International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 3 Number 2 (2014) pp. 296-306**
http://www.ijcmas.com

Original Research Article

Antioxidant activities and phenolic compounds in Bulgarian *Fumaria* species

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Keywords

Antioxidant activity; *Fumaria;* Polyphenolics

A B S T R A C T

Fumaria plants have been traditionally used against skin diseases, as diuretics, laxatives, hepatoprotectants and etc. Their biological activity is associated with the presence of isoquinoline alkaloids, while polyphenols are not investigated well in this context. Polyphenols could increase the added value of medicinal plants extracts by enhancing their antioxidant activity or improving overall biological activities. The presented study evaluated the polyphenolic contents and antioxidant activities of extracts of five Bulgarian *Fumaria* species (*Fumariaceae*): *Fumaria officinalis* L., *Fumaria thuretii* Boiss., *Fumaria kralikii* Jord., *Fumaria rostellata* Knaf. and *Fumaria schrammii* (Asch) Velen. Qualitative and quantitative determinations of phenolic acids and flavonoids were performed by HPLC system with UV-detection. Antioxidant activity of extracts was evaluated by four popular spectrophotometric methods (DPPH, ABTS, FRAP and CUPRAC). The total phenolic content was the highest in extract of *F. officinalis* with quercetin (0.49 ± 0.03 mg/g DW), *p*-coumaric (1.10 ± 0.03 mg/g DW) and ferulic (2.35 ± 0.04 mg/g DW) acids as major compounds. Theses extracts also showed the highest antioxidant activity among the investigated plants. Our data reveal new possibilities to expand applications of *Fumaria* extracts from a local ethnomedicine drug to new industrial fields such as foods and cosmetics preservation.

Introduction

The genus *Fumaria (Fumariaceae*) consists of 60 species widely distributed all over the world and especially in Mediterranean region (Suau et al., 2002;

Jaberian et al., 2013). In the Bulgarian flora, the genus is represented by ten species: *Fumaria officinalis* L., *Fumaria thuretii* Boiss., *Fumaria kralikii* Jord., *Fumaria rostellata* Knaf., *Fumaria schrammii* (Asch.) Velen., *Fumaria parviflora* Lam., *Fumaria densiflora* DC., *Fumaria petteri* Rchb., *Fumaria vaillanti* Loisel. and *Fumaria schleicherii* Soy. - Will. (Assyov *et al*., 2012.

Extracts of *Fumaria* spp. have been traditionally used for treatment of some skin diseases (rashes or conjunctivitis), rheumatism, stomach ache, abdominal cramps, fever, diarrhea, syphilis and leprosy (Şener, 2002; Maiza-Benabdesselam *et al*., 2007). Research revealed that *Fumaria* extracts also possessed strong antihypertensive, diuretic, hepatoprotective and laxative effects, mainly because of the presence of isoquinoline alkaloids (Suau *et al*., 2002). In addition, some species, such as *F. cilicica* Hausskn, *F. densiflora* DC., *F. kralikii*, *F. parviflora* and *F. vaillantii* have been reported to possess significant antioxidant activities, due to their high phenolic contents (Orhan *et al*., 2012; Riaz *et al*., 2012; Jaberian *et al*., 2013).

Antioxidants have been used as important protective agents for human health against oxidative stress disorders, as well as protectors against oxidative damage in food systems. During the last decade, there is a growing demand of natural plant extracts with antioxidant activity because of the increased report for carcinogenic effect of some synthetic antioxidants, currently applied in foods, cosmetics, and pharmacy (Riaz *et al*., 2012). Plant polyphenols are example for natural compounds with strong antioxidant properties (Kulišić *et al*., 2006). The presence of polyphenols in medicinal plants could additionally increase the added value of their extracts by enhancing their antioxidant activity or improving their overall biological activities. Therefore, characterization and evaluation of biological activities of those compounds are critical steps in assessment of herbal extracts for their possible application as food additives or pharmaceuticals. However, there was scanty information concerning polyphenol composition and antioxidant activities of *Fumaria* extracts and according to our best, such data is missing for Bulgarian *Fumaria* ssp. The aim of this study was to analyze the polyphenolic contents and antioxidant activities of extracts of five *Fumaria* species grown in Bulgaria*: Fumaria officinalis, Fumaria thuretii, Fumaria kralikii, Fumaria rostellata* and *Fumaria schrammii.*

Materials and Methods

Plant materials

Aerial parts (leaves, stems and flowers) by several random chosen plants of *F. officinalis* L., *F. thuretii* Boiss., *F. kralikii* Jord., *F. rostellata* Knaf. and *F. schrammii* (Asch.) Velen. were collected from their natural habitats in Bulgaria. *F. rostellata* and *F. thuretii* were collected from Blagoevgrad region, *F. officinalis*, *F. kralikii* and *F. schrammii* were collected from Black sea region, all in May 2012. Identification of the plant species was made through the reference to the Academy of Sciences Herbarium (Herbarium of the Institute of Biodiversity and Ecosystem Research in Sofia (SOM)) and with the Herbarium of Sofia University. Transect method was used for establishing the distribution of *Fumaria* species of the localities. Transects were selected in order to cover the maximum area. The samples were dried in shade at

ambient temperature for 14 days, and powdered by homogenizer. The powder was used for extraction of polyphenols.

Extraction procedure

Each plant sample (0.5 g) was extracted three times with 70% ethanol at 70 ºC in water bath for 15 min. The biomass was removed through filter paper filtration, and the combined ethanol extracts were used for the next analyses.

Total phenolics

The total phenolic contents were measured using a Folin-Ciocalteu assay according to the procedure described by Stintzing *et al*., (2005) with some modifications. Folin-Ciocalteu reagent (1mL) (Sigma) diluted five times was mixed with 0.2 mL of sample and 0.8 mL 7.5% Na_2CO_3 (Sigma). The reaction was 20 min at room temperature in darkness. After reaction time, the absorption of sample was recorded at 765 nm against blank sample, developed the same way but without extract. The results were expressed in mg equivalent of gallic acid (GAE) per g dry weight (DW), according to calibration curve, build in range of 0.02 - 0.10 mg gallic acid (Sigma) used as a standart.

Total flavonoids

Total flavonoids were determined spectrophotometrically by the method described by Kivrak *et al*., (2009). Each obtained extract (0.2 mL) was added to test tubes containing 0.1 mL 10% aluminum nitrate (Sigma), 0.1 mL 1M potassium acetate (Sigma) and 3.8 mL ethanol (Merck). The reaction time was 40 min at ambient temperature. The absorbance was measured at 415 nm. The results were expressed in mg equivalent of quercetin per g dry weight (DW), according to the calibration curve, linear in range of 10-100 µg/mL quercetin as a standart.

HPLC analysis

Qualitative and quantitative determinations of phenolic acids and flavonoids, were performed by using Waters 1525 Binary Pump HPLC systems (Waters, Milford, MA, USA), equipped with Waters 2484 dual λ Absorbance Detector (Waters, Milford, MA, USA) and Supelco Discovery HS C18 column (5 µm, 25 cm × 4.6 mm), operated under control of Breeze 3.30 software. For separation of the phenolic acids, a mobile phase of 2% (v/v) acetic acid (solvent A) and 0.5 % (v/v) acetic acid : acetonitrile (1:1, v/v) (solvent B) was used. The gradient program used was described previously by Marchev *et al*., (2011). Ferulic, sinapic, *p*-coumaric acids (Sigma) were used for creation of standard calibration curves. For separation of flavonoids a mobile phase, consists of 2.0 % (v/v) acetic acid (solvent A) and methanol (solvent B) was used. The elution program was described by Marchev *et al*., (2011). Myricetin, kaempferol, quercetin, hesperidine and apigenin (Sigma) were used for calibration standard curves. The quercetin glycosides rutin and hyperoside were analyzed on the same HPLC system by using gradient of 2% (v/v) acetic acid (Sigma) (solvent A) and acetonitrile (Sigma) (solvent B). The elution program was: 0-15 min 80% A and 20% B, 15-17 min 50% A and 50% B, 17-20 min 80% A and 20% B. The detection was carried out at 370 nm.

DPPH assay

Each analyzed extract (0.15 mL) was mixed with 2.85 mL freshly prepared 0.1

mM solution of 1,1-diphenyl-2-picrylhydrazyl radical (DPPH, Sigma) in methanol (Merck). The reaction was performed at 37 °C in darkness and the absorptions at 517 nm were recorded after 15 min against methanol. The antioxidant activity was expressed as mM Trolox equivalents (TE) per g dry weight (DW) by using calibration curve, build by 0.05, 0.1, 0.2, 0.3, 0.4 and 0.5 mM 6-hydroxy-2,5,7,8-tetramethylchroman- 2- carboxylic acid (Trolox, Fluka) dissolved in methanol (Sigma). Calculations of % inhibition and EC_{50} were determined as described by Kivrak *et al*., (2009).

ABTS assay

The method described by Thaipong *et al.*, (2006) was used after some modifications. ABTS radical was generated by mixing aliquot parts of 7.0 mM 2, 2`azinobis (3)-ethylbenzthiazoline-6-sulfonic acid (ABTS, Sigma) in dd H_2O and 2.45 mM potassium persulfate (Merck) in dd H_2O. The reaction was performed for 16 h at ambient temperature in darkness and the generated ABTS radical is stable for several days. Before analyses, 2.0 mL of generated $ABTS^{\cdot+}$ solution was diluted with methanol at proportions 1:30 (v/v), so the obtained final absorbance of the working solution was about 1.0 ÷ 1.1 at 734 nm. For the assay, 2.85 mL of this $ABTS^{\cdot+}$ solution was mixed with 0.15 mL of obtained extracts. After 15 min at 37 °C in darkness the absorbance was measured at 734 nm against methanol. The antioxidant activity was expressed as mM (TE)/g DW by using calibration curve, build in range of 0.05-0.5 mM Trolox (Fluka) dissolved in methanol (Merck).

Ferric reducing antioxidant power (FRAP) assay

The assay was performed according to method, described by Benzie and Strain (1996) slightly modified as follow: the FRAP reagent was freshly prepared before analyzes by mixing 10 parts 0.3 M acetate buffer (pH 3.6), 1 part 10 mM 2,4,6-tripyridyl-*s*-triazine (TPTZ, Fluka) in 40 mM HCl (Merck) and 1 part 20 mM $FeCl_3.6H_2O$ (Merck) in dd H_2O. The reaction was started by mixing 3.0 mL FRAP reagent with 0.1 mL of investigated extract. Blank sample, prepared with methanol instead of extract was developed as well. The reaction time was 10 min at 37 °C in darkness and the absorbance at 593 nm of sample against blank was recorded. Antioxidant activity was expressed as mM TE/g DW by using calibration curve, build in range of 0.05-0.5 mM Trolox (Fluka) dissolved in methanol (Merck).

Cupric reducing antioxidant capacity (CUPRAC) assay

The assay was performed according to Apak *et al.*, (2006) with some modifications. Reaction was started by mixing 1.0 mL 10 mM $CuCl_2.2H_2O$ (Sigma) in dd H_2O, 1.0 mL 7.5 mM Neocuproine (Sigma) in methanol, 1.0 mL 0.1 M ammonium acetate buffer (pH 7.0), 0.1 mL of investigated extract and 1.0 mL dd H_2O. Blank sample, with methanol instead of extract was developed as well. The reaction was carried out for 20 min at 50 °C in darkness and the sample absorption (450 nm) was recorded against blank. Antioxidant activity was expressed as mM (TE)/g DW by using calibration curve, build in range of 0.05-0.5 mM Trolox (Fluka) dissolved in methanol (Merck).

Statistical analyses

Plant material of each species was collected as average sample from several randomly chosen individual plants from

the same habitat. Three independent extracts were prepared from each sample and each extract was analyzed for polyphenol content and antioxidant activities in triple replication. The presented values are means (n = 3) with standard deviations (± SD). Figures were made by Microsoft Office Excel ® 2000.

Results and Discussion

Analyses of total phenolics and total flavonoids

Fumaria species were traditionally used against liver diseases in folklore medicine of many countries Orhan *et al*., (2012). Study on hepatoprotective activity of different fractions of ethanol extract from *F. indica* (Hausskn.) Pugsley. showed that mainly the alkaloid protopine is responsible for the observed high liver protection effects of this plant (Rathi *et al*., 2008). Growing demand of natural herbs for application in medicine leads to approval of *F. officinalis* for application in treatment of colick pains in Germany (Hentschel *et al*., 1995). However, for deep understanding and correct evaluation of pharmaceutical values, more detailed investigations on composition and biological activities of extracts from different *Fumaria* species should be performed. In this study we investigated phenolic and flavonoid contents and antioxidant activities of five *Fumaria* species (*F. officinalis*, *F. thuretii*, *F. kralikii*, *F. rostellata* and *F. schrammii)*, growing in Bulgaria. All investigated plants showed high total phenolic contents, ranging between 20.20 ± 0.29 (in *F. thuretii*) and 30.30 ± 0.31 mg GAE/g DW (in *F. officinalis*) (Figure 1). As comparison, recent published data showed that total phenolic contents of ethanol extracts from *F. cilicica*, *F. densiflora*, *F. kralikii* and *F. parviflora* collected in Turkey varied in significantly lower range (between 0.05 and 0.09 mg GAE/g dry extract or approximately 0.015 and 0.030 mg GAE/g DW) (Orhan *et al*., 2012). Similar tendency could be observed also in the total flavonoid contents. In our study the concentration of total flavonoids in ethanol extracts of *F. officinalis*, *F. thuretii*, *F. kralikii*, *F. rostellata* and *F. schrammii* ranged between 8.70 ± 0.01 to 16.62 ± 0.03 mg QE/g DW (Figure 1), whereas Orhan *et al*., (2012) reported total flavonoid contents in ethanol extract of Turkish *F. cilicica*, *F. densiflora*, *F. kralikii* and *F. parviflora* to varied between 0.02-0.05 mg QE/g dry extract (or approximately 0.006–0.017 mg QE/g DW). The observed differences in results, reported by Orhan *et al*., (2012) and the data in the current study are most probably due to the different extraction methods applied, instead of geographical and climate differences at which plants were grown. Recently, Jaberian *et al*., (2013) reported that methanol extract of *F. vaillantii* contains more total phenolics, compared to methanol-water extract (10.5 compared to 4.27 mg GAE/g DW), whereas the methanol-water extract was rich in total flavonoids (3.11 compared to 2.07 mg QE/g DW in methanol extract). The reported values of *F. vaillantii* extracts by Jaberian *et al*., (2013) were in the same range as the data we reported in this study. However, it is obviously that Bulgarian *F. officinalis*, *F. thuretii*, *F. kralikii*, *F. rostellata* and *F. schrammii* have higher phenolic and flavonoid contents, compared to *F. vaillantii*.

HPLC analyses of polyphenols

To obtain more detailed data about phenolic profile of ethanol extracts from investigated five *Fumaria* species, we

analyzed the contents of major flavonoids and phenolic acids in them (Table 1). Three flavonols (myricetin, kaempferol and quercetin), two quercetin glycosides (rutin and hyperoside), one flavanone (hesperidin), one flavone (apigenin) and three phenolic acids (*p*-coumaric, ferulic and sinapic acids) were identified and quantified (Table 1).

HPLC analysis showed, that the observed variations in flavonoids compositions were more significant between investigated species (Table 1). Ethanol extract of *F. officinalis* was the richest source of quercetin (0.49 ± 0.03 mg/g DW), *p*-coumaric and ferulic acids (1.10 ± 0.03 and 2.35 ± 0.04 mg/g DW, respectively). High concentration of quercetin (0.51 ± 0.03 mg/g DW) was also found in extract of *F. thuretii*, but this was the only extract in which quercetin glycosides rutin and hyperoside were not detected. The extract of *F. kralikii* showed the highest levels of myricetin (0.49 ± 0.07 mg/g DW), kaempferol (0.14 ± 0.01 mg/g DW), hyperoside (7.58 ± 0.13 mg/g DW), apigenin (0.38 ± 0.03 mg/g DW, whereas the extracts of *F. rostellata* and *F. schrammii* showed the highest content of rutin (9.92 ± 0.11 and 8.39 ± 0.15 mg/g DW, respectively).

Flavanone glycoside hesperidin was not detected in extracts of *F. officinalis*, *F. kralikii* and *F. rostellata*. Flavone apigenin was missing only in extract of *F. schrammii*, whereas no quercetin glycosides (rutin and hyperoside) were detected in extract of *F. thuretii* (Table 1). In contrast, rutin and hyperoside were the major flavonoids in other four investigated species. This fact is of great importance, because these quercetin glycosides possessed several biological activities. Rutin was reported to have cardioprotective and hepatoprotective action (Panchal *et al*., 2011). This quercetin glycoside suppresses hyperglycemia, increases plasma concentrations of insulin and decreases oxidative stress (Ahmed *et al*., 2010, Panchal *et al*., 2011). Other activities such as antibacterial, antitumour, antiinflammatory, antidiarrhoeal, antiulcer, antimutagenic, myocardial protecting, vasodilator and immunomodulatory have been also reported (Janbaz *et al*., 2002; Pereira *et al*., 2008). Hyperoside possess antiviral activity, antinociceptive, antiinflammatory, cardioprotective, hepatoprotective, gastricmucosal-protective and neuroprotective effects (Liu *et al*., 2005; Lin-lin *et al*., 2007; Zeng *et al*., 2011).

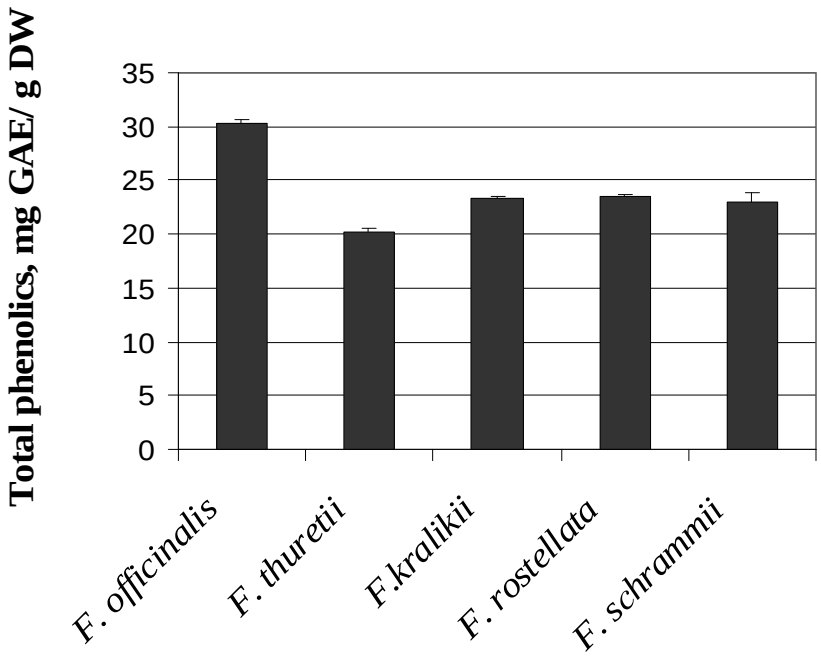
The high content of rutin and hyperoside found in investigated *Fumaria* ssp. defines them as valuable sources of non-alkaloid pharmacologically active compounds.

The phenolic acids patterns in all investigated plants were similar, with slight variations in concentrations (Table 1). The high content of phenolic acids could be considered as prerequisite for expected high antioxidant activities. In addition, it is well known that phenolic acids have some important biological activities. Sinapic acid was reported to have antiinflammatory effect by suppressing production of some proinflammatory mediators (Yun *et al*., 2008) and *p*-coumaric acid could be used in fighting diseases, related to oxidative stress by protecting DNA from oxidative damages (Guglielmi *et al*., 2003).

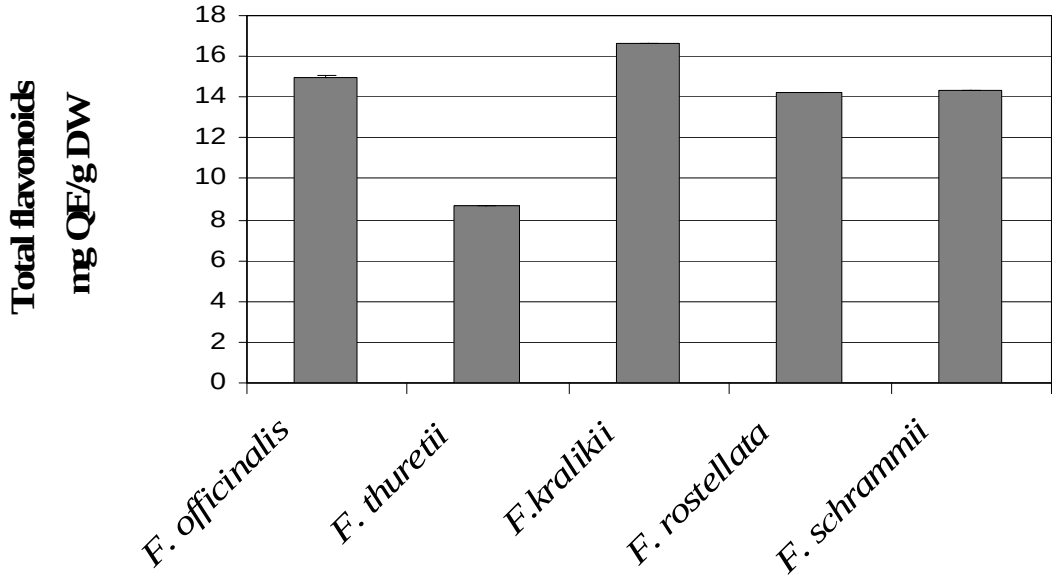
Evaluation of antioxidant activities

To evaluate antioxidant activities of investigated ethanol extracts, their abilities to scavenge DPPH and ABTS radicals, as well as their power to reduce ferric

Figure.1 Total phenolics (A) and total flavonoids (B) contents in ethanol extracts of five Bulgarian *Fumaria* species.



A.



B.

Table.1 HPLC analysis of polyphenols content in ethanol extracts of five Bulgarian *Fumaria* Species

	Compound*	*F.officinalis*	*F. thuretii*	*F.kralikii*	*F.rostellata*	*F.shrammii*
Flavonoids						
Flavonols	Myricetin	0.25 ± 0.01	0.28 ± 0.01	0.49 ± 0.07	0.17 ± 0.03	0.25 ± 0.01
	Kaempferol	0.08 ± 0.01	0.12 ± 0.01	0.14 ± 0.01	0.06 ± 0.01	0.04 ± 0.01
	Quercetin	0.49 ± 0.03	0.51 ± 0.03	0.36 ± 0.02	0.32 ± 0.02	0.14 ± 0.01
Quercetin glycoside	Rutin	6.47 ± 0.13	nd	4.17 ± 0.07	9.92 ± 0.11	8.39 ± 0.15
	Hyperoside	6.51 ± 0.12	nd	7.58 ± 0.13	1.06 ± 0.03	2.78 ± 0.05
Flavanone glycoside	Hesperidin	nd	0.29 ± 0.01	nd	nd	0.26 ± 0.01
Flavone	Apigenin	0.12 ± 0.02	0.17 ± 0.02	0.38 ± 0.03	0.05 ± 0.01	nd
Phenolic acids						
	p-Coumaric acid	1.10 ± 0.03	0.39 ± 0.05	0.50 ± 0.05	0.55 ± 0.05	0.37 ± 0.04
	Ferulic acid	2.35 ± 0.04	1.74 ± 0.03	1.75 ± 0.03	2.25 ± 0.03	2.00 ± 0.04
	Sinapic acid	0.68 ± 0.02	1.05 ± 0.04	3.03 ± 0.05	0.70 ± 0.02	0.38 ± 0.02

*- mg/g DW; nd – not detected

Table.2 Antioxidant activities of ethanol extracts from five Bulgarian *Fumaria* species and some standard phenolic compounds

Plant extracts	**DPPH */****	**ABTS ***	**FRAP ***	**CUPRAC ***
F. officinalis	160.05 ± 3.27 / 2.39 ± 0.04	131.14 ± 6.08	161.48 ± 2.67	625.67 ± 7.44
F. thuretii	82.58 ± 2.91 / 5.15 ± 0.16	100.54 ± 8.87	80.63 ± 3.51	344.55 ± 1.17
F. kralikii	100.23 ± 2.07 / 4.08 ± 0.02	118.51 ± 5.11	108.59 ± 8.55	407.44 ± 7.82
F. rostellata	110.62 ± 2.41 / 3.81 ± 0.03	108.30 ± 8.74	100.31 ± 7.94	395.09 ± 8.19
F. schrammii	119.41 ± 4.80 / 3.44 ± 0.09	113.41 ± 5.46	120.36 ± 2.00	474.67 ± 1.77
Standards***				
Quercetin	3.22 ± 0.12 / 0.19 ± 0.03	7.44 ± 3.42	6.54 ± 0.73	10.64 ± 0.34
Rutin	4.53 ± 0.13 / 0.34 ± 0.01	2.95 ± 0.2	7.91 ± 0.96	13.93 ± 0.30
Ferulic acid	4.23 ± 0.38 / 0.68 ± 0.03	8.36 ± 1.62	9.47 ± 1.44	10.05 ± 0.22
Sinapic acid	4.60 ± 0.45 / 0.53 ± 0.04	6.55 ± 0.39	10.61 ± 1.48	14.74 ± 0.38
p-Coumaric acid	0.77 ± 0.27 / 1.14 ± 0.07	8.21 ± 1.99	5.14 ± 1.31	12.45 ± 1.98

*- values are in mM TE/g DW; **- EC_{50}, mg DW/ml; ***- values are in mM TE/mg

(FRAP) and cupric (CUPRAC) ions were investigated (Table 2). Ethanol extract of *F. officinalis* was the extract with the highest antioxidant activity in all used assays (160.05 ± 3.27, 131.14 ± 6.08, 161 ± 2.67 and 625.67 ± 7.44 mM TE/g DW for DPPH, ABTS, FRAP and CUPRAC methods, respectively), whereas the extract of *F. thuretii* was the extract with the lowest activity (82.58 ± 2.91, 100.54 ± 8.87, 80.63 ± 3.51 and 344.55 ± 1.17 mM TE/g DW for DPPH, ABTS, FRAP and CUPRAC methods, respectively), Table 2. Ethanol extracts of the other three investigated species (*F. kralikii*, *F. rostellata* and *F. schrammii*) had similar antioxidant activities (Table 2). Antioxidant activities of the major phenolic acids and some of flavonoids, detected in *Fumaria* extracts were determined as well (Table 2).

Antioxidant activities of plant extracts were usually explained with the presence of phenolic acids and flavonoids in them (Jaberian *et al*., 2013). There was scanty data concerning antioxidant activities of *Fumaria* extracts in scientific literature. Jaberian *et al*., (2013) reported strong antioxidant activities of methanol and methanol-water extracts of *F. vaillantii*, determined by DPPH method (EC_{50} = 0.1273 and 0.1533 µg/mL extract, respectively). In opposite, Orhan *et al*., (2012) reported that ethanol extracts of *F. cilicica*, *F. densiflora*, *F. kralikii* and *F. parviflora* collected in Turkey, showed extremely low antioxidant activities in both DPPH and FRAP methods. In our study, we decided to evaluate antioxidant activities of ethanol extracts of *F. officinalis*, *F. thuretii*, *F. kralikii*, *F. rostellata* and *F. schrammii* by applying two methods, based on mixed hydrogen atom transfer (HAT) and single electron transfer (SET) mechanisms (DPPH and ABTS) and two methods, based only on SET mechanism (FRAP and CUPRAC). The results showed the existence of correlation between total phenolic and total flavonoid concentrations in investigated extracts ant their antioxidant activities (Figure 1 and Table 2). Thus, the ethanol extract of *F. officinalis* had the highest antioxidant activities and the highest total phenolic and flavonoid contents (30.30 ± 0.31 mg GAE/g DW and 15.01 ± 0.01 mg QE/g DW), whereas the extract of *F. thuretii* had the lowest antioxidant activities and the lower total phenolic and flavonoid contents (20.20 ± 0.29 mg GAE/g DW and 8.70 ± 0.01 mg QE/g DW) (Figure 1 and Table 2). It should be noted that the extract of *F. thuretii* was the only one in which rutin was not detected. Analysis of antioxidant activities of individual flavonoids and phenolic acids showed that rutin, sinapic and ferulic acids were the most active compounds (Table 2). Moreover, our data showed that the extracts with the lowest antioxidant activities (*F. thuretii* and *F. rostellata*) were the extracts without and with the lowest quercetin glycosides contents, respectively (Tables 1 and 2). Relatively high concentrations of rutin, hyperoside, sinapic and ferulic acids in extracts of *F. officinalis*, *F. kralikii*, and *F. schrammii* were the most probable reason for observed high antioxidant activities in those plants, compared to extract of *F. thuretii*.

This study demonstrates for the first time the potential of *Fumaria* species (*F. officinalis*, *F. thuretii*, *F. kralikii*, *F. rostellata* and *F. schrammii*), grown in Bulgaria, as perspective source of valuable flavonoid compounds with high antioxidant activities and health beneficial properties. Our data reveals new possibilities to expand applications of

Fumaria extracts from a local ethnomedicine drug to new industrial fields such as foods and cosmetics preservation. The specific metabolite profiles and the remarkable antioxidant activities defined *F. officinalis*, *F. thuretii*, *F. kralikii*, *F. rostellata* and *F. schrammii* as interesting objects for development of *in vitro* systems for production of valuable flavonoids with antioxidant activities.

Acknowledgement

The authors thank for the financial support of this research by the Bulgarian Science Foundation, Bulgarian Ministry of Education and Science under contract number: DMU 03/77 - 2011.

Conflicts of Interest

The authors declare no conflicts of interest.

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