Journal of International Scientific Publications: Agriculture and Food

Volume 2, ISSN 1314-8591 (Online), Published at: http://www.scientific-publications.net

CONTENT OF BIOLOGICALLY ACTIVE COMPOUNDS IN WATER EXTRACTS OF PERIWINKLE (*CATHARANTHUS ROSEUS* (L.) G. DON)

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Abstract

The experiment was carried out in the year 2013. The experimental plant material consisted of herb (leafy flowered stems) and leaves of periwinkle (Catharanthus roseus (L.) G. Don). The aim of the study was to compare the content of biologically active compounds (L-ascorbic acid, total polyphenols), dry matter, and antioxidant activity of fresh and dried plant material. Moreover, on the base of the dried plant material (herb and leaves of periwinkle), three kinds of water extracts were prepared: infusions, decoctions and macerates; with three times of extraction. In the obtained water extracts content of dry matter, L-ascorbic acid, total polyphenols and antioxidant activity were determined.

On the base of the study results it was shown that fresh leaves of periwinkle had higher biological value in comparison with its herb. However, after drying process higher content of biologically active compounds was noted for the herb. 15 min/h decoction was found to be the most effective method of water extraction of dried perwinkle herb and leaves. Moreover, herb water extracts were characterized by higher content of polyphenols, while leaf water extracts – by higher content of dry matter, L-ascorbic acid and antioxidant activity.

Key words: *Catharanthus roseus, water extract, infusion, decoction, macerate, biologically acive compounds*

INTRODUCTION

The genus *Catharanthus* belongs to family Apocynaceae and it includes eight species. Except *C. pusillus* (Murray) G. Don, all of them are originally endemic to Madagascar (Brun et al. 2001). The most common of *Catharantus* species and widely cultivated all over the world is *C. roseus* (L.) G. Don (perwinkle, Madagascar perwinkle) (Pandey-Rai et al. 2006, Rahmatzadeh & Kazemitabar 2013). It grows to 20-80 cm high and blooms with pink, purple, or white flowers (Swanberg & Dai 2008). Therea are over 100 cultivars of *C. roseus* known (Ku et al. 2013).

The perwinkle plants impart a characteristic smell to their surroundings. It is due to the presence of volatile compounds in their leaves and flowers. The leaves contained 0.05% of essential oils, while flowers - 0.07%. There were 52 leaf essential oil compounds and 41 compounds of the flower essential oil already found (Pandey-Rai et al. 2006).

Catharanthus roseus is one of the most important medicinal and ornamental plants (Rahmatzadeh & Kazemitabar 2013). It contains over 100 different alkaloids from terpenoid indole group (TIAs) (Pandey-Rai et al. 2006, Liu et al. 2011, Rahmatzadeh & Kazemitabar 2013). Vindoline, catharanthine and 3',4'-anhydrovinblastine are major perwinkle alkaloids as well as precursors in the biosynthetic pathways of vinblastine and vincristine (Verma et al. 2008). The physiologically important antineoplastic alkaloids such as vincristine and vinblastine are present in the leaves and the antihypertensive alkaloids are found in the roots such as ajmalicine, serpentine, and reserpine (Mishra et al. 2001, Idrees et al. 2010, Kumar et al. 2012, Rahmatzadeh & Kazemitabar 2013). Moreover, due to the presence of other various phytochemical constituents (i.a. glycosides, terpenoids, flavonoids, phenols, tannins, carbohydrates, saponins, phytosterols, protein and amino acids), *C. roseus* also possess good antibacterial, antifungal, antioxidantant, antidiabetic and antiviral potential (Marcone et al. 1997, Favali et al. 2004, Debnath et al. 2006, Pandey-Rai et al. 2006, Verma et al. 2008, Kingston 2009, Patil & Ghosh 2010, Prasad et al. 2010, Gajalakshmi et al. 2013).

There are several methods of plant material extraction to possess biologically active compounds. One of them is water extraction (Guimarães et al. 2011). The most common forms of the preparation of water extracts are herbal tea infusion or decoction, a procedure which is modified according to the stability of the biologically active substances in the processed medicinal plant (Dadáková et al. 2010).

In opinion of Verma et al. (2008), hot water extraction can be environmentally friendly method for the extraction of perwinkle secondary metabolites such as glycosides and flavonoids. Kim et al. (2004) used a hot water

extraction method to extract secologanin, a monoterpenoid glycoside, which is also the precursor in biosynthesis of *Catharanthus* alkaloids.

The aim of the presented study was to compare the content of biologically active compoundsin perwinkle herb and leaves, and assess the effect of the water extraction method on the antioxidant potential of the obtained infusions, decoctions and macerates.

MATERIAL AND METHODS

The experiment was carried out in 2013 in the Department of Horticulture of West Pomeranian University of Technology in Szczecin. The research material consisted of fresh and dried herb (leafy flowered stems) and leaves of periwinkle (*Catharanthus roseus* (L.) G. Don).

Perwinkle was grown from seedlings produced in the glasshouse. They were transplanted into the open field on 28th June, at a density of 25×20 cm. Plot area was 2.75 m^2. The experiment was set in a randomized block design with four replications. During the growing season the crop management was carried out. It included mainly irrigation, weeding and soil cultivation. For the laboratory research the crop was collected at full-bloom stage (29th July).

The chemical analyses of raw plant material included determination of the content of dry matter (drying at 105°C to constant weight), vitamin C as L-ascorbic acid (by the method of Tillmans), total polyphenols – by spectrophotometer, using gallic acid as reference, and Folin-Ciocalteu reagent (Singleton & Rossi 1965). Scavening effect of perwinkle on DPPH-radical was determined according to the method of Yen & Chen (1995). Raw homogenised material was diluted 1000 times in 99.8% methanol cz.d.a. (0.1 g of sample in 100 mL of methanol). DPPH percent inhibition was calculated according to Rossi et al. (2003).

Part of the raw plant material was dried in a through-flow laboratory dryer set at 35°C. In dried and pulverized material chemical analyses were performed, the same as mentioned above for raw plant material.

Moreover, the dried herb and leaves of perwinkle (cut into about 1 cm pieces) were used to prepare three kinds of water extracts: infusions (with three times of infusion: 5, 10 and 15 minutes), decoctions (three decoction times: 5, 10 and 15 minutes) and macerates (three cold maceration times: 5, 10 and 15 hours). Infusions: 200 mL of boiling distilled water was added to 2 g of the sample and left to stand at room temperature for 5, 10 and 15 minutes. Decoctions: 200 mL of distilled water was added to 2 g of the sample, and heated until boiling. The mixture was left to stand at boiling water for 5,10 and 15 minutes and then at room temperature for another 5 minutes. Macerates: 200 mL of distilled water was added to 2 g of the sample and left for 5, 10 and 15 hours at room temperature for the extraction.

The obtained infusions, decoctions and macerates were filtered using Whatman No. 1 filter paper. In the filtrates content of dry matter, L-ascorbic acid, total polyphenols and antioxidant activity were determined, by the same methods as mentioned above for raw and dried plant material.

Results of the study were subjected to an analysis of variance, and the means were separated by the Tukey's test at p=0.05.

RESULTS AND DISCUSSION

On the base of the study results it was shown that fresh leaves of periwinkle had higher biological value in comparison with its herb (Table 1). Statistically significant differences between these two usable parts of perwinkle were found for the content of dry matter and total polyphenols. *Catharanthus* leaves were characterized by 1.88% higher amount of dry matter and by 184.31 $mg \cdot 100g^{-1}$ DW of higher content of polyphenols, in comparison with the herb.

After drying process the content of biologically active compounds in perwinkle herb and leaves has significantly decreased: L-ascorbic acid - on average by 99.31%, total polyphenols - by 30.48% and antioxidant activity - by 72.89%. Dry matter content has increased and amounted on average for perwinkle dried herb and leaves 91.94%.

It was also found that after the drying process perwinkle herb was characterized by higher content of biologically active compounds in comparison with the leaves. Moreover, water extracts obtained from perwinkle herb contained higher amounts of polyphenols, while leaf water extracts were characterized by higher content of dry matter, L-ascorbic acid and antioxidant activity (Table 2 and 3).

Volume 2, ISSN 1314-8591 (Online), Published at: http://www.scientific-publications.net

Table 1. The effect of drying process on the content of dry matter, L-ascorbic acid, total polyphenols and antioxidant activity of herb and leaves of perwinkle

Usable part of the plant (factor I)	State of the plant material (factor II)	Dry matter (%)	L-ascorbic acid ($mg \cdot 100g^{-1}$ DW)	Total polyphenols ($mg \cdot 100g^{-1}$ DW)	Antioxidant activity (% DPPH)
Herb	raw	14.45	1407.40	3280.76	58.67
	dried	91.90	15.41	2540.58	21.13
	mean	53.17	711.40	2910.67	39.90
Leaves	raw	18.11	2130.53	3804.66	93.01
	dried	91.99	8.98	2385.30	20.00
	mean	55.05	1069.75	3094.98	56.51
Mean for factor II	raw	16.28	1768.96	3542.71	75.84
	dried	91.94	12.19	2462.94	20.56
Mean		54.11	890.58	3002.82	48.20
$LSD_{\alpha=0.05}$ for factor I		1.591	n.s.	103.351	n.s.
$LSD_{\alpha=0.05}$ for factor II		0.082	271.951	591.093	28.540
$LSD_{\alpha=0.05}$ for interaction IxII		0.116	384.596	n.s.	n.s.

On the base of the statistical analysis results it was found that the content of dry matter in herb and leaf water extracts differed significantly according to the type and time of water extraction. Higher contents were noted for decoctions and macerates, respectively on average: 0.40% in herb extracts and 0.41% in leaf extracts. The content of dry matter in infusions was significantly lower and amounted 0.25% in herb infusions and 0.28% in leaf infusions. In the case of herb extracts significantly higher amounts of dry matter were determined after 15 min/h and 10 min/h extraction while in the case of of leaf extracts - after 15 min/h extraction.

L-ascorbic acid content in the water extracts made of dried perwinkle herb differed significantly only according to the type of extraction. Significantly higher amounts of this compound were determined in decoctions and macerates than in infusions. However, there were no significant differences in L-ascorbic acid content between macerates and infusions found.

Decoctions made of perwinkle leaves contained significantly higher amounts of L-ascorbic acid (0.29 $mg \cdot 100g^{-1}$ DW) in comparison with macerates (0.25 $mg \cdot 100g^{-1}$ DW) and in comparison with infusions (0.16 $mg \cdot 100g^{-1}$ DW). Higher amounts of L-ascorbic acid in herbal decoctions in comparison with infusions were also found by Guimarães et al. (2011). Moreover, it was proved on the base of obtained study results that 15 min/h leaf extracts were characterized by the highest content of L-ascorbic acid in comparison with 10 min/h and 5 min/h extracts. It was also found that in the case of infusions and decoctions 15 min/h and 10 min/h time were the most effective for L-ascorbic acid extraction, while for macerates - 15 min/h and 5 min/h time.

Higher amounts of L-ascorbic acid were assessed in leaf water extracts which were also characterized by higher antioxidant activity, in comparison with herb extracts. In the study of Raudsepp et al. (2013), the antioxidant

activity was also positively correlated with the ascorbic acid content in the water infusions made of different plant material.

Content of total polyphenols in perwinkle herb and leaf water extracts differed significantly according to the etraction type, and in the case of herb extracts also according to the extraction time.

Table 2. The effect of the type and time of water extraction on the content of dry matter, L-ascorbic acid, total polyphenols and antioxidant activity in perwinkle herb extracts

Type of the water extract (factor I)	Extraction time (factor II)	Dry matter (%)	L-ascorbic acid ($mg \cdot 100g^{-1}$ DW)	Total polyphenols ($mg \cdot 100g^{-1}$ DW)	Antioxidant activity (% DPPH)
Infusion	5 minutes	0.21	0.12	1.16	8.49
	10 minutes	0.29	0.09	7.15	12.38
	15 minutes	0.27	0.06	4.63	10.15
	mean	0.25	0.09	4.31	10.34
Decoction	5 minutes	0.41	0.22	18.55	37.22
	10 minutes	0.43	0.13	20.55	26.31
	15 minutes	0.45	0.11	21.77	38.96
	mean	0.43	0.15	20.29	34.16
Macerate	5 hours	0.32	0.12	12.12	18.78
	10 hours	0.37	0.13	16.20	26.74
	15 hours	0.39	0.11	14.55	20.54
	mean	0.36	0.12	14.29	22.02
Mean for factor II	5 minutes/hours	0.31	0.15	10.61	21.49
	10 minutes/hours	0.36	0.11	14.63	21.81
	15 minutes/hours	0.37	0.09	13.65	23.22
Mean		0.35	0.12	12.96	22.17
$LSD_{\alpha=0.05}$ for factor I		0.105	0.056	15.468	9.706
$LSD_{\alpha=0.05}$ for factor II		0.049	n.s.	3.489	n.s.
$LSD_{\alpha=0.05}$ for interaction IxII		n.s.	n.s.	n.s.	n.s.

Volume 2, ISSN 1314-8591 (Online), Published at: http://www.scientific-publications.net

Table 3. The effect of the type and time of water extraction on the content of dry matter, L-ascorbic acid, total polyphenols and antioxidant activity in perwinkle leaf extracts

Type of the water extract (factor I)	Extraction time (factor II)	Dry matter (%)	L-ascorbic acid ($mg \cdot 100g^{-1}$ DW)	Total polyphenols ($mg \cdot 100g^{-1}$ DW)	Antioxidant activity (% DPPH)
Infusion	5 minutes	0.25	0.13	2.29	19.96
	10 minutes	0.27	0.17	3.33	25.75
	15 minutes	0.31	0.17	4.37	30.26
	mean	0.28	0.16	3.33	25.32
Decoction	5 minutes	0.42	0.25	9.55	44.68
	10 minutes	0.41	0.32	16.81	48.45
	15 minutes	0.50	0.30	21.07	59.32
	mean	0.44	0.29	15.81	50.82
Macerate	5 hours	0.33	0.27	10.72	27.25
	10 hours	0.41	0.20	13.59	26.69
	15 hours	0.42	0.28	11.07	31.66
	mean	0.38	0.25	11.79	28.53
Mean for factor II	5 minutes/hours	0.33	0.21	7.52	30.63
	10 minutes/hours	0.36	0.23	11.24	33.63
	15 minutes/hours	0.41	0.25	12.17	40.41
Mean		0.37	0.23	10.31	34.89
$LSD_{\alpha=0.05}$ for factor I		0.071	0.016	2.727	12.208
$LSD_{\alpha=0.05}$ for factor II		0.034	0.017	n.s.	6.597
$LSD_{\alpha=0.05}$ for interaction IxII		n.s.	0.030	n.s.	n.s.

The most effective for polyphenol extraction from perwinkle herb were: decoction and maceration (on average 17.29 $mg \cdot 100g^{-1}$ DW), while from the leaves - decoction (15.81 $mg \cdot 100g^{-1}$ DW). In the case of herb extracts the highest results were obtained for 10 and 15 min/h time. Similar results were obtained by Goncalves et al. (2013). The authors compared total phenolic content in water extracts obtained from ten different species of medicinal plants. Hot water extracts were characterized by significantly higher content of total polyphenols in comparison with the cold ones.

It should be also stressed that the content of total polyphenols assessed in perwinkle water extracts was very high: 12.96 mg·100g^{-1} DW in herb extracts and 10.31 mg·100g^{-1} DW in leaf extracts. In the study of Kim et al. (2011) where hot water extracts from 13 different herbs were compared the highest amount of total polyphenols was noted for clove (10.83 mg·100g^{-1} DW), and on average content for all tested species was 2.88 mg·100g^{-1} DW.

The results of antioxidant activity of the perwinkle water extracts were analogous to the previous ones. The highest results were obtained for decoction - 34.16% DPPH in herb extracts and 50.82% DPPH in leaf extracts. The significant effect of the extraction time was found only according to the leaf extracts and the most effective was 15 min/h. It was also found that leaf extracts were characterized by higher antioxidant activity than herb extracts, even though they contained smaller amounts of total polyphenols. In the literature it is usually indicated that phenolic compounds are major contributors to antioxidant activity (Zheng & Wang 2001, Huda-Faujan et al. 2007, Liu et al. 2008, Kim et al. 2011, Raudsepp et al. 2013). Although, it is possible that the DPPH radical scavenging activity of species might be exhibited by the synergistic effect of the combinations of total phenolics and other antioxidant components including antioxidant vitamins and pigments (Chu et al. 2000, Charanjit & Harish 2002, Al-Ismail & Aburjai 2004, Kim et al. 2011). Moreover, in opinion of Heinonen et al. (1998) and Kakhonen et al. (1999) different phenolic compounds may have different responses to Folin-Ciocalteu reagent.

CONCLUSIONS

1. Fresh leaves of periwinkle were characterized by higher biological value in comparison with its herb. However, after drying process higher content of biologically active compounds was noted for the herb.
2. Water extracts obtained from perwinkle herb contained higher amounts of polyphenols, while leaf water extracts were characterized by higher content of dry matter, L-ascorbic acid and antioxidant activity.
3. The most effective, among all of the tested in the experiment methods, for biologically active compound water extraction, was 15 min/h decoction.

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