# Comparison of antioxidant properties of infusions from different parts of *Arnica montana* and *Arnica chamissonis* var. *foliosa* Less.

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**Summary**

*Arnica chamissonis* (AC) was a good source of total phenolics, phenolic acids and flavonoids. Irrespective of raw material the highest reducing powers were observed in AC infusions, whereas the highest chelating power (about 100%) was stated in the rhizomes of AC. Free radicals were the most effectively scavenged by extracts of AC flower heads and herb (about 60% – SASA and 90% – ABTS, respectively). Lipid peroxidation was strongly inhibited by flower heads infusion of both *Arnica* genera and herb infusions of *Arnica montana* (AM) – about 100%. Positive correlations between $ABTS^{+\cdot}$ and $O_2^{\cdot-}$ radical scavenging properties, reducing power and total phenolics content (r=0.97, r=0.79, r=0.83, and r=0.50, respectively), flavonoids content (r=0.92, r=0.77, r=0.66, and r=0.51, respectively) and phenolic acids content (r=0.86, r=0.94 and r=0. 75, respectively) were found. In addition, inhibition of lipid peroxidation was correlated with total phenolics and flavonoids contents (r=0.50, r=0.51, respectively).

***Key words:*** *antioxidant activity, Arnica, free radicals, phenolic compounds*

## INTRODUCTION

Reactive oxygen species (ROS) such as $O_2^{\cdot}$, $H_2O_2$ and OH are incessantly generated inside the human body as a consequence of the exposure to a multitude of exogenous chemicals in our ambient environment and/or a number of endogenous metabolic processes involving redox enzymes and bioenergetics electron transfer [1]. Under normal circumstances, the generated ROS are detoxified by the antioxidants present in the body. However, owing to the ROS overproduction and/or inadequate antioxidant defense, this balance is hampered favouring the ROS upsurge that culminates in the oxidative stress [2]. ROS can readily react with and oxidize most biomolecules including carbohydrates, proteins, lipids and DNA. There is an increasing evidence that the accumulation of ROS in biological system causes oxidative damage to the tissue which affects cellular integrity and functions.

Antioxidants are often divided into different groups according to their mode of action. A common division is between inhibitors of enzymatic and non-enzymatic peroxidation. Since the structural criteria for activity are different for these two inhibition modes, antioxidant activity against enzymatic (e.g. lipoxygenase-catalyzed) and non-enzymatic radical mediated peroxidation processes should be included as a part of the antioxidant investigations [3]. Non-enzymatic peroxidation comes from the structure of active compounds. There are various abilities among these mechanisms: (1) the ability to protect the initiation of a chain reaction, (2) to chelate the intermediate metal ions, (3) to destroy peroxides, (4) to neutralize free radicals and finally (5) their reducing ability [4].

*Arnica* is the most widely employed medicinal plant in the clinics either by itself or in combination with other herbs. However, antioxidant properties of this plant are much less documented. Flowering heads (*Arnicae anthodium),* rhizomes (*Arnicea rhizoma),* leaves *(Arnicae folium)* and green parts (*Arnicae herba)* of arnica are used as pharmaceutical raw materials [5]. Preparations of *A. montana* (AM) flowers have been used in both traditional and homeopathic medicine for topical treatment of post-trauma effects and inflammatory diseases. It is noteworthy that wild-growing *A. montana* plants are under natural protection and does not give stable yields in commercial plantations. Other *Arnica* sp. have been investigated for their pharmaceutical activity and *A. chamissonis* (AC) was confirmed as a substitute for *A. montana.*

In recent literature it is lack of reports concerning antioxidant activity of hydrophilic compounds presented in *Arnica* genus. We compared the antioxidant activity of different parts of a plantation-grown AM and AC, as a novel source of natural antioxidants, potential nutraceuticals and food preventatives.

## MATERIALS AND METHODS

### Plant materials

The genus *Arnica* belongs to the family *Asteraceae*. In the study different parts of cultivated AM and AC were tested. A field experiment was carried out in 2006 on three-year-old plantation of AM grown on grey-brown podsolic soil with granulometrical composition of heavy loamy sand. The soil was characterised by mean content of humus, very low phosphorus, potassium and magnesium content, and was acid in reaction. In autumn 2005, all the plots were subjected to phosphorus-potassium fertilisation in the following doses: 24.0 kg P and 66.4 kg K per ha, whereas 40.0 kg N of nitrogen were applied in two equal doses: in spring, before the beginning of vegetation and after the heads harvest. During the vegetation plants were three times weeded (by hand) and inter-rows were cultivated.

### Chemicals

2,2-Diphenyl-1-picrylhydrazyl (DPPH); Tween 40; 2,2'-azinobis (3 ethylbenzthiazo-linesulfonic acid) diammonium salt (ABTS); linoleic acid; haemoglobin; 3-(2-pirydyl)-5,5-bis(4-phenyl-sulfonic acid)-1,2,4-triazine (ferrozine), nitroblue tetrazolium (NBT), phenazine methosulfate (PMS); nicotinamide adenine dinucleotide (NADH) were purchased from Sigma (St. Louis, USA).

### Extracts preparation

Water (infusion) extracts were prepared following Polish Pharmacopoeia V [6]. Two grams of plant dried material seeds were extracted for 60 min. with hot distilled water, cold off and centrifuged.

### Determination of total phenolic compounds

The amount of total phenolics was determined using Folin-Ciocalteau reagent [7]. The amount of total phenolics was expressed as a gallic acid equivalent (GAE) in mg per ml of sample.

### Determination of phenolic acids content

The amount of total phenolic acids was determined following the Szaufer-Hajdrych [8], phenolic acids content was expressed as a caffeic acid equivalent (CAE) in $\mu$g per ml of sample using the calibration curve.

## Determination of flavonoids content

Total flavonoids content was determined according to the method described by Bahorun [9]. Total flavonoids were calculated as a quercetin equivalent (QE) in mg per ml of sample using the calibration curve.

## Determination of ABTS radicals scavenging activity

The experiments were carried out using an improved ABTS decolorisation assay [10]. $ABTS^{+\bullet}$ was generated by oxidation of ABTS with potassium persulfate. The affinity of test material to quench ABTS free radical was evaluated according to equation:

scavenging% = $[(A_C - A_A)]/A_{C)}] \times 100$;

where: $A_{C(0)}$ – absorbance of control, $A_A$ – absorbance of sample.

## Superoxide anion scavenging activity (SASA)

The superoxide anion scavenging activity was determined according to the method described by Liu et al. [11]. The affinity of test material to quench superoxide anion was evaluated according to equation:

SASA% = $[(A_{(0)} - A_A)] / A_{(0)}] \times 100$;

where: $A_{(0)}$ – absorbance of control, $A_A$ – absorbance of sample.

## Metal chelating activity

Chelating power was determined by the method of Guo et al.[12]. The percentage of inhibition of ferrozine – $Fe^{2+}$ complex formation was given in formula:

% inhibition = $[1-(A_p/A_c)] \times 100$;

where: $A_c$ - absorbance of the control, Ap – absorbance in the presence of the sample.

## Reducing power

Reducing power was determined by the method of Oyaizu [13].

## Inhibition of linoleic acid peroxidation

The antioxidant activity was determined as the degree of inhibition on the haemoglobin-catalyzed peroxidation of linoleic acid according to Kuo et al. [14]. The antioxidative activity of the sample was calculated as:

AA[%] = $(1-(As-A_0)/(A_{100}-A_0)) \times 100$;

where: $A_0$ – absorbance of the control (without haemoglobin), As – absorbance in the presence of the sample, $A_{100}$- absorbance without the sample.

## Statistical analysis

All experimental results were mean ±SD. Data were evaluated by using one-way analysis of variance (Tukey test). The values of $p<0.05$ were regarded as significant.

## RESULTS AND DISCUSSION

Numerous medicinal plants contain large amounts of antioxidants other than vitamin C, vitamin E and carotenoids. The antioxidative effect is mainly bound to the presence of phenolic components such as flavonoids, phenolic acids and phenolic diterpenes. Until now, biochemical studies have shown that herbs of *A. montana* contain various organic compounds, such as flavonoids, phenolic acids (gallic, caffeic and their esters with quinic acid: chlorogenic and cynarine) sesquiterpene lactones, triterpenes, xanthophylls, polyacethylenes, hydroxycumarines, irydoids, pyrrolizidine alkaloids and volatile oils [15,16]. Decoctions or tinctures of *Arnica* yellow flower heads are popular remedies used to treat infections of the skin, bruises and muscle pains [17]. Probably their biological functions are due to, at least partially, their protective effects against oxidation. The chemical constituents of *Arnica* comprise a complex mixture of sesquiterpenolactones (e.g. helenalin), flavonoids and phenolic acids. Anti-inflammatory effects are mainly explainable by the inhibition of transcription factors NF-κB and NF-AT caused by the sesqiuterpenolactones. Yet, flavonoids and phenolic acids are also crucial for several reasons. They show significant antioxidant and antibacterial activities, serve as chemosystematic markers and are used to assure identity and purity of AM, according to European Pharmacopoeia [17].

Flower heads, herb and rhizomes of AM and AC are a good source of bioactive compounds. All raw AC materials contain more phenolics, flavonoids and phenolic acids than AM. Only in rhizomes, total flavonoids content was comparable (about 0.23 QE mg/ml). Irrespective of plant genus, the highest phenolics contents were observed in flower head samples (4.34 mg GAE/ml in AM and 7.63 mg GAE/ml in AC), while the lowest – in rhizome infusions (2.85 mg GAE/ml in AM and 3.83 mg GAE/ml in AC). From the quantitative point of view, the best raw material was the flower heads of AC. It is noteworthy that AC are better source of phenolic compounds than AM, irrespective of plant part (tab. 1).

The mechanisms of actions of natural products from traditional herbal medicines have been focused on their antioxidant activities. What learned from nature could help to overcome the toxicity issues of synthetic antioxidant compounds, such as butylated hydroxyanisole that is widely used in food procession [18] and to develop new chemical entities of antioxidants.

Most of antioxidant potential in herbs is due to the properties of phenolic compounds, which can act as reducing agents, free radical scavengers and hydrogen

donators. Moreover, antioxidants are known to interrupt the free-radical chain of oxidation and to donate hydrogen from the phenolic hydroxy groups, thereby forming stable free radicals which do not initiate or propagate the further oxidation of lipids [4]. The antiradical activities of *Arnica* plants were already studied by Heilmann et al. [19] and Cassels et al. [20], which resulted in promising data. Thus, other antioxidant activities of *Arnica* (free radical scavenging abilities, reducing and chelating power, and the ability of lipid peroxidation inhibition) were assayed in this studies.

It is common to evaluate the antioxidant activity of plants using several methods to measure various oxidation products. In our work, five methods, which include $ABTS^{+\cdot}$ and $O^{2-}$ radicals scavenging activity, ferric reducing ability, chelating power and ability to inhibit lipid peroxidation were used to evaluate the antioxidant activity of AM and AC infusions.

The reductive ability can also determine the antioxidant capacity of the samples. In order to examine the reducing power of extracts, the $Fe^{3+}$ to $Fe^{2+}$ reduction in the presence of the extracts was investigated. All analysed samples possessed high reducing power. Irrespective of raw material slightly higher reducing abilities were observed in the AC infusions. The highest reducing activity was stated in the herb and flower heads infusions (from both *Arnica* genera), while the lowest – in the rhizomes. It should be noted that both Arnica genera possessed comparable reducing ability, a level of reducing power depends on using row material (fig. 1).

Intermediate metal ions play an important role in the Fenton reaction. This process is closely bound to the formation of free radicals, especially hydroxy- and peroxy-radicals. Inhibition of this reaction can be performed by deactivating or chelating iron ions [5].

Transition metals, such as ion can stimulate lipid peroxidation by generating hydroxyl radical trough Fenton reaction and accelerate lipid peroxidation by decomposing lipid hydroperoxides into peroxyl and alkoxyl radicals, therefore drive the chain reaction of lipid peroxidation. The chelating activities for ferrous ion of the extracts were assayed by the inhibition of formation of red-colored ferrozine and ferrous complex.

As shown in fig. 2 the formation of red-colored complex was most effectively inhibited in the presence of AC rhizomes infusion, indicating the highest chelating activity (about 100%). AC flower heads infusion and all samples obtained from AM showed chelating power ranged from about 60% to about 45%, respectively. In all AM samples no significant differences were observed, iron chelating abilities were about 50% (fig. 2).

Formation and accumulation of reactive ROS is believed to be one of the mechanisms of myocardial damage by ischemia/reperfusion. During reperfusion, xanthine oxidase converts oxygen into superoxide anion, which in turn dismutates into $H_2O_2$, and generates hydroxyl radicals trough Fenton reaction, resulting in cell damages [2].

Superoxide anion radical, as the precursor of the more reactive oxygen species including hydroxyl and peroxynitrite radicals, is very harmful to the cellular components in a biological system. The superoxide anion radical scavenging activities of the tested infusions from AM and AC assayed by the PMS-NADH system were shown in fig. 3. Superoxide scavenging abilities of extracts obtained from flower heads both Arnica genera and AC herb infusions was not significantly different and reached about 60%. Lower activity was observed in the case of rhizomes infusion, however AM rhizomes extract had higher scavenging power than AC sample.

Most of antioxidant potential in herbs and spices is due to the properties of phenolic compounds, which can act as free radical scavengers [21]. Another antioxidant activity screening method, applicable for both lipophilic and hydrophilic antioxidants – ABTS radical cation decolourisation assay, showed that infusion from flower heads and herbs of AC exhibited the highest antiradical power (about 90%). Infusion from AC flower heads and herbs was significantly more effective than analogical samples of AM. Only in rhizomes infusion case, the activity of both analysed samples was relatively low and comparable (about 35%, see fig. 4).

Cell homeostasis is closely bound with the correct functionality of the cell membrane. Membrane lipids peroxidation can negatively influence signalling cascades and finally cause the pathological process. In chronic diseases, such Alzheimer's or Parkinson's disease, significantly increased LPO products were observed [22]. Oxidative damage of membranes can influence many things such as destroying ion channels or active transport mechanisms as well as deactivating membrane enzymes.

In the haemoglobin-catalysed peroxidation of linoleic acid assay, linoleic acid served as a model lipid. Peroxidation was induced by haemoglobin and the damage was assayed following the thiocyanate method of Kuo et al. [14].

As can be seen in figure 5, infusion of flower heads of both *Arnica* genera and AC herbs caused total inhibition of lipid peroxidation (100% – in model system). Infusion obtained from AC herbs showed significantly lower activity (about 80%). In the samples obtained from rhizomes of AC and AM only slight ability to inhibition of lipids peroxidation was found ( about 30 and 10 %, respectively).

Many studies have shown that the phenolic contents in plants can be correlated with their antioxidant activities [23]. Małolepsza and Urbanek [24] suggest that flavonoid complexes included in plant samples provide better results than single compounds. The analysis of the correlation between total phenolic compounds, flavonoids and phenolic acids content and antioxidant activities showed significant dependence in the free $ABTS^{\cdot+}$ and $O^{2-}$ radical neutralizing properties, reducing power and lipid peroxidation inhibition and the total phenolic contents ($r=0.97$, $r=0.79$, $r=0.83$ and $r=0.50$, respectively) and flavonoids concentration ($r=0.92$, $r=0.77$, $r=0.66$ and $r=0.51$, respectively). Beside of this, the positive correlations between total phenolics acids content and reducing power, $O^{2-}$ and $ABTS^{\cdot+}$ free radicals neutralizing properties were found ($r=0.86$, $r=0.75$ and $r=0.94$, respectively, see tab. 2). These results indicate that biological active compounds of *Arnica* seeds associate themes together and show synergistic or antago-

nistic effects. It was also clear that the antioxidant potential of the biological sample is not restricted to the concentration of active compounds but is also closely bound to the mutual relationships between them. In earlier studies, Sherwin [25] defined the benefits that come from using the mixture of antioxidant compounds. He claims that it is possible to complement the active mechanisms of biological compounds and reduce methodological problems such as varying solubility and colour of a simple compound solution.

## CONCLUSION

In conclusion, biologically active compounds from different parts of AM and AC possessed a wide spectrum of antioxidant activities evaluated with use of five testing systems. From the results it can be concluded, that AC extract showed antioxidant properties comparable to AM, presumably due to differences in acontent and structure of antioxidant components. However, samples of both *Arnica* genera could be used as a natural antioxidant source and a possible substitution of artificial antioxidants should be considered. Besides their natural properties, AC is easy to obtain, cheap and effective. Therefore, it would be interesting to do further studies using *Arnica* extracts as food additives and cosmeceuticals in order to increase the shelf life of products especially by preventing lipid peroxidation and antiradical activity.

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## PORÓWNANIE WŁAŚCIWOŚCI ANTYOKSYDACYJNYCH NAPARÓW Z RÓŻNYCH CZĘŚCI *ARNICA MONTANA* I *ARNICA CHAMISSONIS* VAR. *FOLIOSA* LESS.

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**Streszczenie**

Koszyczki kwiatowe, ziele i kłącza *Arnica montana* L. (AM) i *Arnica chamissonis* var. *foliosa* Less. (AC) okazały się dobrym źródłem związków fenolowych. Wyższą zawartość związków fenolowych ogółem, fenolokwasów i flawonoidów miały próby z AC. Próby te charakteryzowały się również najwyższym potencjałem redukcyjnym. Najwyższą zdolnością (około 100%) do chelatowania charakteryzował się napar z kłączy AC. Wolne rodniki były efektywnie neutralizowane przez napary z kwiatów i ziela AC (odpowiednio o około 60% – $O_2^-$ i 90% – ABTS). Utlenianie lipidów było silnie hamowane przez napary z kwiatów obu gatunków arniki i z ziela AM (około 100%). Wykazano pozytywną korelację pomiędzy zdolnością do neutralizacji wolnych rodników ABTS i $O_2^-$, zdolnością do redukcji i hamowania peroksydacji lipidów a zawartością związków fenolowych ogółem (odpowiednio r=0,97; r=0,79; r=0,83 i r=0,50) i flawonoidów (odpowiednio r=0,92; r=0,77; r=0,66 i r=0,51).

***Słowa kluczowe:*** *właściwości przeciwutleniające, Arnica, wolne rodniki, związki fenolowe*