in *C. annuum*. In contrast of these findings, Maksimova et al. (2016) reported strong cytotoxic potential of isolated capsaicin and weak cytotoxicity of ethanolic extract of *C. annuum* as a result of the presence of other bioactive compounds in *Capsicum* fruits that prevent the cytotoxic effects of the extracts on neuroblastoma cells. Therefore, isolated compounds may have a more potent activity than crude extracts in the successful targeting of cancerous cells.

Correlation analysis

A positive relation was obtained between total polyphenols and the antioxidant activity of extracts expressed as radical scavenging activity in DPPH assay (R^2 = 0,9008) and ferric reducing capacity in FRAP assay (R^2 = 0.9634) (Fig. 3). This observation is supported with previous research on the antioxidant effects of phenolic compounds, mainly due to multiple mechanisms like neutralizing free radicals and certain structural characteristics such as multi-hydroxylation of the phenol ring (Ademoyegun et al., 2011; Jing et al., 2012; Natella et al. 1999; Sawai and Sakata, 1998). However, no significant correlation was observed between the total polyphenols and the cytotoxic potential, suggesting of more complex mechanisms involved and a need to conduct a broader analysis with more samples in order to determine the exact role of polyphenols in the cytotoxicity of herbal extracts.

Conclusion

Overall, all samples were identified as rich source of bioactive compounds with prominent antioxidant capacity compared to synthetic standards. *Capsicum annuum var. annuum* was described as sample with prominent chelating ability and rich source of polyphenols. Plant species were also characterized with potent cytotoxic properties of which *Foeniculum vulgare* and *Allium schoenoprasum* exhibited moderate cytotoxicity, whereas *Capsicum annuum var. annuum* exhibited strong cytotoxicity in the Brine shrimp lethality assay. However, the presence of polyphenols in the aforementioned extracts was only correlated to the antioxidant properties of the samples, while no evident correlation was demonstrated to their cytotoxicity. Therefore, further studies are necessary in order to clarify the role of polyphenols in the cytotoxic mechanisms and to identify the compounds that are predominantly responsible for their bioactivity.

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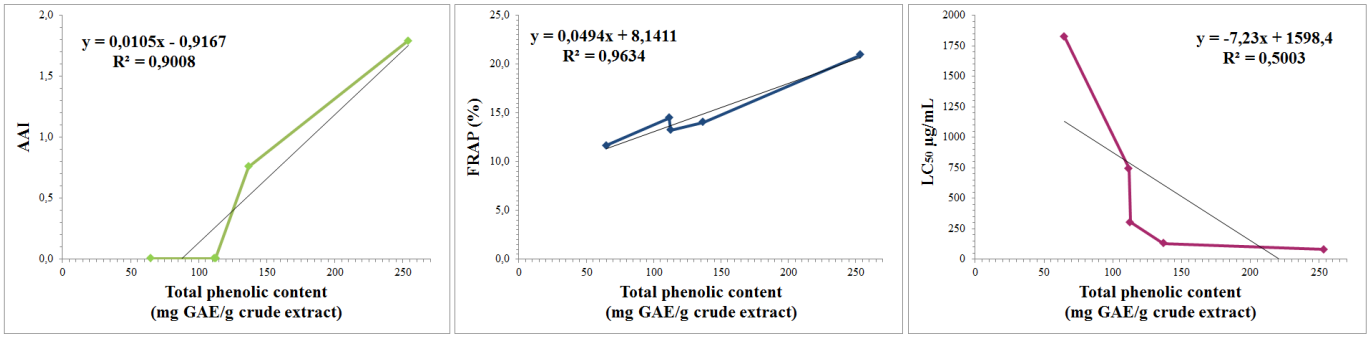

Figure 3. Correlation between A. TPC and AAI (DPPH); B. TPC and % FRAP capacity; C. TPC and LC_{50} (BSLA)

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