Environmental Engineering and Management Journal July 2019, Vol. 18, No. 7, 1553-1560
http://www.eemj.icpm.tuiasi.ro/; http://www.eemj.eu

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ANTIOXIDANT ACTIVITY AND PHENOLICS CONTENT OF *Capsella bursa-pastoris* AND *Marrubium vulgare* DEPENDING ON ENVIRONMENTAL FACTORS

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Abstract

In this work we aimed to examine the influence of the main abiotic factors (temperature, rainfall, relative humidity, atmospheric pressure) on the synthesis of active compounds (phenolics, flavonoids), with antioxidant and therapeutic effect of two herbs - *Capsella bursa-pastoris* (shepherd's purse) and *Marrubium vulgare* (horehound) collected from two different areas of Romania: Dobrogea (Constanta) and Muntenia (Bucharest). The studies have shown that biogenesis and accumulation of secondary metabolites that gives the therapeutic properties of medicinal plants is influenced by biotic and abiotic factors. In this work we aimed to determine the profile of concentrations of biologically active compounds from the studied herbs depending on the harvest time period and variability of the environmental factors. The medicinal herbs had harvested in autumn (September, 2013) and in spring (May – June, 2014); the alcoholic and aqueous extracts were analyzed in terms of composition: in main biologically active compounds (phenolics, flavonoids) and antioxidant activity as DPPH and ABTS, and spectral data analysis by FTIR. Experimental data obtained showed a significantly higher phenolics and flavonoids content and also a higher antioxidant activity in herbs harvested from the Dobrogea (Constanta) area, for the herbs studied.

Key words: abiotic factors, antioxidant activity, *Capsella bursa-pastoris*, flavonoids, *Marrubium vulgare,* phenolics

Received: December, 2017; Revised final: June, 2018; Accepted: July, 2019; Published in final edited form: July, 2019

1. Introduction

The secondary metabolites from plants are sources for additives and flavors used in pharmaceuticals and alimentary industry. These metabolites are vital in the plant's adaptation process to the environment and to overcoming stress conditions and can generate free radicals. These can react with certain kind of molecule: DNA, proteins and lipids resulting into membranes changes which further determines cell and tissue's injuries (Abdi and Ali, 1999).

Environmental factors such as temperature, fertility, light intensity, the rich mineral content in the water and CO_2 influence the growth of plants and secondary metabolite production (Ramakrishna and Aswathanarayana, 2011; Vincze et al., 2018). Variations in the aforementioned elements impact the quality and properties of wild and cultivated extracts for additional developmental applications (Szakiel et al., 2011).

Examples in reference to the influence of abiotic factors on the secondary metabolic synthesis from herbs are shown in Table 1.

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Table 1. The influence of the abiotic factors on the level of secondary metabolites

Abiotic factors	*Plant species*	*Effect*	*References*
Lower temperature UV-B irridiation	*Malus sp.*	Higher levels of anthocyanins	Ban et al. (2007) Ubi et al. (2006) Lin-Wang et al. (2011)
Long exposure to light	*Panax quinquefolius*	Ginsenosides in high amount	Fournier et al. (2003)
Higher altitudes and lower temperatures	*Arnica montana*	Phenolics derivatives	Albert et al. (2009)
Solar radiation exposure	*Vaccinium myrtillus*	Activation of flavonoid biosynthesis	Jaakola et al. (2004)
Drought	*Quercus ilex*	Decrease of monoterpene synthesis	Lavoir et al. (2009)

Plants belonging to the same species but occurring in different geographical zones may differ significantly in their content of particular secondary metabolites, both qualitatively and quantitatively (Szakiel et al., 2011). The study on the interaction between plant secondary metabolism and environmental factors may be helpful to the cultivation and standardization of traditional medicinal herbs and may provide a new approach to increasing target secondary metabolites.

Capsella bursa-pastoris (Shepherd's Purse) (fam. Brassicaceae-Cruciferae) is a herbaceous plant species which occurs very frequently in the spontaneous flora of Romania. The plant is known for its curative qualities from antiquity, when it was used as a laxative and aphrodisiac.

In folk medicine it is used as a uterine tonic, hemostatic and healing. For medicinal purposes, shepherd's purse are harvested in spring (April-May) and has many therapeutic uses: tonics, stimulants, hemostatic, healing, astringent, utero-tonic, coronary vasodilators, hypotensive, anti-inflammatory analgesic and anti-ulcer (Kuroda and Takagi, 1969).

Marubium vulgare (Horehound) (fam. Laminaceae*)* is a plant of wild flora, widespread in Europe. In Romania it is spread much in the steppe of Oltenia, Muntenia, Moldova and all over in Dobrogea. For therapeutic purposes use leaves, flowers and young stems, harvested between June and September. Many of the effects ascribed to traditional medicine of the horehound have been confirmed in recent years of scientific research and clinical studies, as: antioxidant (Berrougui et al., 2006), analgesic (Meyre-Silva et al., 2005), anti oedematogenic (Stulzer et al., 2006), antidiabetic type II (Herrera-Arellano et al., 2004) and in neurological disorders very recently (Orhan et al., 2010). This species is less used in phytotherapy. There are not data regarding the influence of abiotic factors on the phenolics and flavonoids amount of *Capsella bursa-pastoris* and *Marubium vulgare*.

Since the cellular antioxidative status plays an important role in the capacity of plants to cope with oxidative stress induced by environmental factors, the aim of the current work was to compare the plant secondary metabolites (phenolics and flavonoids) production and the antioxidant activity from the same species harvested from different geographical regions: Muntenia and Dobrogea, in two seasons as well as associations with some environmental factors.

2. Materials and method

2.1. Preparation of plant sample

The medicinal herbs - *Capsella bursa-pastoris* and *Marubium vulgare* - were harvested in autumn (September, 2013) and in spring (May – June, 2014) from two different Romania areas: Dobrogea (Constanta) and Muntenia (Bucharest). The aerial parts of plants (leaves, stems and inflorescences) were dried at the oven to 40ºC then were ground in a mill grinder (GRINDOMIX).

We have obtained two types of extracts: aqueous, 8% weight with distilled water at 60ºC (8 grams the ground herb at 100 mL distilled water) and hydro-alcoholic extracts, 8% weigh with 50% ethanolic solution (8 grams the ground herb at 100 mL ethanolic solution with 50% concentration). All extracts were prepared by sonication for 1 h and then filtered through filter paper. Clear extracts obtained were stored at 4ºC until bioanalysis extracts was performed.

2.2. Total phenolic content

The total phenols'content determination from plant extracts was performed by the Folin-Ciocalteu spectrophotometric method (Singleton et al., 1999). In brief, at 2.5 mL sample were added 2.5 mL Folin – Ciocalteu reagent, shaken and filtered, then 1 mL of filtrate was mixed with 9.5 mL sodium carbonate 20% and after 2 minutes the absorbance was read at 760 nm. The phenolic content in the tested samples was calculated based on the calibration curve obtained between 0-100 mg/L gallic acid (GAE), with R^2 =0.9969.

2.3. Total flavonoid content

The total flavonoid content was determined according Lin method (aluminum chloride based method), with slight modifications (Lin and Tang, 2007). Briefly, at 5 mL of the extract were added 7.5 mL of MeOH and filtered. At which point, 1 mL of the resultant sample was transferred to a 5 mL bottle, then 1 mL of 10% sodium acetate solution, 0.6 mL of 2.5% aluminum chloride hexahydrate solution and 0.5 mL MeOH were added. After 15 min, the absorbance of the mixture was metered at 430 nm and the flavonoid content expressed in mg rutin equivalent (RE)/L was measured using the calibration curve in the 0-120 mg/L concentration range and $R^2 = 0.991$.

2.4. Evaluation of the antioxidant activity of the extract was carried out spectrophotometrically using two comparative modes:

2.4.1. DPPH assay

Briefly 100 μL extract were mixed with 1 mL DPPH (2,2-diphenyl-1-picrylhydrazyl) solution (0.25 mM) and 1.9 mL methanol. The absorbency was measured at 517 nm, after 3 min with slight modifications from the method by (Bondet et al., 1997). The results were calculated using (Eq. 1):

$$\text{radical scavenging activity (\%)} = \left[\frac{A_B - A_A}{A_B}\right] \times 100 \qquad (1)$$

where: AB = control absorbance and AA = sample absorbance

2.4.2. ABTS assay

Briefly 100 L sample were mixed with 2500 μL ABTS (2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt) solution (0.175 mM obtained in potassium persulfate solution 0.25 mM) and 400 μL distilled water. The absorbency was measured at 731 nm, after 3 minutes (Rice-Evans and Miller, 1994) and were expressed as the equivalente Trolox (TEAC). The calculation of the results was done according to the formula (Eq. 2):

$$TEAC_{sample} = C_{trolox} \cdot f \cdot \frac{A_{sample} - A_{blank}}{A_{trolox} - A_{blank}} \qquad (2)$$

where: A blank = control absorbance; A sample = sample absorbance; A Trolox = Trolox absorbance; C_{Trolox}= Trolox concentration; f = dilution factor.

2.5. Fourier transform infrared radiation (FTIR) analysis

FTIR spectra were obtained from KBr pellets prepared using 1.0 mg of powdered flower samples. The pellets were analyzed in the absorption mode of FTIR and all spectra were recorded with a resolution of 4 cm^{-1}, 64 scans and the wave number ranged from 4000 to 400 cm^1 using a FTIR spectrophotometer (Bruker TENSOR 27 instrument, using the OPUS software version 6.0).

2.6. Weather data

Data on weather conditions in the successive years of study were obtained from the website of http://rp5.ru and http://freemeteo.com. Results are presented as means for each month in the period from January to December.

2.7. Statistical analysis

The Statistical Analysis was done using test Microsoft Office Excel 2007.

3. Results and discussions

3.1. Determination of total phenolics and flavonoids contents

The results regarding bioactive compounds (phenolics, flavonoids) of vegetal extracs were presented in Fig. 1 and Fig. 2.

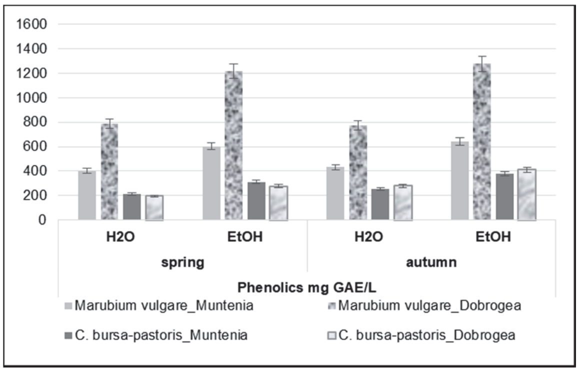

Fig. 1. Total phenolics'content in *Marrubium vulgare* and *Capsella bursa-pastoris* extracts

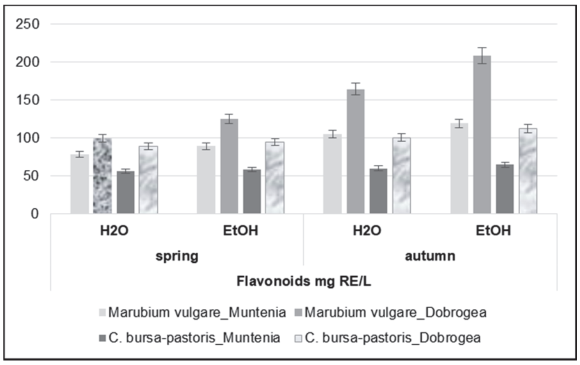

Fig. 2. Flavonoids' content in *Marrubium vulgare* and *Capsella bursa-pastoris* extracts

It can be observed a seasonal variation in the phenolics and flavonoids' content at the studied plants: these compounds content was generally low in spring compared with autumn. A possible explanation would be that the synthesis of secondary metabolites increases in autumn to improve plant tolerance to cold weather. Our results are in agreement with other studies (Bilger et al., 2007; Nenadis et al., 2015).

The efficiency of extractive phenolics compounds from plant material is largely dependent on the solvents and their different concentrations used (Jakopic et al., 2009). As expected, the content of phenolics and flavonoids was significantly higher in hydro-alcoholic extracts than those aqueous extracts due to higher polarity of the ethanol-water mixture than water.

The results showed that the phenolics and flavonoids' content was significantly higher plants harvested from Dobrogea region than those from Muntenia region. The phenolics and flavonoids' content in *Marrubium vulgare* hydroalcoholic extracts was of 98% and 60%, respectively, higher than of plants harvested from the Dobrogea region. The similar data have been obtained for *Capsella bursa-pastoris* extracts.

There was a positive correlation between secondary metabolites studied and some environmental factors (relative humidity, temperature and precipitation) of two harvesting areas, as presented in Tables 2 and 3.

The high amount of metabolites from harvested plants from Dobrogea region can be directly correlated with higher humidity, and precipitation factor (days with precipitation were 247, but total precipitation was 1085 mm, in 2013-2014), but values moderate of the temperature for the studied period, while in Muntenia region were 210 days with precipitation and total precipitation was 1281 mm. The average temperature was lower in Muntenia region compare to the same months in Dobrogea region. A direct correlation between increasing light intensity and drought and polyphenol content was also reported in previous studies (Larson, 1988; Chalker-Scott and Fnchigami, 1989; Ashihara et al., 2008).

Elemental factors, such as soil contents, temperature, rainfall and the intensity of ultraviolet radiation can affect the concentrations of phenolics compounds (Kouki and Manetas, 2002; Monteiro et al., 2006).

Other studies have revealed that the quantity of phenolics in plants and antioxidant activities, depend on biological factors (genotype, organ and ontogeny), as well as, environmental (temperature, watering volume and light intensity) variables (Lisiewska et al., 2006). UV radiation (300–400 nm) increased flavonoids in the roots of pea plants (Shiozaki et al., 1999); UV-B was also shown to induce the production of flavonoids in silver birch and grape leaves (Tegelberg et al., 2004).

Table 2. Weather conditions in 2013-2014 (monthly means) for Dobrogea (Constanta) region*

Month	***Air temperature (ºC)***		***Precipitation: Total precipitation (mm)***		***Precipitation: Days with precipitation***		***Atmospheric pressure (mm Hg)***		***Relative humidity (%)***		***The average value of the wind speed (m/s)***	
	2013	**2014**	**2013**	**2014**	**2013**	**2014**	**2013**	**2014**	**2013**	**2014**	**2013**	**2014**
January	2.9	3.5	37	65	20	15	758.1	762	87	91	2.3	2.2
February	4.8	3.4	12	18	14	11	760.4	763.3	86	92	2.3	1.6
March	5.7	8.1	12	48	15	14	757.3	760.5	77	79	2.3	2.5
April	12.4	3.0	27	30	10	19	760.8	758.1	73	87	2.0	2.3
May	18.9	17.0	36	77	8	14	757.7	758.2	76	77	1.9	1.8
June	22.0	21.0	75	160	6	15	757.8	758.6	71	73	2.0	2.1
July	23.5	23.8	43	47	12	12	759.4	759.5	68	59	1.8	0.8
August	24.5	24.5	24	32	6	6	760.1	752.7	66	55	1.6	0.8
September	18.5	20.0	76	21	12	8	759.1	760.5	65	71	2.2	2.1
October	12.8	13.7	154	61	6	11	764.4	763.9	82	79	2.0	2.2
November	10.9	-	21	-	10	-	762.0	-	84	-	1.9	-
December	2.3	-	9	-	3	-	768.2	-	84	-	2.2	-

**Weather archive on site: http://rp5.ru and http://freemeteo.com*

Table 3. Weather conditions in 2013-2014 (monthly means) for Muntenia (Bucharest) region*

Month	***Air temperature (ºC)***		***Precipitation: Total precipitation (mm)***		***Precipitation: Days with precipitation***		***Atmospheric pressure (mm Hg)***		***Relative humidity (%)***		***The average value of the wind speed (m/s)***	
	2013	**2014**	**2013**	**2014**	**2013**	**2014**	**2013**	**2014**	**2013**	**2014**	**2013**	**2014**
January	-0.6	-0.1	52	62.3	15	11	753.2	753.9	90	92	1.5	3.6
February	3.0	2.7	46	2.2	13	3	754.6	754.7	88	90	1.7	3.4
March	5.7	8.2	51	56.5	12	10	750.8	753.0	71	72	1.7	5.3
April	14.4	12.2	30	113	11	15	762.1	752.5	59	73	1.5	5.4
May	19.7	17.0	49	105	9	8	751.8	752.4	58	67	1.1	2.8
June	22.0	19.9	113	153	13	18	752.3	753.3	63	72	1.0	4.0
July	20.6	22.4	26	44	2	10	754.0	751.3	58	74	1.0	1.8
August	23.7	24.6	26	43	6	9	753.8	751.9	60	67	1.8	2.2
September	16.4	19.0	71	47	8	7	752.5	755.0	68	69	2.0	4.2
October	11.4	12.0	85	73	8	13	756.8	758.4	81	88	2.8	4.1
November	8.1	-	32	-	7	-	753.7	-	87	-	3.3	-
December	-0.4	-	1.0	-	2	-	759.9	-	88	-	3.0	-

**Weather archive on site: http://rp5.ru and http://freemeteo.com*

3.2. Antioxidant activity analysis

Results obtained by DPPH assay for antioxidant activity of the extracts are showed in Fig. 3 and by ABTS assay are showed in Fig. 4. It can observed that the extracts of plants harvested from Dobrogea region had a antioxidant activity significantly higher than similar plants harvest from Muntenia region, by both methods applied. For example, in the case of the hydro-ethanolic *M. vulgare* extract from Dobrogea region the $TEAC_{ABTS}$ value was three times higher than similar plants from Muntenia region (673.4 µmol Trolox/L versus 203.3 µmol Trolox/L). Antioxidant activity values obtained by the two methods presented a good correlation with the total phenolics content values, this is consistent with the reports of other studies (Ksouri et al., 2008, Nenadis et al., 2015).

3.3. FT-IR spectral analysis

Another method applied to highlight the differences in chemical composition of each plants studied, was the FT-IR spectrocopy. The FT-IR spectral analysis for *M. vulgare* and *Capsella bursa-pastoris* harvested from these geographical regions of Romania was showed in Fig. 5 and Fig. 6.

FT-IR spectra of these plants from the two regions can be compared to the FT-IR spectrum of Gallic acid, which shows main peaks at the following wavelengths: 3400.7, 2960.40, 2931.51, 2865.75, 1639.70, 1509.58, 1420.99, 1153.78, 1097.08, 777.58, 663.54, 601.28, 462.89 cm^{-1}. A broad band at 3400-33295 cm-1 belongs to the stretching vibration of phenolic hydroxyl group (-OH). The broad band at 2924-2925 cm-1 indicates the presence of aromatic (C-H) group, whereas the appearance of two medium and weak bands at 1652 cm^{-1} and 1655 cm^{-1} indicate the stretching vibration of aromatic (C=C) group, characteristic of phenolics. Additionally, it was observed that other peaks attributed to other functional groups of the glycoside composition, lipids etc.

It can observed that both plants have a similar chemical composition, but secondary metabolites content is higher at plants harvested from Dobrogea region.

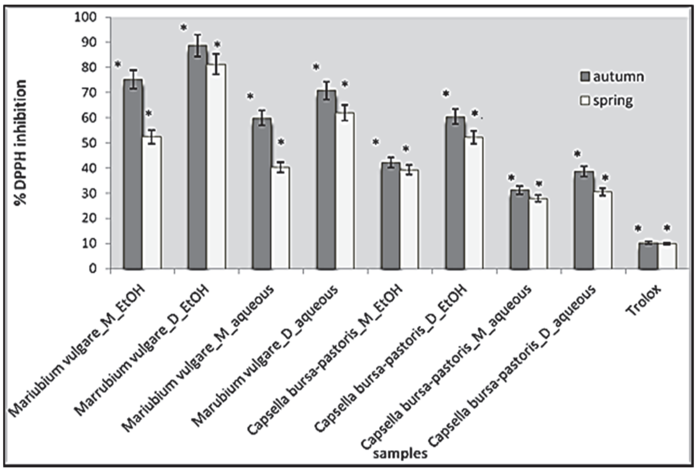

Fig. 3. Antioxidant activity of extracts analyzed by DPPH assay (* $p<0.05$, compared radical scavenging activity (by DPPH) with the phenolics content of extract; * $p<0.05$, compared radical scavenging activity (by DPPH) with the flavonoids content of extract; M= Muntenia; D= Dobrogea)

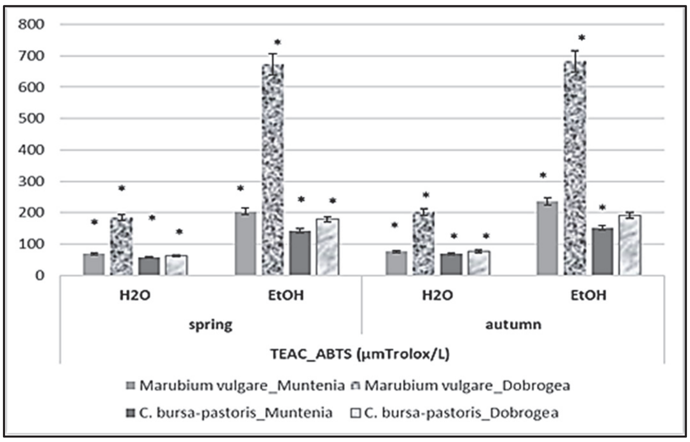

Fig. 4. Antioxidant activity of *Marrubium vulgare* and *Capsella bursa-pastoris* extracts by ABTS assay (*The data represent the means ± SD of triplicate samples * $p<0.05$, compared radical scavenging activity (by ABTS) with the phenolics content of extract; * $p<0.05$, compared radical scavenging activity (by ABTS) with the flavonoids content of extract)

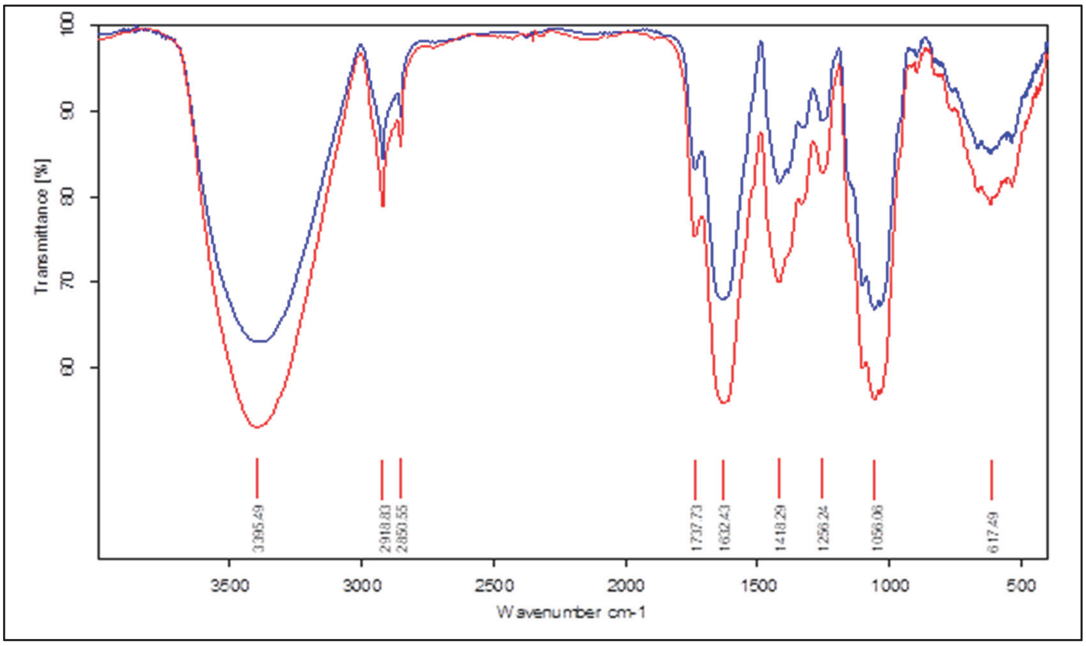

Fig. 5. FT-IR spectra of *Mariubium vulgare* harvested from Muntenia (1) and Dobrogea (2)

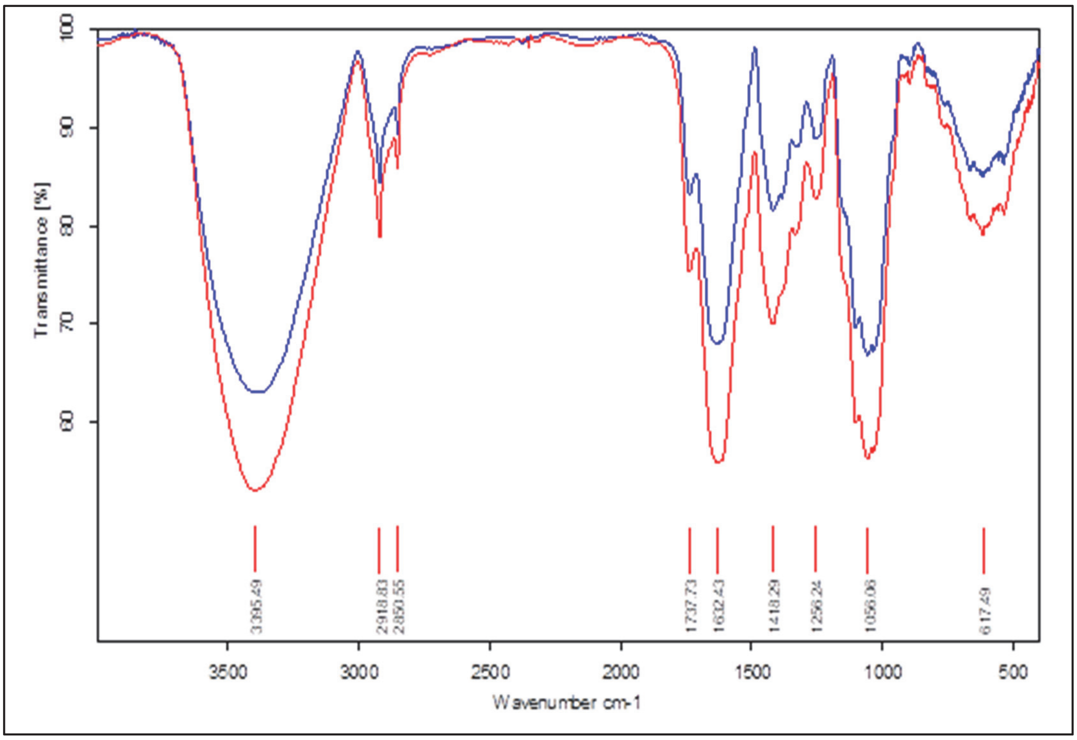

Fig. 6. FT-IR spectra of *Capsella bursa-pastoris* harvested from Muntenia (1) and Dobrogea (2)

4. Conclusions

The content of phenolics and flavonoids with antioxidant activity in medicinal plants from spontaneous flora (*Capsella bursa-pastoris* and *Marrubium vulgare*), from Dobrogea and Muntenia regions varies accordingly under the influence of different environmental factors. The effects of a number of abiotic factors (light intensity, temperature, relative humidity, wind speed) have been presented in this study.

Experimental data obtained showed a higher content of phenolics and flavonoids significantly correlated with a higher antioxidant activity at plants harvested from the Dobrogea region (Constanta). Such comparative investigations are performed to offer information about the optimum harvest period in order to obtain the highest quality of medicinal herbs containing phenolics and flavonoids as active constituents with potential uses in the pharmaceutical industry.

However, agricultural technologies of domestication of wild plant species and intensification of crop plant production can be developed based on existing knowledge of the influence of abiotic factors on plant secondary metabolism.

Conflict of interest

The authors of this article declare that there is no conflict of interest.

Acknowledgments

This research was supported by the Romanian National Center for Program Management – PN 09-360101/2012 project.

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