

Effect of storage on the antioxidant properties of *Plantago lanceolata* L. and *Plantago major* L. alcoholic extracts

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ABSTRACT

Introduction: Plant extracts are used, among others, for the production of cosmetic goods. However, storing them for too long may degrade valuable substances and reduce antioxidant activity. The *Plantago lanceolata* L. and the *Plantago major* L. are applied as agents in the treatment of skin diseases, where they show an anti-inflammatory as well as an antibacterial effect.

Materials and methods: In this study, plant material was extracted using 4 solvents: ethanol, methanol, propan-1-ol as well as propan-2-ol (all at 3 different concentrations). The ultrasound-assisted extraction was applied for 30–60 min. After the extraction, antioxidant activity of a part of the extracts was immediately evaluated – using the 2,2-diphenyl-1-picrylhydrazil

(DPPH) method – while the remaining part of the extracts was stored for 6 months and then evaluated for antioxidant potential.

Conclusions: The most beneficial antioxidant properties were found in the extracts of dried leaves for both *P. lanceolata* and *P. major* in 70% (v/v) isopropanol and concentrated methanol, determined immediately after their preparation. In the case of *P. major*, dried leaves and a longer preparation time led to a significant increase in antioxidant activity. Both species, *P. lanceolata* and *P. major*, seem to be valuable plants with high antioxidant activity for improving everyday health. Cosmetic preparations containing extracts from these plants seem to be a good solution to provide effective anti-aging protection.

Keywords: plant extracts; DPPH; alcohol solvent; ultrasound-assisted extraction.

INTRODUCTION

The *Plantago lanceolata* L. and the *Plantago major* L., belonging to the plantain family (*Plantaginaceae*), are plants commonly found in Poland and large parts of Europe, North Africa and Western Asia. Both species are quite short plants, with a shortened stem forming a rosette, on which the characteristic leaves are concentrated. In the case of *P. major* they are broad-leaved, whole-sided or sparsely toothed, while *P. lanceolata* has parallel leaves, lanceolate, whole-sided, with few teeth. Both species are among weeds growing on field roads, meadows, pastures, roadsides, lawns and agricultural wastelands. Plantago leaves have been used in medicine since antiquity. Both *P. major* and *P. lanceolata* are used externally. They have, among others, anti-inflammatory effects and accelerate the regeneration of the damaged epidermis. Forever, expectorant, anti-inflammatory as well as antibacterial activities can be observed and they can also be used as an auxiliary in upper respiratory tract diseases [1, 2, 3]. The leaves of *P. lanceolata* are obtained mainly in a natural state, but due to an increase in demand with depleting natural resources, the plant was introduced to field crops [1].

The main ingredients of these species which have an impact on therapeutic effect include iridoid glycosides, tannins, organic acids, mucous substances, pectin, vitamins and mineral salts [1, 4, 5, 6, 7]. Due to their valuable chemical composition, these plants have high antioxidant properties, which has been confirmed by many studies [2, 8, 9, 10, 11].

In recent years, increasing attention has been paid to the high antioxidant activity of plants, used as natural ingredients in many cosmetic preparations. Moreover, “phytocosmetics” have recently enjoyed great popularity [12].

The interest in cosmetic preparations containing plant raw materials with strong antioxidant properties is growing year on year. This is due to the fact that oxidative stress, caused by an excess of reactive oxygen species (ROS), plays an important role in the aging process – not only of the skin, but of the entire body. Too much ROS leads to, among others, a disturbance of cellular metabolism through the violation of protein, lipid and DNA structures, and, as a consequence, to premature skin aging. Moreover, the skin is constantly exposed to external factors such as UV radiation, ionizing radiation, temperature or contact with heavy metals [13, 14]. Extracts from some plants, including *P. major* and *P. lanceolata*, contained in cosmetic preparations can contribute to a beneficial effect on the skin due to their high antioxidant properties. Both species of plantain are used primarily in anti-inflammatory preparations [11, 15]. Moreover, *P. lanceolata* extracts have the ability to absorb UV radiation, which means they can be applied as a potential natural sunscreen [16].

The aim of the study was to assess the antioxidant potential of alcoholic extracts from dried and fresh leaves of *P. lanceolata* and *P. major*, prepared in 4 solvents by applying a method belonging to “green techniques”, i.e., ultrasound-assisted extraction. Four low-molecular aliphatic alcohols with various molecular masses were applied as solvents.

To evaluate the stability of the extracts, the effect of a 6-month storage on their antioxidant capacity was also evaluated.

MATERIALS AND METHODS

2,2-diphenyl-1-picrylhydrazil (DPPH) was sourced from Sigma Aldrich, USA; methanol, ethanol, propan-1-ol and propan-2-ol (all of AR quality) were from Chempur, Piekary Śląskie, Poland.

The plant material consisted of fresh and dried leaves of *P. major* and *P. lanceolata*. The leaves of the 1st herb were collected in Szczecin, while the leaves of the 2nd plant came from the Bytów district (north of Poland). Both species were harvested at the end of May 2019. The plant material was dried for 2 weeks, in an airy, dark room at room temperature.

The plant material was extracted using 4 solvents, at 3 concentrations: ethanol – 40% (v/v), 70% (v/v), 96% (v/v), methanol – 40% (v/v), 70% (v/v), 99.5% (v/v), propan-1-ol – 40% (v/v), 70% (v/v), 99.5% (v/v) and propan-2-ol – 40% (v/v), 70% (v/v), 99.7% (v/v). The ultrasound-assisted extraction was applied for 30–60 min. After the extraction, the antioxidant activity of a part of the extracts was immediately evaluated while the remaining part of the extracts was evaluated after 6-months of storage at room temperature in the dark.

The antioxidant activity of the extracts using the DPPH reagent was measured as described previously [17]. Absorbance measurements were performed with the Spectroquant Pharo 300 Merck spectrophotometer at 517 nm using 1 cm cells. The stock DPPH solution was prepared by dissolving 0.012 g of DPPH radical in 100 cm³ 96% (v/v) ethyl alcohol. To obtain the working solution, the stock solution was diluted with 70% (v/v) ethanol to obtain absorbance in the range of 0.980-1.020. To determine the antioxidant activity of the extracts, 2500 µL of the working solution and 132 µL of the studied extract were mixed and incubated for 10 min at room temperature in the dark. Absorbance was then measured at 517 nm against 70% (v/v) ethanol. Based on the obtained results the radical scavenging activity (RSA) was calculated according to the formula:

$$\text{RSA [\%]} = (1 - A/A_0) * 100\%$$

where A – the absorbance of test sample; A₀ – the absorbance of the control.

The results are presented as arithmetic means ± standard deviations (SD). The significant differences between the individual groups was determined by the Wilcoxon test ($\alpha = 0.05$). Calculations were made using Microsoft Excel 2010 and Statistica 12 (Statsoft) software.

RESULTS

The antioxidant activity of the extracts is shown in figures in this part. Both fresh and dried *P. major* and *P. lanceolata* leaf extracts showed antioxidant potential.

The range of antioxidant activity for all tested extracts varied depending on storage time. In the case of fresh *P. major* leaves tested immediately after extraction, the antioxidant activity ranged from 5.09 ± 8.29 %RSA for extracts in concentrated propan-1-ol (60 min) to 72.85 ± 1.42 %RSA for those prepared in 40% (v/v) methyl alcohol (30 min). The antioxidant activity tested after 6 months of storage of these extracts ranged from 9.74 ± 0.86 %RSA for extracts prepared in 70% (v/v) ethanol (30 min) to 67.22 ± 0.5 %RSA for extracts prepared in 40% (v/v) methanol (30 min) – Figure 1.

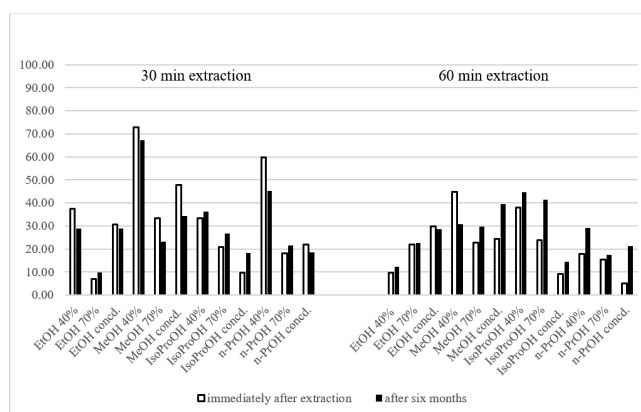


FIGURE 1. The antioxidant activity of alcoholic extracts of fresh *Plantago major* evaluated immediately after extraction and after 6 months of storage (n = 3)

The antioxidant potential of extracts from dried *P. major* leaves, analyzed immediately after extraction, ranged from 1.58 ± 1.11 %RSA for extracts in concentrated propan-2-ol to 82.53 ± 1.66 %RSA in 70% (v/v) propan-2-ol, both after a 60 min extraction. The antioxidant potential of the same samples determined after half a year in storage ranged from 5.53 ± 1.04 to 79.03 ± 0.3 %RSA for extracts prepared in concentrated propan-2-ol and 70% (v/v) isopropyl alcohol after a 30–60 min extraction, respectively – Figure 2.

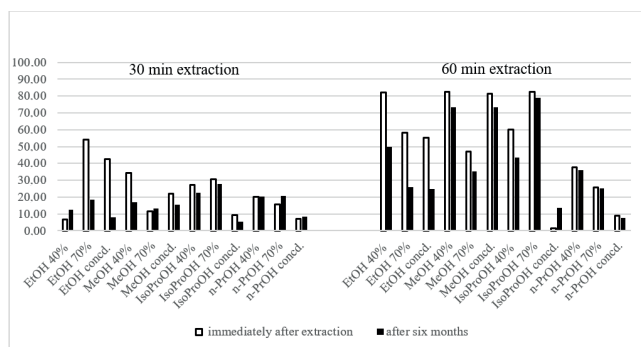


FIGURE 2. The antioxidant potential of alcoholic extracts of dried *Plantago major* leaves, determined immediately after extraction and after 6 months of storage (n = 3)

The extracts from fresh leaves of *P. lanceolata* evaluated immediately after their preparation showed antioxidant capacity ranged from 13.95 ± 3.90 %RSA for extracts in concentrated

propan-2-ol (60 min) to 64.33 ± 1.22 %RSA for samples extracted with concentrated methanol (60 min). After a 6-month storage of the extracts, the activity ranged from 6.95 ± 2.10 %RSA for extracts in concentrated propan-1-ol (60 min) to 73.22 ± 3.65 %RSA for those in 40% (v/v) methanol (60 min) – Figure 3.

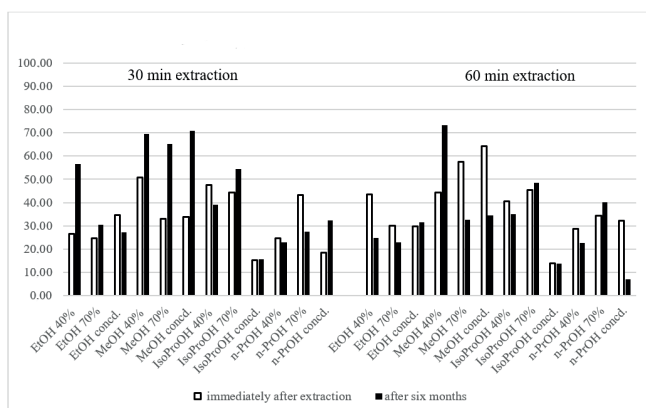


FIGURE 3. The antioxidant activity of alcoholic extracts from fresh *Plantago lanceolata* leaves, evaluated immediately after extraction and after 6 months storage (n = 3)

Antioxidant activity of dried *P. lanceolata* leaf extracts evaluated immediately after their preparation ranged from 14.88 ± 0.8 %RSA in concentrated propan-1-ol (30 min) to 87.77 ± 0.46 %RSA in concentrated methanol (30 min). After a 6-month storage of the extracts, the antioxidant activity ranged from 11.71 ± 3.51 %RSA in concentrated propan-1-ol (30 min) to 74.75 ± 1.53 %RSA in concentrated methyl alcohol (60 min) – Figure 4.

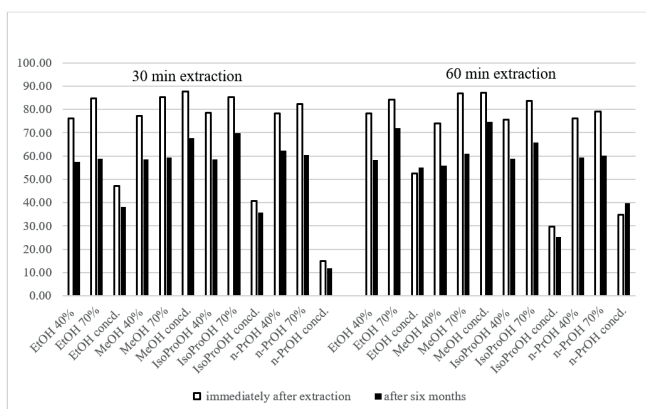


FIGURE 4. Antioxidant potential of *Plantago lanceolata* alcohol extracts from dried leaves, evaluated directly after extraction and after 6 months storage (n = 3)

Tables 1 and 2 present an analysis of statistical differences between the antioxidant activity of individual extracts, evaluated by the Wilcoxon test. Table 1 shows the significant differences between the extracts determined immediately after preparation and after 6 months of storage. Table 2 compares the antioxidant activity of the extracts between the 2 different *Plantago* species. In all analyzed cases, significant differences were only found in extracts from dried leaves. The antioxidant capacity of the extracts determined immediately after

TABLE 1. The statistically significant differences in antioxidant activity of *Plantago major* and *Plantago lanceolata* extracts, determined immediately after extract preparation and after 6-months of storage, evaluated by the Wilcoxon test

The plant material analysed	Z	p
Extracts from fresh <i>P. major</i> leaves	0.943	0.346 (NS)
Extracts from dried <i>P. major</i> leaves	2.857	0.004
Extracts from fresh <i>P. lanceolata</i> leaves	0.257	0.797 (NS)
Extracts from dried <i>P. lanceolata</i> leaves	4.114	<0.0001

NS – not significant differences

TABLE 2. Statistically significant differences of antioxidant activity between *Plantago major* and *Plantago lanceolata*, determined by the Wilcoxon test

The plant material analysed	Z	p
Fresh leaf, extracts evaluated immediately after extraction	2.117	0.030
Dried leaf, extracts evaluated immediately after extraction	3.493	0.000
Fresh leaf, 6-month storage	2.086	0.037
Dried leaf, 6-month storage	3.942	0.000

NS – not significant differences

preparation and after 6-months of storage differ significantly for both sets of *Plantago* raw material. Moreover, statistically significant differences of antioxidant activity between the *P. lanceolata* and *P. major* extracts prepared from both fresh and dried raw material and determined after 6-months were observed.

DISCUSSION

Plantago lanceolata L. and *P. major* L. have a broad spectrum of activity and are used primarily in the cosmetology and pharmaceutical industry. The health properties of both species are helpful in the treatment of many disorders, including those of the skin and respiratory tract. The main active ingredients of the raw materials show anti-inflammatory, shielding, antibacterial and astringent activity. They are also useful in the acceleration of wound healing and regeneration of the epidermis. Both species are characterized by a high RSA [11], which makes them useful in the cosmetic industry [18, 19].

The antioxidant activity of *P. lanceolata* and *P. major*, measured by the DPPH method, was confirmed in our study but was dependent on the method of extract preparation. Four solvents of different concentrations and 2 preparation times using ultrasound-assisted extraction were applied. The effect of storing extracts for 6-months in the dark at room temperature on antioxidant potential was also evaluated. In the case of *P. major*, the antioxidant potential of fresh leaf extracts, measured immediately after extract preparation, was highest for extracts prepared in 40% (v/v) methanol (30 min) – 72.85 ± 1.42 %RSA, while for dried leaves, in 70% (v/v) propan-2-ol (60 min) – 82.53 ± 1.66 %RSA. Similarly, for *P. lanceolata* extracts the highest activity was observed for extraction in concentrated

methanol for fresh and dried leaves: 64.33 ± 1.22 %RSA (60 min) and 87.77 ± 0.46 %RSA (30 min), respectively. A high activity of *P. lanceolata* and *P. major* extracts measured by the DPPH method was also reported by Wang et al., Kobeasy et al., Kantawong et al., and Kalantari et al [2, 9, 10, 11, 20]. Selamoglu et al. found that both species have a higher ability to scavenge free radicals compared to the leaves of other plants such as *Robinia pseudoacacia*, *Platanus orientalis* and *Aesculus hippocastanum*. In their study, the antioxidant activity of ethanol extracts from *P. lanceolata* and *P. major* was 84.25 and 90.25 %RSA, respectively [21]. Pereira et al. performed a comparative analysis of extracts from roots, leaves and flowers of the buck's-horn plantain (*P. coronopus* L.). The authors compared aqueous extracts with those prepared in organic solvents such as methanol, ethyl acetate, and hexane. The highest %RSA evaluated by the DPPH and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) methods was observed in *P. coronopus* roots extracted with methanol and ethyl acetate [22]. Kobeasy et al. analyzed the antioxidant potential of *P. major* extracts. The main purpose of their study was to compare *P. major* L. with *Cyamopos tetragonoloba* L. (guar beans) in terms of composition and antioxidant capacity. In the initial phase, the authors extracted plants using ethanol, hot and cold water as extractants, after which the extracts were freeze-dried. The highest amount of flavonoids was found in *P. major* leaves, which was confirmed by the DPPH method. Moreover, ethanol extracts of *P. major* leaves had higher antioxidant properties than *C. tetragonoloba* extracts [9].

The applied solvent for the extraction of plant material may play an important role in the determination of antioxidant activity [23, 24]. The polarity of the applied solvent is very important as it can influence the transfer mechanisms of a single electron or hydrogen atom during the evaluation of antioxidant potential [25]. Four solvents were used in our study: ethanol, methanol, propan-1-ol and propan-2-ol. It was shown that extracts prepared in concentrated methanol (*P. lanceolata*) and in 70% (v/v) isopropanol (*P. major*) have the highest potential. A high antioxidant activity of methanol extracts of *P. lanceolata* leaves is also reported by Duda-Chodak et al. [26].

The stability of plant alcoholic extracts depends on the species of plant used to prepare the extract. Plant extracts contain a large group of biologically active compounds, among others, hydrophilic vitamin C, flavonoid and phenolic acid derivatives with different lipophilicity and more lipophilic carotenoid dyes or chlorophylls [27]. In our study, an attempt was also made to estimate possible changes in the antioxidant potential of the extracts after a 6-month storage period. Only the antioxidant activity of the extracts of dried *P. lanceolata* and *P. major* evaluated immediately after preparation differed significantly. Significant differences in the antioxidant activity between the studied species were also shown. However, it can be observed that the change of antioxidant activity after storage is dependent on the solvent used for extraction. In some cases, an increase of antioxidant activity was found, while in others there was a decrease of this parameter. In the case of fresh *P. major* leaf extracts, the highest antioxidant activity decreased from 72.85

to 67.22 %RSA, while in extracts obtained from dried leaves of these species – from 82.52 to 79.02 %RSA. A similar trend was observed for the most valuable extracts from *P. lanceolata*: after 6-months of storage, the antioxidative activity decreased from 64.33 to 34.44 %RSA (fresh leaves) and from 87.77 to 67.69 %RSA (dried leaves). In contrast, an increase of antioxidant potential after 6-months of storage was found in some samples. The reason for the change in antioxidant activity in stored plant extracts could be the decomposition of some valuable components, as well as the formation of new compounds with a higher RSA [28]. According to the literature, in the majority of studies, a reduction of antioxidant capacity after long-term storage of plant extracts was observed [27, 29]. Zielonka-Brzezicka et al. found that a one-year storage of ethanol extracts from fresh and dried leaves and fruits of raspberries and blackberries could have different effects on the antioxidant activity measured by the DPPH method. For example, the antioxidant activity of extracts from fresh raspberry leaves decreased from 99.32 to 85.71 %RSA. This contrasted with dried raspberry leaves and dried blackberry fruits extracts which saw an increase in antioxidant activity. Where an increase of antioxidant capacity was found, it should be added that in their study, the extracts were stored, similarly as in the presented study, at room temperature and in a dark place [30].

CONCLUSIONS

To sum up, the most beneficial antioxidant properties were found for extracts of dried leaves, both *P. lanceolata* and *P. major* in 70% (v/v) isopropanol and concentrated methanol determined immediately after their preparation. It is rather to indicate the appropriate extraction time to obtain such extracts. The exception to this were the samples prepared from *P. major* dried leaves for which a longer preparation time led to a significant increase of antioxidant activity. In some cases, prolonged storage time led to a change in antioxidant potential. Therefore, it seems essential to extend the study to evaluate the effect of storage time on antioxidant capacity to assess if this parameter changes after longer amounts of time.

Both species, *P. lanceolata* and *P. major*, seem to be valuable plants with high antioxidant potential, for everyday health prevention. Cosmetic preparations containing extracts from these plants seem to be a good solution to provide effective anti-aging protection.

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