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Department of Horticulture[1], Department of Fruit, Vegetables and Cereals Technology[2], Wroclaw University of Life and Environmental Sciences

ANITA BIESIADA[1], ANNA SOKÓŁ-ŁĘTOWSKA[2], ALICJA Z. KUCHARSKA[2]

Antioxidant activity of Calendula officinalis, Arnica chamissonis and Echinacea purpurea as dependent on the part of plant and method of material preparation

Aktywność antyoksydacyjna *Calendula officinalis*, *Arnica chamissonis* i *Echinacea purpurea* w zależności od części rośliny i metody przygotowania surowca

INTRODUCTION

Chemical compounds of an antioxidant character protect organism cells against injuries caused by free radicals [20]. It is believed that substances such as polyphenols, flavonoids, isoflavones, anthocyanins and catechins can be classified as the compounds featuring the strongest antioxidant properties [11, 18]. Weaker antioxidant properties are possessed by carotenoids or vitamins C and E [20]. In the last ten years antioxidant activity has been investigated with the use of different methods, among others, through the capability of iron chelation according to FRAP method (Ferric reducing ability of plasma), as well as by neutralizing stabile free radicals using synthetic radicals DPPH (1,1' diphenyl-2-pikrylohydrazil) and ABTS (2,2'-azynobis-3-etylobenzotiazolin-6-sulfonic acid) [9]. The final result, however, is highly affected by the quality of raw material and the kind of substance used for extraction [13]. The content of bioactive substances in herbs can vary among others due to plant species variability, its growth phase, the country of origin, and seasonal environmental variability (biotic and abiotic factors) [15, 24].

Royal marigold (*Calendula officinalis* L.) is an annual decorative plant whose breeding varieties of dark orange, full flowers are cultivated as herbal raw material. Most often used material of this plant is marigold flower head (*Flos Calendulae*) containing, among others, triterpenes, flavonoids, carotenoids, polyacetylenes, volatile oils and phenolic acids [4]. Therapeutic effect of marigold flower heads is partly connected with antioxidant properties of some of its components [2]. Also marigold herb has been applied in phytotherapy. Preparation of Calendula herb is used as a stimulant for circulation, promotion of healing, as a releasing, lancing and purging agent, as well as for gastric hemorrhage, ulcers, spasms, swelling of the glands, jaundice and anemia. Topically they are used for putrid or cancerous abscesses, wounds, bleeding and eczema [11]. Arnica chamissa

(*Arnica chamissonis* Less.), due to its high usability for cultivation, is often treated as herbal raw material replacing mountains arnica, a plant under protection and troublesome for cultivation. It is a perennial plant whose basic herbal material is a flower head (*Anthodium Arnicae*) containing, among others, flavonoids, sesquiterpene lactones, triterpenes, volatile oil, irydoids, phenolic acids - in free and bonded form, amines and phytosterols [10, 23]. Apart from flowers, leaves herbs of arnica are also used for therapeutic purposes [6, 8, 23]. Purple coneflower (*Echinacea purpurea* Moench.) is a perennial plant originating from North America. It is one of the best known as far as its pharmacological activity is concerned. Preparations made from purple coneflower constitute a group of best–selling immunostimulatory remedies [4]. Herbal raw material (*Herba Echinaceae*) contains, among others, aromatic volatiles, glycosides, amides, inulin and polyacetylenes. Due to its valuable properties, all three plants have become an object of interest among cosmetologists. The aim of research was the assessment of antioxidant activity level of extracts from leaves and flowers of royal marigold, arnica chamissa and purple coneflower, as well as the effect of the way of material preparation on the mentioned properties.

MATERIAL AND METHODS

Field experiment was conducted in experimental station belonging to Department of Horticulture at Wrocław University of Environmental and Life Sciences in the years 2008-2009. Material for examination included leaves and fully developed flower heads which, in the case of purple coneflower and arnica, were collected from two–year old plants, while in royal marigold from annual plants. In the course of the experiment the content of polyphenols, carotenoids and chlorophyll a+b, as well as the level of antioxidant activity of the examined herb were estimated. The material for chemical analyses was collected raw during harvest period, which for marigold and arnica took place in the first half of June and for coneflower in the half of July.

The weighed amounts of fresh material were crushed with the use of BOSCH MSM 6700/01 blender and extracted 80% methyl alcohol at room temperature for 24 hours. The samples prepared in that way underwent determination of phenolic compounds content by Folin-Ciocalteau method [19], antiradicals activity in relation to radicals DPPH (1,1-difenyl-2-pikrylohydrazyl) according to [24], ABTS (2,2'azynobis-(3etylobenzotiazolin-6-sulfonis acid) according to [16] and reducing power FRAP [1]. Dry matter was determined according to gravimetric method, following PN-90/A-75101/03, chlorophyll a + b content and sum of carotenoids according to [17].

In the samples of fresh material of marigold, arnica and coneflower flowers, after their drying in a dark place in natural conditions, polyphenols content and antioxidant activity,were determined, counted over dry matter. Significance of differences was estimated by ANOVA test and scheduling of homogeneous groups was done using Duncan test, introducing Statistica v.7.1.program.

RESULTS AND DISCUSSION

The content of total polyphenols as well as carotenoid and chlorophyll pigments differed in flower heads and leaves of the examined plants (Tab.1, 3). Polyphenols content measured in mg per 1g of raw

material was higher in leaves than in flowers in two analysed species of arnica chamissa and purple coneflower, while inverse relationship was recorded for marigold, where polyphenols content in flower heads was slightly higher. In arnica chamissa the quantity of polyphenols was very high, about six times more than in the two remaining species, both in leaves and in flowers and it was comparable to previously recorded for plant from *Lamiaceae* family [5]. Drying process evidently increased contents of polyphenols in materials of the three examined species from *Asteraceae* family. Similar results were earlier reported for plants from *Lamiaceae* family [5, 7]. According to [14] insoluble phenolics are the components of cell walls, while soluble phenolics are compartmentalized within the plant cell vacuoles. So, drying process could help in the release of these insoluble parts from tissue by damaging polyphenol-carbohydrates complexes, polyphenol-proteins or high-molecular weight phenolics [14]. Similarly, in experiments made by [3] and [25], dried herbs exhibited significantly higher total phenolics than fresh material.

Table 1 Content of polyphenols, chlorophyll a+b and carotenoids in leaves and flowers of *Arnica chamissonis*, *Echinacea purpurea* and *Calendula officinalis* (mg·1g^{-1} f.m.)

Species	Part of plant	Polyphenols	Chlorophylls	Carotenoids
Arnica chamissonis	Flower	10.35±0.29	0.28±0.03	0.26±0.03
	Leaf	19.73±0.81	0.96±0.10	0.16±0.03
Echinacea purpurea	Flower	1.59±0.38	0.55±0.01	0.07±0.01
	Leaf	3.09±0.92	3.78±0.14	0.15±0.02
Calendula officinalis	Flower	1.21±0.04	0.29±0.03	0.88±0.11
	Leaf	1.00±0.03	1.01±0.08	0.11±0.01

Table 2. Antioxidant activity of leaves and flowers of *Arnica chamissonis*, *Echinacea purpurea* and *Calendula officinalis*

Species	FRAP µM Trolox·g^{-1} f.m.		DPPH µM DPPH·g^{-1} f.m.		ABTS µM Trolox·g^{-1} f.m.	
	Leaf	Flower	Leaf	Flower	Leaf	Flower
Arnica chamissonis	121.68 b	76.63 c	1.62 b	3.03 a	29.66 a	17.24 c
Echinacea purpurea	117.17 b	160.68 a	0.65 d	1.13 c	17.96 c	24.41 b
Calendula officinalis	62.18 d	27.69 e	0.15 e	0.12 e	18.01 c	17.07 c

Table 3. Content of phenolics dry matter, and antioxidant activity in fresh and dry flowers of *Arnica chamissonis*, *Echinacea purpurea* and *Calendula officinalis*

Material		Dry matter %	Phenolics mg GAL·g^{-1} d.m.	DPPH µM DPPH·g^{-1} d.m.	FRAP µM Trolox·g^{-1} d.m.	ABTS µM Trolox·g^{-1} d.m.
Arnica chamissonis	fresh	13.8	46.9	4.2	317.4	73.2
	after drying	90.0	65.8	5.3	346.3	30.6
Echinacea purpurea	fresh	23.1	14.7	1.1	141.6	25.0
	after drying	91.3	33.9	4.4	293.8	55.2
Calendula officinalis	fresh	23.7	9.0	0.1	28.0	17.8
	after drying	90.7	11.5	0.5	42.1	15.5

The content of chlorophyll was in all examined plants considerably higher in leaves than in flowers. The highest amount of chlorophyll was recorded in leaves of purple coneflower, while the lowest quantity of this pigment was found in arnica chamissa. In purple coneflower inflorescence the highest level of chlorophyll was determined and in the remaining two species the level of this pigment showed twice lower values. Carotenoids content in leaves and flower heads of the examined plants did significantly vary. In arnica and marigold more carotenoids were present in blossom, while in purple coneflower their higher amount was recorded in leaves. The highest level of carotenoids was assayed in marigold blossom. These pigments, however, occurred in less diversified level in leaves than in blossom and their quantity was considerably lower than that in leaves of *Lamiaceae* family plants [5].

Among the three examined plants stronger antioxidant properties, measured by FRAP and ABTS tests, characterized the extracts from arnica chamissa and purple coneflower in comparison to royal marigold (Tab. 2). On the basis of analyses it was possible to state that antioxidant activity of the examined plant raw material depended on the method of its determining. In FRAP test, in arnica chamissa, and royal marigold higher antioxidant activity featured extracts from leaves. However, when ABTS test was applied, only arnica leaves were characterized by considerable higher antioxidant activity, while in royal marigold this prevalence was insignificant and in purple coneflower higher antioxidant activity was characteristic for blossom both for FRAP and ABTS test. Application of DPPH test proved that inflorescence of arnica chamissa and purple coneflower showed stronger antioxidant activity than their leaves. The results of chemical analyses point to significant relationship between polyphenols level in raw material and its antioxidant activity. The only exception concerned the material consisting of purple coneflower, where higher level of polyphenols, chlorophyll and carotenoids in leaves did not translate into their increased antioxidant activity as compared to flowers. According to [22] a positive relationship between TEAC (ABTS and FRAP) values and total phenolics content, was found only in family groups with many representative herbs within *Labiatae* and *Compositae*. Antioxidant activity of the assessed dry materials was higher, especially when FRAP and ABTS tests were used. In earlier investigation the results varied to a considerable degree. In selected plants from *Lamiaceae* family decreased antioxidant activity of raw material after its drying was recorded [5] but [3, 13 and 25] stated higher TEAC capacity in dry herbs than in fresh samples. They concluded that the disruption of cell walls of fresh tissue may also activate the release of oxidative and hydrolytic enzymes that could destroy the antioxidants compounds in the product.

CONCLUSIONS

In arnica chamissa and royal marigold higher antioxidant activity, measured by FRAP and ABTS tests featured leaves, while in purple coneflower the mentioned values were determined in blossom. In DPPH test inflorescence of purple coneflower and arnica chamissa was characterized with stronger antioxidant activity in comparison to leaves. The content of chlorophyll in coneflower blossom was twice higher than in flower heads of arnica chamissa and royal marigold. Coneflower leaves contained three times more chlorophyll as compared to marigold and arnica leaves. Higher content

of carotenoids was recorded in blossom of royal marigold and arnica chamissa, while in purple coneflower their more advantageous source proved to be leaves. Among three species of medicinal plants the most considerable content of polyphenols was determined in leaves, which constituted the amount higher by a third than in blossom. Significantly highest polyphenols content, counted over dry matter, was recorded in material dried in natural conditions. Also antioxidant activity of the assessed dry materials showed higher values, particularly when FRAP and ABTS tests were applied.

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SUMMARY

Antioxidant activity as well as phenolics, carotenoids and chlorophyll contents were estimated in flowers and leaves of arnica chamissa, royal marigold and purple coneflower. Higher antioxidant activity was observed in flowers of arnica chamissa and royal marigold and in leaves of purple coneflower. After drying of raw material in natural conditions, the content of phenolics and antioxidant activity was higher than in fresh material.

Keywords: parts of plants, DPPH, ABTS, FRAP, phenolics, arnica chamissa, coneflower, royal marigold

STRESZCZENIE

Oceniono aktywność antyoksydacyjną oraz zawartość polifenoli, karotenoidów ogółem oraz chlorofilu a+b w kwiatach i liściach trzech gatunków roślin leczniczych arnice łąkowej, jeżówce purpurowej i nagietku lekarskim. Większą aktywność antyoksydacyjną oraz zawartość polifenoli, karotenoidów i chlorofilu odnotowano w kwiatostanach arniki i nagietka lekarskiego oraz w liściach jeżówki purpurowej. Po wysuszeniu w warunkach naturalnych stwierdzono większą zawartość polifenoli i aktywność antyoksydacyjną w kwiatach badanych roślin w porównaniu do świeżego surowca.

Słowa kluczowe: części rośliny, DPPH, ABTS, FRAP, polifenole, arnika łąkowa, jeżówka purpurowa, nagietek lekarski