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*Full Length Research Paper*

# Composition and biological activity of *Rhaponticum carthamoides* extracts obtained from plants collected in Poland and Russia

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***Rhaponticum carthamoides* is a medicinal plant indigenous to Siberia. Although its root tissue is a well-known source of biologically active compounds, recent studies have focused on the above-ground part of the plant due to its easier accessibility. An *R. carthamoides* plantation was recently established in Northern Poland. Composition and antioxidant activity were compared for *R. carthamoides* leaf extracts obtained from plants grown in two plantations: in Poland (Lubiewice) and in Russia (Koriazma); the latter plantation has growing conditions similar to those in the *R. carthamoides* natural habitat. Plant secondary compounds were extracted with chloroform, methanol or water. As indicated by gas chromatography-mass spectrometry (GC-MS), the content of most compounds analyzed was higher in extracts from plants grown in Lubiewice (Poland). Antioxidative activity (as measured by diphenyl 1-picrylhydrazyl free radical (DPPH) and ferric reducing antioxidant power (FRAP) assays) and cytotoxic activity (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay) were higher in extracts from the Polish material.**

**Key words:** Antioxidant activity, cytotoxic activity, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), diphenyl 1-picrylhydrazyl free radical (DPPH), ferric reducing antioxidant power (FRAP), gas chromatography-mass spectrometry (GC-MS).

## INTRODUCTION

The number of known plant-derived active substances has already surpassed 50,000 (Kohlmünzer, 2000). Importance of these compounds as components of cosmetics and pharmaceuticals is growing, though in the same time the cosmetics and pharmaceutical industries require raw material of high value and standardized composition. However, plant biochemical profiles may differ, depending on the place of origin due to various climate, photoperiod and soil conditions (Castro et al., 2001; Szentmihályi et al., 2004).

*Rhaponticum carthamoides* (Willd) Iljin (synonyms: *Leuzea carthamoides*, maral's root) has a long story of use in Siberian folk medicine, where its roots, already in the times of first Russian settlers, were applied to treat overstrain and common weakness after illnesses (Yance, 2004). Systematic scientific studies of *R. carthamoides* extracts (RCEs) activity and composition, verifying the

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**Abbreviations: DW,** dry weight; **DPPH,** diphenyl 1-picrylhydrazyl free radical; **FRAP,** ferric reducing antioxidant power; **GC-MS,** gas chromatography-mass spectrometry; **MTT,** 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide.

traditional use, began in 1940-ties, mainly in the former Soviet Union and Japan. Petkov et al. (1984) reported that water-ethanol extract from *R. carthamoides* roots increased rats physical performance (forced-swim test) and mice learning ability (maze test). Todorov et al. (2000) observed anabolic effect of the root RCE in mice and ascribed it to the presence of 20-hydroxyecdysone. Recently, Maslov and Guzarova (2007) described cardioprotective and anti-arrhythmic effect of RCE.

Along with the research, related to the physiological effects, the analysis of plant extracts composition was being run. Initially, *R. carthamoides* gained attention as a rich source of ecdysteroids, compounds with potentially adaptogenic and anabolic activity (Yance, 2004). 20-hydroxyecdyson is the main *R. carthamoides* ecdysteroid, however, almost 50 others have been isolated so far (Kokoska and Janovska, 2009), of which 17 were characterized only during the last 4 years (Budesinky et al., 2008).

In addition to ecdysteroids, several other biologically active compounds have been isolated from its above- and underground organs. Over 30 terpenoids have been identified in volatile oils (here: sesquiterpenes constituting nearly 70% of the volume) obtained from *R. carthamoides* roots and rhizomes (Havlik et al., 2009). Additionally, the underground part contains phytosterols, triterpenoid alcohol (carthamenyl) (Grimshaw et al., 1981) and thiophene polyacetylenes exhibiting antifungal (Chobot et al., 2003) and phototoxic (Chobot et al., 2006) activity. In *R. carthamoides* seeds, *N*-feruloserotonins were detected (Pavlik et al., 2002), compounds showing mild antinoceptive activity (Yamamotová et al., 2007). A range of polyphenols and phenolic acids were also indentified in *R. carthamoides* underground tissue, e.g. stilbene derivative (Hajdu et al., 1998), gentisic acid, *o*-hydroxyphenylacetic acid (Skiba and Weglarz, 2003), accompanied by a number of flavonoid glycosides, mainly the derivatives of quercetin and isorhamnetin (Sharaf et al., 2001; Varga et al., 1990).

Flavonoid glycosides (mainly containing quercetin, patuletin and campherol as aglycone part) were also found in *R. carthamoides* herb (Koleckar et al., 2010; Miliauskas et al., 2005; Varga et al., 1990), together with phenol acids-protocatechuic (Skiba and Węglarz, 2003) and chlorogenic (Miliauskas et al., 2005). Polyphenols (mainly flavonoid glycosides) are believed to be the class of compounds responsible for high antioxidative and anti-free-radical potential of RCEs, described by Miliauskas et al. (2005). On the other hand the ability of methanol RCE to inhibit the platelet aggregation, induced by adenosine diphosphate (ADP) or collagen, should be ascribed to free flavonoids forms (Koleckar et al., 2008). Flavonoids and caffeic acid derivatives seem to be also responsible for the RCEs inhibitory effect towards NK-κB (Peschel et al., 2011).

Complex and rich chemical composition as well as a wide range of activities exhibited, makes *R. carhamoides* interesting also from the commercial point of view.

Currently, a number of preparations based on *R. carthamoides*-derived compounds, mainly designed for sportsmen, are commercially available. An *R. carthamoides* plantation was recently established in Lubiewice, Poland, with the intention of producing *R. carthamoides* preparations for the Polish market (FITOSTAR, personal information, http://fitostar,pl/preparat_rapontik).

This study presents a preliminary comparison of the composition of extracts isolated, from *R. carthamoides* plants growing under conditions similar to those in their natural habitat (Russian Federation) and from *R. carthamoides* growing in the plantation recently established in Lubiewice, Poland.

We decided to concentrate on the *R. carthamoides* above-ground parts (leaf extracts), though traditionally roots and rhizomes constituted the pharmaceutical raw material. However, it should be underlined, that this approach means eliminating the plant and precluding the possibility of annual harvest, without renewing the plantation. There is also a growing number of records demonstrating high biological activities of constituents obtained from *R. carhamoides* herb, mainly antioxidative and immunomodulatory (Kokoska and Janovska, 2009) and pointing to the above ground part as a more abundant source of polyphenolic constituents, than roots and rhizomes (Skiba and Weglarz, 1999). Therefore, using the *R. carthamoides* aerial parts is scientifically warranted and might be interesting from the economical point of view.

## MATERIALS AND METHODS

### Plant Material

Plant material, supplied in the form of *R. carthamoides* air-dried powdered leaves, was a generous gift from Professor N. P. Timofeev and FITOSTAR™. Leaves were obtained from plants grown in two locations.

1) Koriazma (Arkhangelsk region, Russian Federation): leaves were harvested in the summer of 2003 and 2006. Samples collected at different growth stages were pooled together. Further in the text are referred to as the “K-rus” material.
2) Lubiewice (Kujawsko-Pomorskie voivodeship, Republic of Poland): material was collected every 5 to 7 days during the summer of 2006 (soil class IV and V; 18 samples in total) and 2007 (flowering and vegetative shoots; 6 samples in total). Further in the text referred to as the “L-pol” material.

Detailed description of climate and photoperiod data of both locations is as shown in Table 1.

### Soil class definitions

Class IV soils (the average arable land): yields of crops grown on these soils are significantly lower than the soils of the upper. These soils are very susceptible to fluctuations in groundwater levels.

**Table 1.** Comparison of the climate conditions in Lubiewice (Republic of Poland) and Koriazma (Russian Federation).

| Site of sample collection | Koriazma | Lubiewice |
|---|---|---|
| Meteorological station location | Kotlas | Chojnice and Sliwice |
| Coordinates of the sample collection site | 61.32ºN<br>47.15ºE | 53.47ºN<br>18.07ºE |
| Climate type | Temperate continental (subarctic) | Temperate continental (warm) |
| Vegetation period (days) | 10 - 110 | 200 |
| Average air temperature | -13.7ºC (Jan), + 17.1ºC (Jul) | -1.2ºC (Jan), +19ºC (Jul) |
| Annual precipitation (mm) | 35 (January), 77 (July), 558 (Summer) | 55 (January), 63 (July), 616 (Summer) |
| Reference | http://pogoda.ru.net/climate/22887.htm; The average values of air temperature and precipitation are calculated using data for years 1971-2000 | Data were kindy provided by Instytut Meteorologii i Gospodarki Wodnej. The average values of air temperature and precipitation are calculated using data for years 2006-2008 |

Class V soils (the weak arable soil): this class includes rocky or sandy soils with low levels of humus. They are poor in organic substances.

**Extraction procedure**

Powdered leaves from both locations were sonicated for 30 min in chloroform, methanol or water (1 g dry weight/20 ml solvent). Plant residue was removed by filtration through double Whatman filter paper (no. 1). The solvents were evaporated at room temperature, using a rotary evaporator, and the extracts were further diluted in methanol (1 g dry weight (DW)/4 ml methanol).

**Cytotoxic activity (MTT assay)**

HL-60 cells (human leukaemia) were obtained from the Division of Cell Biology, Intercollegiate Faculty of Biotechnology UG & MUG, Gdansk. Cells were grown in RPMI medium (Sigma Aldrich), supplemented with 10% foetal bovine serum (FBS, GIBCO$^{TM}$), antibiotics (GIBCO$^{TM}$), 1% L-glutamine (GIBCO$^{TM}$) and non-essential amino acids (GIBCO$^{TM}$).

Cells were seeded in the 96-well plates at $2 \times 10^5$ cells/ml density and grown at 37°C in 5% $CO_2$. On the following day, plant extracts were added at the following concentrations: chloroform extract (10 µg/ml); methanol extract (50 µg/ml); water extract (100 µg/ml). Cells were incubated with the extracts for 24 h in conditions as described earlier. At 2 h prior to culture termination, 10 µl of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT; Sigma) solution (5 mg/ml phosphate buffered saline (PBS), pH = 7.4) was pipetted into the wells. Following incubation, the medium was aspirated and formazan crystals were dissolved in 99.9% dimethyl sulfoxide (DMSO; Sigma).

The colour intensity was measured spectrophotometrically at 570 nm wavelength with a multiplate reader (Wallace Victor, 1420 Multilabel Counter). The test was performed in five independent replicates. The results were calculated using the following formula:

$$\text{Viabililty (\%)} = [(Abs_{570sample} - Abs_{570blank})/(Abs_{570control} - Abs_{570blank})] \times 100\%$$

where $Abs_{570}$ = absorbance at 570 nm; control = non-treated cells.

**Antioxidant activity**

***Inhibition of linoleic acid peroxidation***

The ability of *R. carthamoides* leaf extracts to inhibit linoleic acid peroxidation was determined with a *β*-carotene-linoleate model system. The test was performed as described by Taga et al. (1984) with modifications suggested by Zhang and Hamauzu (2003). Briefly, 0.03% w/v *β*-carotene (Fluka) solution in chloroform was prepared and mixed with linoleic acid (Fluka) and Tween 20 (Koch Light Laboratories Ltd.) in the following proportions: 3 ml:40 µl:400 µl. Subsequently, chloroform was evaporated and 100 ml of water was added in order to form emulsion. 3 ml aliquots were mixed with 200 µl RCEs. Test tubes were incubated at 50°C for 120 min. Absorbance was measured at 470 nm at time points 0 and 120 min. The analysis was performed in triplicates.
Antioxidant activity (AA) was calculated according the formula:

$$AA = 100 \times [1-(A_0-A_t)/(A^c_0-A^c_t)].$$

$A_0$, $A^c_0$ is the absorption measured at the beginning of incubation for the RCE and control respectively; $A_t$, $A^c_t$ is the absorption measured after 120 min incubation for the RCE and control respectively.

***Molybdenum reduction***

The procedure of Prieto et al. (1999) with minor modifications was followed to determine the ability of *R. carthamoides* leaf extracts to reduce molybdenum ions ($Mo^{+6}$) that were present in the form of phosphomolybdate. 0.1 ml extract (2.5 mg/ml) was added to 0.9 ml reaction mixture (0.6 M sulfuric acid, 28 mM sodium phosphate, 4 mM ammonium molibdate). Samples were incubated for 90 min at 95°C, cooled down to room temperature, diluted 20 times with distilled water and the absorption was measured at 695 nm (Beckman DU®640 SpectroPhotometer). The percentage of reduced molybdenum ($Mo^{+5}$) was estimated using a standard curve, obtained with the use of ascorbic acid as a reducing agent. The analysis was performed in triplicates.

***DPPH assay***

The ability of the extracts to scavenge the DPPH free radical was quantified as described by Konczak-Islam et al. (2003). Serial dilutions of RCEs (concentration range from 1 to 1000 µg/ml) were

pipetted into 96-well plate. The stock solution of DPPH stable free radical was prepared in methanol. Aliquots of DPPH were added into the wells to the final concentration of 0.1 mM. After 2 min incubation, absorption was measured at 517 nm (Wallac Victor, 1420 Multilabel Counter).

The results were calculated using the following formula:

$$\text{RSA (\%)} = \frac{\text{Abs}_{517}\,\text{control} - \text{Abs}_{517}\,\text{sample}}{\text{Abs}_{517}\,\text{control}} \times 100$$

where RSA (%) is the radical scavenging activity; $\text{Abs}_{517}$ is the absorption at 517 nm; control is non-reduced DPPH

Results are expressed as $IC_{50}$, which is the concentration of extract been able to scavenge 50% of the DPPH free radical. The analysis was performed in duplicates (L-pol material from summer 2006 and K-rus material) or triplicates (L-pol material from summer 2007).

#### *FRAP assay*

Total antioxidant potential of the extracts was evaluated using the FRAP assay (ferric reducing antioxidant power), according to the protocol of Benzie and Strain (1996). Briefly, working solution consisting of 0.3 M acetate buffer, 10 mM 2,4,6-tripyridyl-s-triazine (TPTZ; Sigma) in 40 mM HCl and 20 mM $FeCl_3$ $6H_2O$, in the following proportions: 10:1:1, was prepared. Serial dilutions of 30 µl RCEs was pipetted into microplates and mixed with aliquots (170 µl) of working solution. Concentration range of RCEs was 0.15 to 150 µg/ml. After 20 min incubation, absorption was measured at 593 nm (Wallac Victor, 1420 Multilabel Counter).

A standard curve for ascorbic acid was constructed in order to estimate the percent of iron reduced and to determine the $IC_{50}$ value, that is, concentration of RCE at which 50% of ferric ions are reduced. The analysis was performed in duplicates (L-pol material from summer 2006 and K-rus material) or triplicates (L-pol material from summer 2007).

### Composition analysis

#### *Gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS)*

Samples of the extract (0.25 mg DW/ml) were dried under nitrogen flow. Dried samples (1 mg) were then silylized with 100 µl of a mixture of 99% bis(trimethylsilyl)acetamide and 1% chlorotrimethyl-silane (Sigma Aldrich) for 24 h at 25°C.

The analyses were carried out on a Clarus 500 (Perkin Elmer) gas chromatograph equipped with a split ratio of 1:30 for the injection port and direct connection to a flame ionization detector (FID). The RTX 5 column (30 m × 0.25 mm i.d., film thickness 0.1 µm) was used. The carrier gas was argon (99.999% purity). For reliable analysis, the injector and detector temperatures were 310°C. The following temperature program was applied: 100 to 310°C, rate 4°C $min^{-1}$.

Mass spectra (70 eV) were recorded on a SSQ 710 mass spectrometer (Finnigan). The samples were introduced through a Hewlett-Packard 5890 gas chromatograph equipped with the same columns and under the same chromatographic conditions as for the GC analysis but with helium as carrier gas (99.999% purity). The injector and transfer line temperature was 310°C. The ion source was maintained at 220°C. Compounds identification was carried out using mass spectral data base and where available it was confirmed by comparison of both retention times and mass spectra of authenticated standards. Qualitative analysis employed the following standards: decanoic acid, hexadecanoic acid, octadecenoic acid, octadecanol, cholesterol, stigmasterol, *β*-sitosterol, campesterol, *α*-amyrin, *β*-amyrin and chlorogenic acid. Standards for ecdysteroid (20-hydroxyecdysone), flavonoids (patuletin, isorhamnetin, quercetin), lignan (matairesinol) and terpenoids (menthol, *β*-caryophyllene) were also used. The quantification of the compounds was based on their peak areas from the GC analysis, compared to the peak areas of the internal standards (alcohols and triterpenols: heneicosanol, free fatty acids and sterols: 19-methylarachidic acid). All standards (purity 98 to 99%) were obtained from Sigma–Aldrich Poland (Poznań, Poland). The results were expressed as mean ± standard deviation. Triplicate injections were made for each sample.

### Statistical analysis

One-way analysis of variance was performed with the use of GraphPad Prism 4 program in order to compare samples. $p<0.01$ value (unless stated otherwise) was considered as statistically significant.

## RESULTS

Extracts isolated from *R. carthamoides* herbs, collected in Koriazma (Russian Federation) and Lubiewice (Republic of Poland) were compared, focusing on both extract composition (GC-MS analysis) and biological activities (cytotoxic and antioxidative). Influence of factors such as climate, soil type and leaf source (vegetative versus flowering shoots) were considered.

Cytotoxic analysis of *R. carthamoides* extracts was performed taking under consideration the source of material. In case of samples collected in Lubiewice (L-pol material), the differences between the cytotoxicity of the extracts isolated from plants harvested at different time points (24. 05 - 07. 07) and growing on different soil types (IV or V) were analyzed. Subsequently, cytotoxicity of the extracts obtained from the Polish material was compared with those isolated from plants collected in Koriazma (K-rus material).

Neither the soil type nor the harvest time point seemed to influence the extract cytotoxic activity, which was statistically indistinguishable between all tested L-pol samples (Figure 1). In the same time, the mean values of the cell survival were ca. twice lower, indicating markedly higher activity, in case of L-pol material, compared to its K-rus counterparts (chloroform extracts: 31.2 versus 65.6%; methanol extracts 40.7 versus 80.7%; water extracts 44.3 versus 90.7%). However, only L-pol water extracts were significantly more active from K-rus samples both from 2003 and 2006 year. In case of chloroform and methanol extracts, the difference was more pronounced when samples from 2003 were compared.

The antioxidant activity of chloroform, methanol and water extracts from leaves collected in both locations in 2006 was evaluated with four methods: *β*-carotene bleaching, phosphomolybdenum reduction, DPPH and FRAP assays. In case of *β*-carotene bleaching and

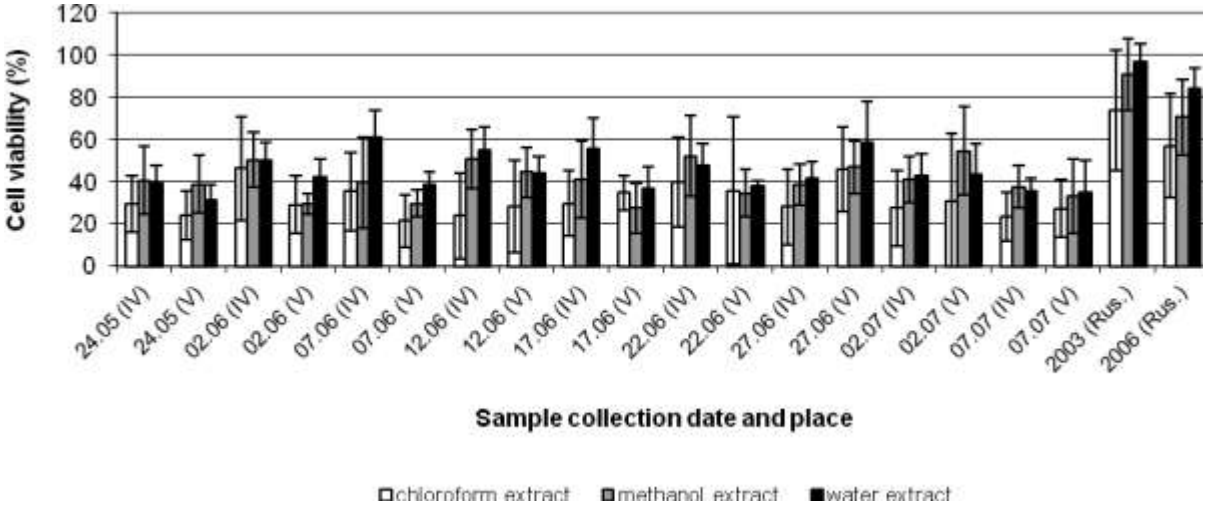


**Figure 1.** Cytotoxic activity exhibited by *R. carthamoides* extracts obtained from the plants grown in two locations: Koriazma and Lubiewice against HL-60 cells. Samples collected in Lubiewice differed in the harvesting time point (24/05/2006 to 07/ 07/2006) and soil class (IV versus V). Extract concentrations as follows: chloroform extracts – 10 µg/ml, methanol extract – 50 µg/ml, water extract – 100 µg/ml.

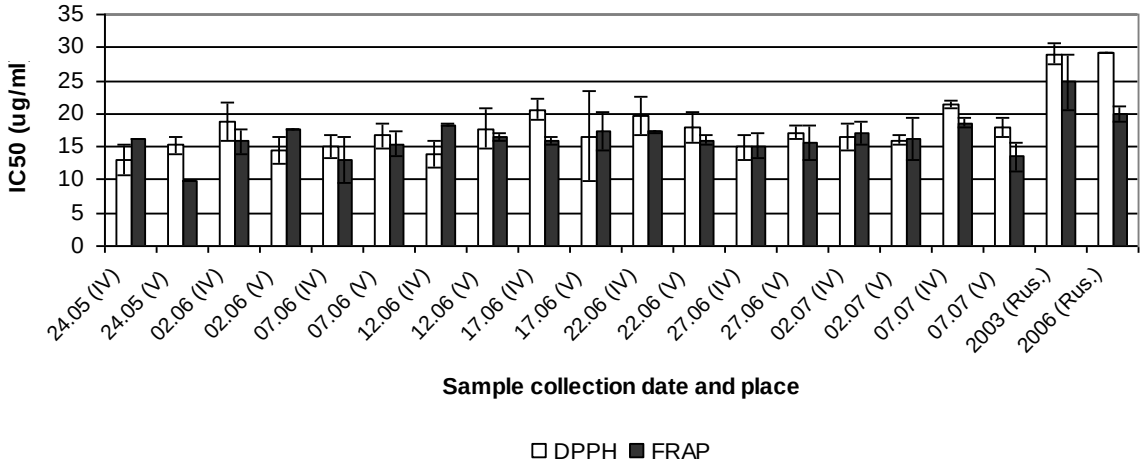


**Figure 2.** Antioxidant activity (DPPH and FRAP assays) of methanol leaf extracts from *R. carthamoides* plants growing in two locations: Koriazma and Lubiewice. Samples collected in Lubiewice differed in the harvesting time point (24. 05 to 07. 07. 2006) and soil class (IV vs. V).

phosphomolybdenum reduction tests, all L-pol samples, irrespective of harvesting time point, solvent used and soil type were undistinguishable among themselves and from their K-rus counterparts (data not shown). Results of the DPPH and FRAP assays were presented only for the methanol extracts, as their activity turned to be higher than water and chloroform ones. Again, soil type and harvest time point did not influence extract activity (except for FRAP analysis for samples harvested on 24. 05; Figure 2). Antioxidant activity of all L-pol samples was higher than samples isolated from K-rus material. $IC_{50}$ values were 16.8±2.3 versus 29.1±0.1 (DPPH assay) and 15.9±2.1 versus 22.3±3.4 (FRAP) for L-pol and K-rus material, respectively.

Based on antioxidative activity results obtained for *R. carthamoides* leaves collected in 2006, we performed analyses of the material collected in Lubiewice, Poland in 2007, using DPPH and FRAP methods only, due to the high sensitivity, excluding in the same time water and chloroform extracts, as less active. This time we used shoot type (flowering versus vegetative) as a distinction criterion. Our data suggest, that neither shoot type nor harvest time point influences the extract activity (Figure 3).

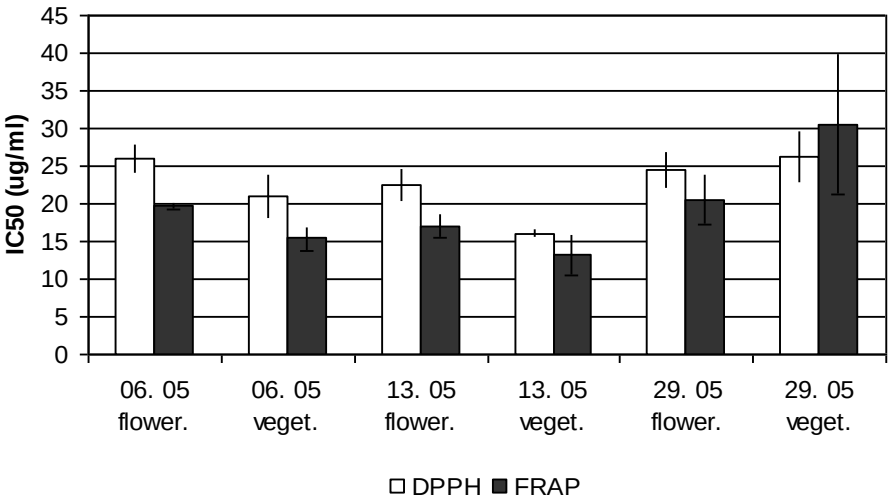


**Figure 3.** Antioxidant activity (DPPH and FRAP assays) of methanol leaf extracts from *R. carthamoides* plants growing in Poland. Leaves were collected during late spring 2007. Samples differed in the harvest time point and shoot type (vegetative vs. flowering). Veget.: Vegetative; flower.: Flowering.

The composition of chloroform, methanol and water extracts isolated from the K-rus material was analyzed by the means of GC-MS. Phytosterols, triterpenoids (amyrin isomers), fatty alcohols and saturated fatty acids were detected in chloroform and methanol extracts, whereas chlorogenic acid was found only in methanol and water extracts. Since the methanol turned to be capable of extracting all the types of compounds of interest and the composition of methanol extract was the most complex, only methanol extracts were used for the comparison analysis between K-rus and L-pol material (Table 2).

Thorough analysis of methanol extract obtained from plants grown in Poland (L-pol material) revealed the presence of 29 compounds. Eight of these compounds were detected as the major compounds: stigmasterol, *β*-sitosterol, *α*- and *β*-amyrin, chlorogenic acid and alcohols $C_{24}$, $C_{26}$ and $C_{28}$. 19 of 29 compounds were identified in smaller quantities. Plants grown in Lubiewice, Poland (L-pol material) also contained 2 compounds present in traces: alcohol $C_{29}$ and fatty acid $C_{13}$. 29 compounds were identified in plants grown in Koriazma, Russia. Three principal compounds were: chlorogenic acid and fatty acids $C_{16}$ and $C_{17}$. The remaining compounds were present in smaller amounts. Plants grown in Russia also contained 6 compounds present in traces (campesterol, alcohols $C_{29}$ and $C_{30}$ and fatty acids $C_{13}$ and $C_{14}$) (Table 2).

Generally speaking, the comparison analysis showed that most of the compounds of interest are synthesized in higher amounts in L-pol samples, with the exception of fatty acids content (Table 2). Regarding polar compounds, the level of chlorogenic acid was also significantly higher in L-pol as compared to K-rus material (Table 2). Concerning polyphenols, compounds of known antioxidative potential, the content of flavonoid aglycon forms (patuletin, isorhamnetin, quercetin) was below the detection limit (data not shown). The latter finding is not surprising, as the major portion of flavonoids in plants exist in the form of glycosides, that is, bound to a sugar moiety (Adom and Liu, 2002; Maimoona et al., 2011).

## DISCUSSION

*R. carthamoides* was used in Siberian folk medicine for many years, and several preparations obtained from this plant are now commercially available. However, only recently *R. carthamoides* was introduced to Poland, and therefore we consider it important to confirm the biochemical profiles of plants grown on the newly established plantations and under typical growing conditions. Here, we present a preliminary analysis of *R. carthamoides* leaf extracts from plants collected in Lubiewice and Koriazma. These locations differ in climate and photoperiod, with the climate in the Russian location being harsher, with shorter photoperiod and lower average temperatures (Table 1).

The first step, before proceeding to the comparison of plant material from the two distant locations, was confirming the homogeneity of L-pol material (that is, ruling out the potential differences in extracts activity caused by seasonal variations and variations caused by soil class and shoot type). Here, our results are somewhat in line with the work presented by Skiba and Weglarz (1999), who studied the changes in polyphenols content in *R. carthamoides* roots, rhizomes and leaves depending on the time of plant material collection. They observed annual fluctuations in flavonoid content in

**Table 2.** Composition of leaf extracts of *R. carthamoides* growing in two locations (in Lubiewice, Poland and Koriazma, Russia) as indicated by GC-MS analysis. Analysis was carried using the plant material collected in the summer of 2007 (Poland) or 2006 (Russia).

| Compound identified | Retention time | K-rus material | | | L-pol material |
|---|---|---|---|---|---|
| | | Chloroform extract | Methanol extract | Water extract | Methanol extract |
| | | Compound content (µg/g DW) | | | |
| **Phytosterols** | | | | | |
| Campesterol | 55.64 | 0.2±0.24 | - | - | 26.90±2.1 |
| Stigmasterol | 56.08 | 2.0±0.24 | 33.8±3.53 | - | 198.24±8.5 |
| *β*-sitosterol | 57.04 | | 131.8±8.46 | - | 344.80±22.7 |
| In total | - | ~2.2 | ~165 | - | ~ 569.9 |
| *β*-amyrin | 57.24 | 14.4±1.07 | 56.4±4.23 | - | 941.64±46.0 |
| *α*-amyrin | 57.81 | 18.71±1.31 | 63.5±3.53 | - | 597.55±38.2 |
| Chlorogenic acid | 53.4 | - | 632.4±28.91 | 4358.4±201.84 | 2024.88±108.3 |
| **Fatty alcohols** | | | | | |
| $C_{22}$ | 42.86 | 4.3±0.36 | 7.8±2.12 | - | 35.40±2.8 |
| $C_{24}$ | 46.59 | 30.7±2.02 | 24.0±2.82 | - | 198.95±7.8 |
| $C_{25}$ | 48.30 | 6.8±0.48 | traces | - | 8.50±1.4 |
| $C_{26}$ | 50.10 | 74.9±6.07 | 107.2±7.76 | - | 853.14±53.1 |
| $C_{27}$ | 51.64 | 1.5±0.24 | 7.1±1.41 | - | 51.68±4.2 |
| $C_{28}$ | 53.27 | 6.4±0.48 | 19.7±2.12 | - | 283.91±14.9 |
| $C_{29}$ | 54.98 | - | traces | - | traces |
| $C_{30}$ | 56.33 | - | traces | - | 22.66±2.1 |
| In total | - | ~124.6 | ~166 | - | ~1454.2 |
| **Saturated fatty acids** | | | | | |
| $C_{12}$ | 20.84 | 1.7 | 15.5±2.12 | - | 0.71±0.7 |
| $C_{13}$ | 23.94 | - | traces | - | traces |
| $C_{14}$ | 27.08 | - | traces | - | 2.12±0.7 |
| $C_{15}$ | 29.58 | 9.6 | 17.6±2.12 | - | 1.42±0.7 |
| $C_{16}$ | 32.25 | 397.5 | 617.6±43.71 | - | 130.98±5.7 |
| $C_{17}$ | 34.37 | 5.5 | 232.7±17.63 | - | 2.12±0.7 |
| $C_{18}$ | 36.70 | 24.4 | 73.3±4.94 | - | 25.49±2.1 |
| $C_{19}$ | 38.87 | 0.1 | traces | - | 0.71±0.7 |
| $C_{20}$ | 40.77 | 5.1 | 18.3±2.12 | - | 4.96±0.7 |
| $C_{21}$ | 42.86 | 4.5 | traces | - | 0.71±0.7 |
| $C_{22}$ | 44.64 | 4.4 | 22.6±2.12 | - | 6.37±1.4 |
| $C_{23}$ | 46.59 | 30.8 | traces | - | 2.12±0.7 |
| $C_{24}$ | 48.23 | 6.4 | 33.1±2.82 | - | 8.50±1.4 |
| $C_{26}$ | 51.60 | - | 8.5±1.41 | - | 4.25±0.7 |
| $C_{28}$ | 54.91 | - | traces | - | 2.12±0.7 |
| In total | - | ~490 | ~1039 | - | ~192.6 |

*R. carthamoides* leaves, with samples gathered in IV and X having significantly lower level of compounds of interest, then those collected in VI, VIII and IX. As our L-pol samples were harvested not earlier than May, it is plausible that the harvest time chosen by us coincides with the optimal harvest time reported previously (Skiba and Weglarz, 1999). The same researchers compared polyphenols (flavonoids and phenolic acids) content in the basal and stem leaves, finding only minor differences in their biochemical profiles. This finding supports our observation, concerning the fact that the type of shoots (vegetative versus flowering) does not have any effect on the leaves content (Skiba and Weglarz, 1999).

Seasonal variations of the antioxidants content in plant

tissues, and therefore the antioxidative potential of a plant extracts were reported also by other researchers. For instance, Xu et al. (2011) analyzed the content of phenolic compounds (that is, flavonoids, anthocyanins) in grape seeds and skins of winter versus summer berries. Amount of polyphenols and antioxidant potential of grape extracts (DPPH, FRAP, ABTS assays) turned to be higher in the winter berries, suggesting the positive role of low temperatures in polyphenols accumulation. This finding was in accordance with the results obtained by Albert et al. (2009), who found out that the ratio of *ortho*-diphenolics (polyphenols being the most active antioxidants) in *Arnica montana* flowering heads, compared with other phenolics, increases with the altitude above the sea level. This effect was initially ascribed to higher ultraviolet bands (UVB) exposition, as polyphenols absorb UV radiation and may act as photoprotectants, but the thorough analysis revealed that it was the temperature, which played the most significant role. This may be explained by the findings of Swiderski et al. (2004) who described a correlation between flavonoid glycosides content in *Rhododendron* species and resistance to low temperatures. They postulated that the cytoplasmic level of these compounds might delay the formation of cell-structure damaging crystals (Swiderski et al., 2004). In case of our study, however, the higher antioxidant potential was observed in extracts from plants growing in the warmer regions (Lubiewice, Poland). This finding suggests the plausible role of other environmental factors.

Differences in the lipophylic compounds content were also observed for plants grown under different conditions. Higher amount of phytosterols (namely stigmasterol and *β*-sitosterol) were detected in L-pol material; in addition, L-pol samples contained low amounts of campesterol, non-detected in the K-rus counterpart. This finding should be emphasized as some of the phytosterols are known to exhibit various biological activities, e.g. stigmasterol, used as a starting material in the production of synthetic progesterone (Boeckman et al., 1977) or *β*-sitosterol, which was proved to act as an inflammatory agent by inhibiting prostaglandin release in macrophages (Awad et al., 2004).

A range of other compounds, being of minor importance from the pharmaceutical/cosmetic point of view, was also taken under consideration when comparing the biochemical profiles of *R. carthamoides* growing in both locations. In general, higher amount of fatty alcohols was detected in the L-pol material. At the same time the content of fatty acids was higher in the K-rus.

## Conclusions

The present study shows the possibility of obtaining high-quality raw material from *R. carthamoides*, cultivated in Central Europe, that is, in the conditions different from its natural habitat. It was also demonstrated, that in the level of different secondary metabolites only minor differences occurred between samples harvested at different time points, from different soil class fields and flowering versus vegetative shoots. This finding means that homogenous plant material might be collected over a long time span during the vegetation period and from a vast area, resulting in a higher yield.

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