ACTA SCIENTIARUM POLONORUM *Acta Sci. Pol., Hortorum Cultus 10(3) 2011, 15-27*

COMPARISON OF *in vitro* LIPOXYGENASE, XANTHINE OXIDASE INHIBITORY AND ANTIOXIDANT ACTIVITY OF *Arnica montana* AND *Arnica chamissonis* TINCTURES

Urszula Gawlik-Dziki, Michał Świeca, Danuta Sugier, Joanna Cichocka

University of Life Sciences in Lublin

Abstract. Preparations of *Arnica* have been used for treatment of post-trauma effects and inflammatory diseases. The aim of the study was to determine and compare the antioxidant activities and *in vitro* lipoxygenase (LOX) and xanthine oxidase (XO) inhibitory activity of different parts of *A. montana* (AM) and *A. chamissonis* (AC) with reference to the polyphenolic compounds content. Based on IC_{50} values could be concluded that active compounds of AM acted mainly as a XO inhibitors while LOX was more effective inhibited by AC samples. The highest antiradical activity was observed in the cases of AM flower heads and AC herbs tincture. All studied materials exhibited high chelating power (with IC_{50} ranged from 10.96 to 11.84 mg/ml) and ability to inhibition of lipid peroxidation (IC_{50} ranged from 10.00 to 15.09 mg/ml). *Arnica* tinctures possess high antioxidant abilities and XO and LOX inhibitory activity that might be helpful in preventing or slowing the progress of free radical dependent diseases. Additionally, results clearly showed that *A. chamissonis* might be a valuable herbal raw material.

Key words: arnica, inflammation, enzyme inhibition, phenolics, antioxidants

INTRODUCTION

Oxidative damage, caused by reactive oxygen species (ROS), has been frequently suggested to be associated with the pathogenesis of various conditions such as aging, arthritis, cancer, inflammation and heart diseases in human body. ROS such as $O_2^{\cdot-}$, H_2O_2, and $\bullet OH$ 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 [Zhao et al. 2006]. Creating ROS is bound; *inter alia*, with of lipoxygenase (LOX) and xanthine oxidase (XO) activity. LOX catalyzes oxygenation of

Corresponding author – Adres do korespondencji: Urszula Gawlik-Dziki, Department of Biochemistry and Food Chemistry, University of Life Sciences in Lublin, Skromna Str. 8, 20-704 Lublin, Poland, tel. (+ 48) 81 462 33 27; fax: (+48) 81 462 33 24; e-mail: urszula.gawlik@up.lublin.pl

polyunsaturated fatty acids containing a *cis, cis*-1,4-pentadiene system to hydroperoxides. The lipoxygenase pathway of arachidonic metabolism produces reactive oxygen species, and these reactive forms of oxygen and other arachidonic acid metabolites may play a role in inflammation and tumor promotion. Inhibitors of arachidonic acid metabolism also inhibited tumor promotion in animal models [Juntachote and Berghofer 2005]. XO is the enzyme that catalyses the metabolism of hypoxanthine and xanthine into uric acid. It is responsible for the medical condition known as gout, which caused by the deposition of uric acid in the joints leading to painful inflammation. XO also serves as an important biological source of oxygen – derived free radicals that contribute to oxidative damage to living tissues involved in many pathological processes such as inflammation, atherosclerosis, cancer and aging. Inhibitors of XO may be potentially useful for the treatment of gout or other XO-induced diseases [Ferraz Filha et al. 2006].

For years preparations of *A. montana* flowers have been used in the both traditional and homeopathic medicine for topical treatment of post-trauma effects and inflammatory diseases. In the *Arnica* case the anti-inflammatory and antioxidant effects are mainly explainable by the inhibition of transcription factors NF-κB and NF-AT by sesquiterpene lactones and flavonoids [Liang et al. 2006]. Main flavonoid present in *Arnica* is quercetin. Quercetin has displayed the ability to prevent the oxidation of low-density lipoproteins (LDL) by scavenging free radicals and chelating transition metal ions. As a result, quercetin may aid in the prevention of certain diseases, such as cancer, atherosclerosis, and chronic inflammation [Hollman and Katan 1997; Murota and Terao 2003].

Arnica flowering heads, applied externally, exhibit antibacterial, antifungal, anti-inflammatory and analgesic activities; may also reduce pain, swelling and discoloration from bruises. Alcoholic, as well as oily extracts, are prepared and used in gels, creams, ointments or as arnica oil [Wagner and Merfort 2007]. Despite the fact that *A. montana* is the most widely employed medicinal plant in the clinics either by itself or in combination with other herbs, enzyme inhibitory and antioxidant properties of this plant are less documented. Wild-growing *A. montana* plants are under natural protection and does not give stable yields in commercial plantations. For this reason many trials were undertaken to find other *Arnica* sp. with comparable properties. Hence, the objective of the present study is to determine and compare of the *in vitro* LOX and XO inhibitory activity and mechanism of inhibition of different parts of plantation – grown *A. montana* (AM) and *A. chamissonis* (AC) plants with reference to their antioxidant activities.

Abbreviations used: AMF – *Arnica montana* flower heads; ACF – *Arnica chamissonis* flower heads; AMH – *Arnica montana* herbs; ACH – *Arnica chamissonis* herbs; AMR – *Arnica montana* rhizomes; ACR – *Arnica chamissonis* rhizomes.

MATERIALS AND METHODS

In the study different parts of cultivated *A. montana* and *A. chamissonis* were tested. A field experiment was carried out on three-year-old plantation. *A. montana* and *A. chamissonis* grown on grey-brown podsolic soil with granulometrical composition of heavy loamy sand. Immediately after harvest, heads, seeds and herbs of *A. montana* and

A. chamissonis were dried at 35°C. Rhizomes with roots were collected, separated from the aboveground parts, cleaned and finally dried at 40°C. Ethanolic extracts (tinctures) were prepared follow Polish Pharmacopoeia V [1999]. Two grams of plant material was filling with 100 ml of 70% ethanol and left in darkness by 2 weeks.

The amount of total phenolics was determined using Folin-Ciocalteau reagent [Singleton and Rossi 1965]. To 0.5 ml of the sample were added 0.5 ml H_2O, 2 ml Folin--Ciocalteau reagent (1:5 H_2O) and, after 3 min, 10 ml of 10% Na_2CO_3 and the contents were mixed and allowed to stand for 30 min. Absorbance at 725 nm was measured. The amount of total phenolics was calculated as gallic acid equivalents (GAE).

To determine the contents of phenolic acids the absorbance at 490 nm of color complexes made by the compounds studied with Arnov's reagent [Szaufer-Hajdrych 2004].

Total flavonoids content was determined according to the method described by Bahorun et al. [2004].

Quercetin from analyzed samples was extracted according to Polish Pharmacopoeia VI [1999]. Amount of quercetin was determined using Varian ProStar HPLC System separation module (Varian, Palo Alto, CA) equipped with Varian ChromSpher C18 reverse phase column (25 mm × 4.6 mm) column and ProStar 325 UV-Vis detector. The column thermostat was set at 35°C. The mobile phase consisted of 1% acetic acid (solvent A) and methanol (solvent B) and flow rate 0.8 ml/min. The gradient elution was used as follow: 0 min, 15% B; 17 min, 55% B; 36 min, 20% B; 37 min, 100% B; 45 min, 100% B; 46 min, 15% B. Quercetin was detected at 370 nm and identified by comparing retention time with that of standard.

Lipoxygenase inhibitors activity was determined spectrophotometrically according to the method described by Axelroad et al. [1981]. LOX activity was determined spectrophotometrically at temperature 25ºC by measuring the increase of absorbance at 234 nm over a 2 min period. Quercetin, main bioactive flavonoid from Arnica, was used as a positive control.

The xanthine oxidase inhibitors activity was measured spectrophotometrically, based on the procedure reported by Sweeney et al. [2001]. Allopurinol, a known inhibitor of XO, was used as a positive control at a final concentration of 10 μg/ml in the assay mixture. To determine the mode of inhibition by studied extracts, Lineveawer-Burk plot analysis was performed. The free radical scavenging activity was measured according to Brand-Williams et al. [1995].

The free radical scavenging activity was measured according to Brand-Williams et al. [1995]. The 40 μl of methanolic extracts was mixed with 3.92 ml 6×10^{-5} mol/L solution of $DPPH^{\bullet}$ in methanol. Absorbance was measured immediately and after 10 min of incubation. The affinity of test material to quench DPPH free radical was evaluated according to equation:

$$\text{scavening}\,\% = \frac{A_{C(0)} - A_{A(t)}}{A_{C(0)}} \cdot 100$$

where: $A_{C(0)}$ – absorbance of control at 0 min.,
$A_{A(t)}$ – absorbance of sample after 10 min.

Chelating power was determined by the method of Guo et al. [2001]. The extract samples (0.5 ml) was added to a 0.1 ml of 2 mM $FeCl_2$ solution and 0.2 ml 5 mM ferrozine and the mixture was shaken vigorously and left standing at room temperature for 10 min. Absorbance of the solution was then measured spectrophotometrically at 562 nm. The percentage of inhibition of ferrozine – Fe^{2+} complex formation was given below formula:

$$\% \text{ inhibition} = \left(1 - \frac{A_A}{A_C}\right) \cdot 100$$

where: A_C – absorbance of control,
A_A – absorbance of sample

The ability to inhibition of lipid peroxidation was determined as the degree of inhibition on the haemoglobin-catalyzed peroxidation of linoleic acid according to Kuo et al. [1999].

Reducing power was determined by the method of Oyaizu [1986]. As 100% of reducing power absorbance of positive control sample (1.32 mM quercetin) was taken. All experimental results were mean ± S.D. of three parallel measurements and data were evaluated by using one-way analysis of variance (Tukey test). P values < 0.05 were regarded as significant.

RESULTS AND DISCUSSION

As being present previously in *Arnica* genus the antioxidative effect is mainly bound to the presence of phenolic components, such as flavonoids, phenolic acids and phenolic diterpenes.

Irrespective of *Arnica* genus, the highest phenolics, flavonoids and phenolic acids content was determined in the flower heads, however their amounts were significant higher in AC case. Content of phenolic compounds and phenolic acids were comparable in both *Arnica* genuses only in the rhizomes case, whereas flavonoids content was significant higher in the *A. chamissonis* samples. Regardless of *Arnica* genus, the highest quercetin content was determined in the tinctures of flower heads, notwithstanding three times more was determined in the *A. chamissonis* sample. Total quercetin amount in the tinctures from AMH and AMR did not differ significantly, while in the *A. chamissonis* case, the lowest was determined in the rhizomes tinctures (table 1).

It is a well-known fact that the active constituents of *Arnica* are sesquiterpene lactones (e.g. helenalin, dihydrohelenalin), flavonoids (e.g. quercetin, patuletin) and phenolic acids (e.g. caffeic, chlorogenic acid) [Spitaler et al. 2008]. The profile of *phenolic acids mainly* comprising chlorogenic and caffeic acids; flavonoids were mainly represented by quercetin and kaempferol. Spitaler et al. [2008] show that secondary metabolites profile of flowering heads of *A. montana* cv. ARBO is closely bound with the place of cultivation. By conducting experiments on potted plants, they excluded the differences in phenolic contents of plants grown at different altitudes, showing they were related primarily to differences in soil composition at these sites. They also suggest that altitu-

dinal changes in the phenolics profile can be caused by UV radiation increase and temperature decrease (phenolics can act as UV-absorbing and antioxidant compounds in higher plants). Differences in the secondary metabolite profiles can also be due to different cultivation conditions.

Table 1. Content of total phenolics (TPC), phenolic acids (TPAC), flavonoids (TFC) and quercetin (QC), n = 9

Tabela 1. Całkowita zawartość związków fenolowych (TPC), kwasów fenolowych (TPAC) flawonoidów (TFC) i kwercetyny (QC), n = 9

		TPC GAE mg/g d.w.	TPAC CAE µg/g d.w.	TFC QE mg/g d.w.	QC mg/g d.w.
A. montana	AMF	116.90±1.00^{b}	73.14±2.33^{bc}	113.22±8.72^{e}	3.20±0.12^{c}
	AMH	55.44±1.68^{c}	51.77±2.13^{a}	34.40±2.76^{c}	0.8±0.07^{a}
	AMR	110.88±4.01^{a}	69.80±0.67^{b}	7.68±1.00^{a}	0.9±0.01^{a}
A. chamissonis	ACF	200.40±7.35^{e}	128.92±5.62^{e}	203.07±12.36^{f}	9.10±0.73^{d}
	ACH	130.26±7.35^{d}	105.21±7.68^{d}	71.47±6.46^{d}	1.42±0.62^{b}
	ACR	109.55±5.34^{ab}	83.50±3.31^{c}	16.03±0.67^{b}	0.9±0.05^{a}

Different literal in the same column indicates statistical differences ($p < 0.05$).
Różne litery w tych samych kolumnach oznaczają różnice istotne statystycznie ($p < 0,05$).

Several reports have indicated that flavonoids were able to inhibit LOX and XO activity and act as anti-inflammatory factors. In our studies the most effective LOX inhibitors were ACF and ACH tinctures. Their LOX inhibitory activity were comparable – IC_{50} about 5.6 mg/ml In contrast to this, tincture of AMF were the least effective LOX inhibitor (IC_{50} = 8 mg/ml). ACR tinctures were as good as AMH and AMR preparations (IC_{50} about 6 mg/ml). The inhibition mechanisms of the *Arnica* tinctures were studied by kinetic analysis using double-reciplotal plotting. Lineweaver-Burk plots of reactions in the presence and absence of the *Arnica* tinctures in a LOX reaction mixture are shown in Figure 1 A–C. Obtained data indicate that all preparations from *A. montana* and from *A. chamissonis* flowers heads inhibited lipoxygenase by acting as uncompetitive inhibitors (mixed type of inhibition). The mode of LOX inhibition for ACH tincture is of competitive, whereas ACR tincture is of noncompetitive (Fig. 1 A–C). Quercetin, used as a positive control, acts as noncompetitive inhibitor (Fig. 1 D). In our work, XO activity was the most effective inhibited by both studied tinctures of herbs, IC_{50} average 1.1 mg/ml and 1.16 mg/ml for AMH and ACH tinctures, respectively. Significantly lower inhibitory effects were observed when studied extract of rhizomes from AC and flower heads both studied species, the lowest activity was found for ACF (IC_{50} = 1.36 mg/ml) (table 2). Lineweaver-Burk plots of reactions in the presence and absence of the *Arnica* tinctures in a XO reaction mixtures are shown in Fig. 2 A–C. The data indicates that the mode of XO inhibition for the AMF, AMR, ACF and ACR tinc-

tures is of competitive type, whereas tinctures of both *Arnica* herbs inhibited XO by binding either with the free enzyme or the enzyme-substrate complex – mode of inhibition is of mixed type (between uncompetitive and non-competitive type of inhibition).

Table 2. Xanthine oxidase (XOI) and lipoxygenase (LOXI) inhibitory activity of tinctures from different parts of *A. montana* and *A. chamissonis*, n = 9

Tabela 2. Zdolność do inhibicji oksydazy ksantynowej (OXI) I lipoksygenazy (LOXI) przez nalewki z różnych części *A. montana* i *A. chamissonis*, n = 9

	IC_{50}, mg d.w/ml						µg/ml	
	A. Montana			*A.chamissonis*			Allopurinol Allopurynol	Quercetin Kwercetyna
	AMF	AMH	AMR	ACF	ACH	ACR		
XOI	1.26±0.11^{b}	1.11±0.07^{c}	1.20±0.08^{a}	1.36±0.05^{d}	1.16±0.03^{a}	1.30±0.12^{b}	17.68±0.97	-
LOXI	8.01±0.52^{c}	6.30±0.31^{a}	6.11±0.72^{a}	5.53±0.20^{b}	5.61±0.51^{b}	6.07±0.82^{a}	-	100±2.06

Different literal in the same verse indicates statistical differences ($p < 0.05$).
Różne litery w tych samych wierszach oznaczają różnice istotne statystycznie ($p < 0,05$).

Results of our studies confirmed those obtained by Cassels et al. [1999]. They indicated that extract from *A. chamissonis* had an average of 40% higher inhibition of LOX activity and reduction of superoxide and hydroxyl free radicals on a tissue dry weight basis than *A. montana*.

In conclusion, both *Arnica* tinctures exhibited a high LOX and XO inhibitory activity and therefore contain bioactive constituents useful in the treatment LOX and XO induced diseases and inflammation.

Most of the antioxidant potential in herbs is due to the properties of phenolic compounds, which can acts as reducing agents, free radical scavengers and hydrogen donators [Oktay et al. 2003]. Thus, other antioxidant activities of both *Arnica* (free radical scavenging abilities, reducing and chelating power, and ability to lipid peroxidation inhibition) were assayed in this work.

The highest antiradical activity was observed for AMF and ACH (IC_{50} = 20.00 mg/ml); on the other hand the lowest activity was determined with extract of AMH (IC_{50} = 36.50 mg/ml) (table 3). As being present tinctures obtained from different parts of AC were possessed of a high antiradical activity, comparable or higher than theses determined for AM samples. In recent studies is lack of informations detailing and comparing antioxidative and health-promoting abilities of *Arnica montana* and *Arnica chamissonis*. The antiradical activities of *Arnica* plants were already studied by Heilmann et al. [1995] and Cassels et al. [1999] that resulted in promising data. The investigations of Heilmann et al. [1995] showed that compounds isolated from *A. chamissonis* ssp. *foliosa* possess a significant radical scavenging activity at a low concentration, thus indicating their therapeutic relevance. Lipids, in ours studies, were very well protected by all *Arnica* extracts, except tincture of AMF. Taking into account abilities to protect

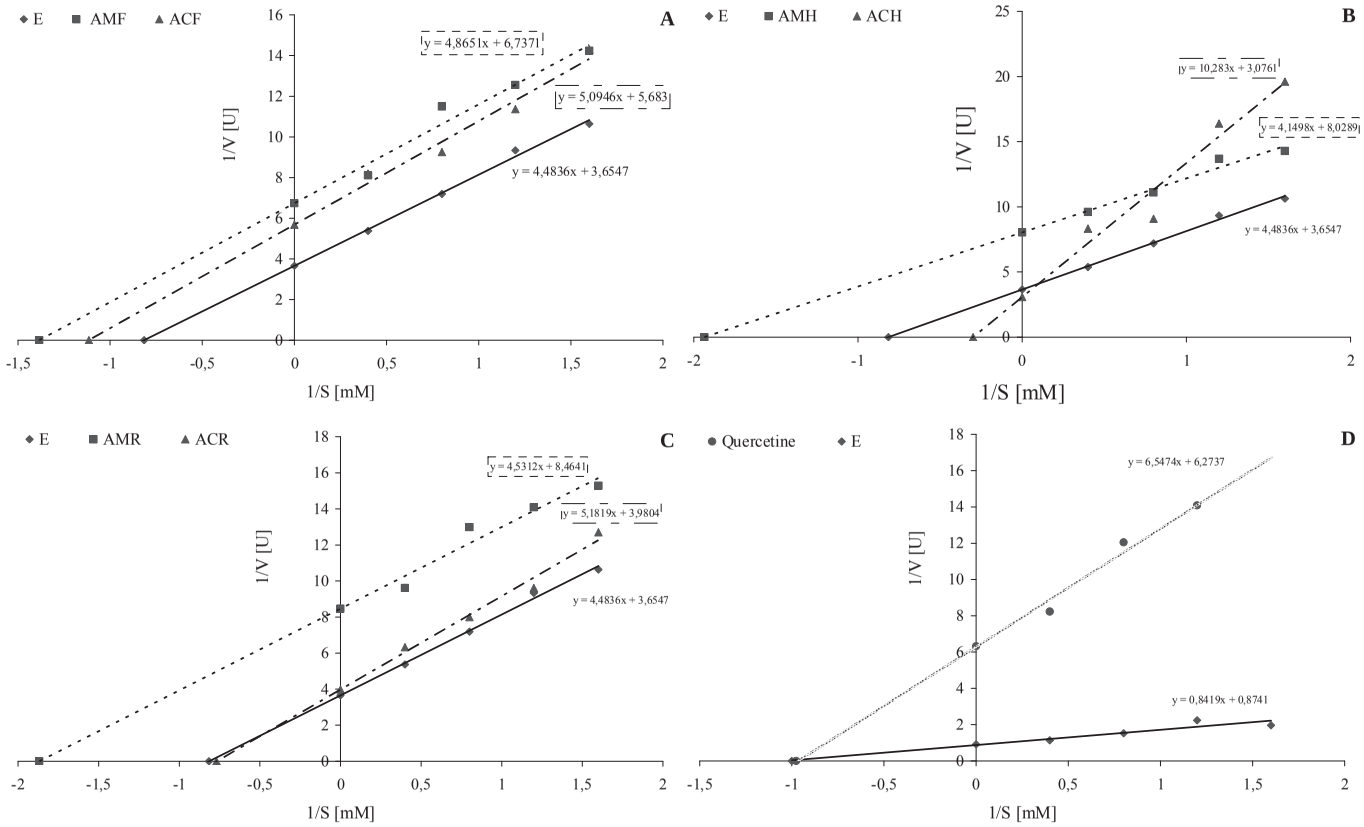

Fig. 1. Inhibition of LOX by *A. montana* and *A. chamissonis* tinctures; A – flower heads, B – herbs, C – rhizomes, D – positive control (quercetin)
Rys. 1. Inhibicja LOX przez nalewki z *A. montana* i *A. chamissonis*, A – koszyczki, B – ziele, C – kłącza, D – kontrola pozytywna (kwercetyna)

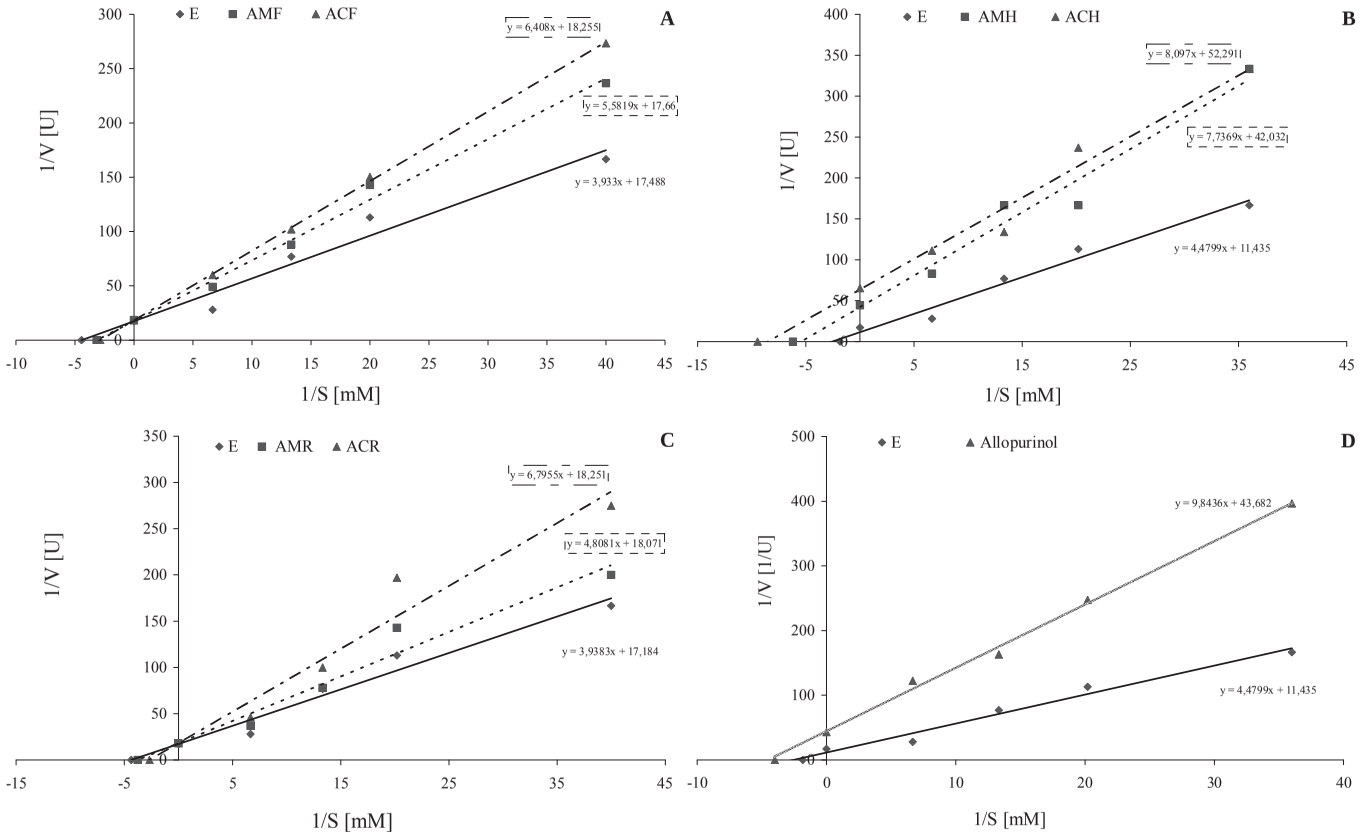

Fig. 2. Inhibition of XO by *A. montana* and *A. chamissonis* tinctures; A – flower heads, B – herbs, C – rhizomes, D – positive control (allopurinol)
Rys. 2. Inhibicja XO przez nalewki z *A. montana* i *A. chamissonis*, A – koszyczki, B – ziele, C – kłącza, D – kontrola pozytywna (allopurynol)

lipids against oxidation should be noted that both AM and AC were a very well source of natural lipids preventers, the IC_{50} (mg/ml) ranged from 10.01 to 15.09. Intermediate metal ions play an important role in the Fenton reaction. This process is closely bound with the formation of free radicals, especially hydroxy- and peroxyradicals. Inhibition of this reaction can be performed by inactivation or chelating of iron ions [Kaur et al. 2006]. Fe^{2+} ion were bound in the highest degree by tinctures obtained from AM and AC rhizomes. It is also note worthy that chelating powers of AMF, AMH and ACH were not significantly different (tab. 3).

Table 3. Antioxidant activity of *Arnica* tinctures, expressed as IC_{50} (mg/ ml), n = 9
Tabela 3. Aktywność antyoksydacyjna nalewek z arniki wyrażona jako IC_{50} (mg/ml), n = 9

Sample – Próba		AA^{DPPH}	LPO	CHP	RP*
A. Montana	AMF	20.00[a]	15.09[b]	16.98[a]	11.84[e]
	AMH	36.50[d]	10.02[a]	16.83[a]	11.78[d]
	AMR	30.50 [c]	11.04[a]	14.68[b]	11.56[c]
A. chamissonis	ACF	24.00[b]	10.01[a]	15.170[c]	11.12[b]
	ACH	20.00[a]	10.05[a]	17.69[a]	10.96[a]
	ACR	24.00 [b]	10.00[a]	12.59[d]	11.02[ab]
Quercetin		4.00	0.20	0.20	0.20

AA^{DPPH} – antiradical activity, LPO – inhibition of linoleic acid peroxidation, CHP – chelating power, RP – reducing power
AA^{DPPH} – aktywność przeciwrodnikowa, LPO – zdolność do hamowania peroksydacji kwasu linolowego, CHP – zdolność do chelatowania, RP – zdolność do redukcji
*as 100% of reducing power taken absorbance of positive control sample (1.32 mM quercetin)
* jako 100% przyjęto absorbancję kontroli pozytywnej (1,32 mM kwercetyny)
Different literal in the same column indicates statistical differences ($p < 0.05$).
Różne litery w tych samych kolumnach oznaczają różnice istotne statystycznie ($p < 0,05$).

The reductive ability can also determine the antioxidant capacity of the samples. Reducing power of tested samples was relatively high, the highest reducing abilities were determined for ACH and ACR. Irrespective of the part of plant materials used for tincture preparation AC were a better source of natural compounds with reducing abilities.

Many studies have shown that phenolic contents of raw plant material can be correlated with their antioxidant activities. Thus, our work tried to demonstrate the synergistic action of antioxidant compounds and mutual influence of different mechanisms of antioxidant protection. Statistical analysis showed that antiradical activity, chelating and reducing power were positively correlated with total phenolics content (r = 0.68, 0.54 and 0.54, respectively). Antiradical activity and reducing power were also correlated with phenolic acids content (r = 0.54 and 0.75, respectively) (table 4). Negative correlation was found between ability to LOX inhibition and total phenolics, total flavonoids

Table 4. Correlation coefficients (*r*) between biological activities and total phenolics (TPC), total flavonoids (TFC), total phenolic acids (TPAC). Statistically significant results with $p < 0.05$ were marked with bold fonts

Tabela 4. Współczynniki korelacji (*r*) pomiędzy aktywnościami biologicznymi a całkowitą zawartością związków fenolowych (TPC), flawonoidów (TFC), kwasów fenolowych (TPAC). Wyniki istotne statystycznie oznaczono pogrubioną czcionką

	TPC	TFC	TPAC	QC
Antiradical activity Aktywność przeciwrodnikowa	**0.68**	0.42	**0.54**	0.29
Chelating power Zdolność do chelatowania	**0.54**	-0.29	0.05	-0.09
Reducing power Zdolność do redukcji	**0.54**	0.14	**0.75**	0.12
Lipid peroxidation inhibition Zdolność do hamowania peroksydacji lipidów	0.05	-0.21	0.24	-0.16
LOX inhibitory activity Zdolność do inhibicji LOX	**-0.68**	**-0.56**	-0.44	**-0.67**
XO inhibitory activity Zdolność do inhibicji XO	-0.06	-0.45	0.12	-0.45

Table 5. Correlation coefficients (*r*) between various biological activities. Statistically significant results with $p < 0.05$ were marked with bold fonts.

Tabela 5. Współczynniki korelacji (*r*) pomiędzy rozmaitymi aktywnościami biologicznymi. Wyniki istotne statystycznie oznaczono pogrubioną czcionką

	Antiradical activity Aktywność przeciw-rodnikowa	Chelating power Zdolność do chelatowania	Reducing power Zdolność do redukcji	Lipid peroxidation inhibition Zdolność do hamowania peroksydacji lipidów	XO inhibitory activity Zdolnoścć do hamowania OX
Antiradical activity Aktywność przeciwrodnikowa	-	-0.18	0.39	**-0.50**	**-0.50**
Chelating power Zdolność do chelatowania	-0.18	-	0.38	0.35	-0.26
Reducing power Zdolność do redukcji	0.39	0.38	-	**0.58**	-0.02
Lipid peroxidation inhibition Zdolność do hamowania peroksydacji lipidów	**-0.50**	0.35	**0.58**	-	**0.53**
XO inhibitory activity Zdolność do hamowania OX	**-0.50**	-0.26	-0.02	**0.53**	-
LOX inhibitory activity Zdolność do hamowania LOX	-0.32	0.16	**0.54**	**0.83**	**0.57**

and quercetin concentration (table 4). The ambiguous relationships between the antioxidant activity and total phenolics are often explained by mutual influence of different antioxidants in the mixture and different method of measuring antioxidant activity. As being present in these studies, ability to LOX and XO inhibition was negatively corre-

lated with antiradical activity (r = -0.55). It is interesting that positive correlation was found between ability to inhibition of lipid peroxidation and capacity to LOX and XO inhibition (r = 0.83 and 0.54 respectively). Moreover, ability to LOX inhibition was positively correlated with reducing power (r = 0.54) and ability to XO inhibition (r = 0.57). Apart from that ability to inhibition of lipid peroxidation was positively correlated with reducing power (r = 0.58). Lipoxygenase, an enzyme that specially introduces oxygen into free fatty acids, contains iron within its molecular structure [Juntachote and Berghofer 2005] and was already proved that some inhibitors acted as Fe^{2+} chelators. However, ours studies showed that for *Arnica* tinctures the inhibition of lipoxygenase was effected by other mechanism (statistical analysis shows not significant positive correlation between these activities) (table 5). Positive correlations between ability to inhibition of lipid peroxidation and antiradical activity were found by Gawlik-Dziki et al. [2009]. Similarly to these studies they also found significant relationships between abilities to lipid peroxidation inhibition and reducing power. It is noteworthy that correlations in the antioxidant activities in the same samples marked with the same methods are observed exceptionally seldom, and the lack of such correlation is stated most often [Erel 2004].

Results obtained indicate that biological active compounds associate themes together and show synergistic effects. It was also clearly pointed out that the biological activity of sample does not come only from the concentration of active compounds, but is closely bound to mutual relationships between them. In the earlier studies Sherwin [1990] defined benefits that come from using the mixture of antioxidant compounds. He claims that it is possible to complement mechanisms of action of biological compounds and reduce methodological problems, such different solubility and color of simple compound solution. All the above remarks allow researchers to increase precision of studies and obtain more authoritative results.

CONCLUSION

In conclusion, the studies suggest that *Arnica* tinctures possess high antioxidant abilities and XO and LOX inhibitory activity that might be helpful in preventing or slowing the progress of free radical dependent diseases. The data (Lineweaver-Burk plots of LOX and XO inhibition) indicates that bioactive compounds of different parts of *Arnica* plant act in different mechanism. Differences in the mode of inhibition seem to confirm the thesis about the mutual interactions of bioactive compounds and give the reason to investigate of whole extracts (complex of active phytochemicals) not only single compound, isolated from plant raw material. Additionally, results clearly showed that *A. chamissonis* might be a valuable herbal raw material.

REFERENCES

Axelroad B., Cheesborough T. M., Laakso S., 1981. Lipoxygenases in soybeans. Methods Enzymol. 71, 441–451.

Bahorun T., Luximon-Ramma A., Crozier A., Aruoma O.I., 2004. Total phenol, flavonoid, proanthocyanidin and vitamin C levels and antioxidant activities of Mauritian vegetables. J. Sci. Food Agric. 84, 1553–1561.

Brand-Williams W., Cuvelier E., Berset C.M., 1995. Use of free radical method to evaluate antioxidant activity. LWT. 28, 25–30.

Cassels A.C., Walsh C., Belin M., Cambornac M., Robin J.R., Lubrano C., 1999. Establishment of a plantation from micropropagated *Arnica chamissonis* a pharmaceutical substitute for the endangered *A. montana.* Plant Cell Tiss. Org. Cult. 56, 139–144.

Erel O., 2004. A novel automated direct measurement method for total antioxidant capacity using a new generation, more stable ABTS radical cation. Clin. Biochem. 37, 277–285.

Ferraz Filha Z.S., Vitolo I.F., Fietto L.G., Lombardi J.A., Saude-Guimaraes D.A., 2006. Xanthine oxidase inhibitory activity of Lychnophora species from Brazil ("Arnica"). J. Ethnopharmacol. 107, 79–82.

Gawlik-Dziki U., Dziki D., Baraniak B., Lin R., 2009. The effect of simulated digestion in vitro on bioactivity of wheat bread with Tartary buckwheat flavones addition, LWT. 42, 137–143.

Guo J.-T., Lee H.-L., Chiang S.-H., Lin H.-I., Chang C.-Y., 2001. Antioxidant properties of the extracts from different parts of broccoli in Taiwan. J. Food Drug Anal. 9, 96–101.

Heilmann J., Merfort I., Weiss M., 1995. Radical scavenger activities of different 3`,4`-duhydrixyflavonols and 1,5-dicaffeoylquinic acid studied by inhibition of chemiluminescence. Planta Med. 61, 435–38.

Hollman P.C.H., Katan M.B.,1997. Absorption, metabolism and health effects of dietary flavonoids in man. Biomed. Pharmacother. 51,305–310.

Juntachote T., Berghofer E., 2005. Antioxidative properties and stability of ethanolic extracts of Holy basil and Galangal. Food Chem. 92, 193–202.

Kaur G., Alam M.S., Jabbar Z., Javed K., Athar M., 2006. Evaluation of antioxidant activity of *Cassia siamea* flowers. J. Ethnopharmacol. 108, 340–348.

Kuo J.-M., Yeh D.-B., Pan B., 1999. Rapid Photometric Assay Evaluating Antioxidative Activity in Edible Part Material. J. Agric. Food Chem. 47, 3206–3209.

Liang M.C., Bardhan S., Pace E.A., Rosman D., Beutler J.A., Porco J.A. Jr, Gilmore T.D., 2006. Inhibition of transcription factor NF-kappaB signaling proteins IKKbeta and p 65 through specific cysteine residues by epoxyquinone A monomer: correlation with its anti-cancer cell growth activity. Biochem. Pharmacol. 71(5), 634–45.

Murota K., Terao J., 2003. Antioxidative flavonoid quercetin: implications of its intestinal absorption and metabolism. Arch. Biochem. Biophys. 417, 12–17.

Oktay M., Gulcin I., Kufrevioglu, O. I., 2003. Determination of in vitro antioxidant activity of fennel (*Foeniculum vulgare*) seed extracts. LWT. 36, 263–271.

Oyaizu M., 1986. Studies on products of browning reaction – Antioxidative activities of products of browning reaction prepared from glucosamine. Japan. J. Nutr. 44, 307–315.

Polish Pharmacopoeia VI., 1999. Warszawa PTF, 390–392.

Sherwin E.R., 1990. Antioxidants. In: *Food Additives*, eds Branen, A.L., Davidson, P.M., & Salminen, S. Marcel Dekker Inc, New York, USA, 144–145.

Singleton V.L., Rossi J.A., 1965. Colorimetry of total phenolics witch phosphomolybdicphodphotungstics acid reagents. Am. J. Enol. Vitic. 16, 144–158.

Spitaler R., Winkler A., Lins I., Yanar S., Stuppner H., Zidorn C., 2008. Altitudinal variation of phenolic contents in flowering heads of *Arnica montana* cv. ARBO: a 3 year comparison. J. Chem. Ecol. 34, 369–75.

Sweeney A., Wyllie S.G., Shalliker R. A., Markhan J.L., 2001. Xanthine oxidase inhibitory activity of selected Australian native plants. J. Ethnopharmacol. 75, 273–277.

Szaufer-Hajdrych M., 2004. Phenolic acids in leaves of species of the *Aquilegia* genus. Herba Pol. 50, 10–14.

Wagner S., Merfort I., 2007. Skin penetration behaviour of sesquiterpene lactones from different Arnica preparations using a validated GC-MSD method. J. Pharm. Biomed. Anal. 43, 32–38.

Zhao G.-R., Xiang Z.-J., Ye T.-X., Yuan Y.-J., Guo Z.-X., 2006. Antioxidant activities of *Salvia miltiorrhiza* and *Panax notoginseng*. Food Chem. 99, 767–774.

PORÓWNANIE ZDOLNOŚCI DO HAMOWANIA AKTYWNOŚCI LIPOOKSYGENAZY I OKSYDAZY KSANTYNOWEJ ORAZ AKTYWNOŚCI ANTYOKSYDACYJNYCH *in vitro* PRZEZ NALEWKI Z *Arnica montana* I *Arnica chamissonis*

Streszczenie. Preparaty otrzymane z arniki są szeroko używane w leczeniu stanów pourazowych oraz chorób o podłożu zapalnym. Celem pracy było określenie i porównanie aktywności antyoksydacyjnych oraz zdolności do hamownia aktywności lipooksygenazy (LOX) i oksydazy ksantynowej (XO) przez nalewki z różnych części *A. montana* (AM) i *A. chamissonis* (AC) w aspekcie zawartości związków polifenolowych. Na podstawie wartości IC_{50} sądzić można, iż aktywne składniki AM działają głównie jako inhibitory OX, podczas gdy LOX była skuteczniej hamowana przez próby otrzymane z AC. Najwyższą aktywnością przeciwrodnikową charakteryzowały się nalewki z koszyczków AM oraz ziela AC. Wszystkie badane próby wykazały wysoką zdolność do redukcji (IC_{50} od 10,96 do 11,84 mg/ml) i peroksydacji lipidów (IC_{50} od 10,00 do 15,09 mg/ml). Nalewki z arniki wykazały wysoką aktywność antyoksydacyjną oraz zdolność do inhibicji OX I LOX, co może wskazywać na ich użyteczność w zapobieganiu lub łagodzeniu przebiegu chorób o podłożu wolnorodnikowym. Ponadto otrzymane rezultaty wskazują na fakt, iż AC może być wartościowym surowcem zielarskim.

Słowa kluczowe: arnika, stan zapalny, inhibicja enzymów, polifenole, antyoksydanty

Accepted for print – Zaakceptowano do druku: 21.04.2011