Effect of High Relative Humidity on Dried *Plantago lanceolata* L. Leaves during Long-term Storage: Effects on Chemical Composition,Colour and Microbiological Quality

Sándor Gonda,[a] * László Tóth,[b] Gyöngyi Gyémánt,[c] Mihály Braun,[c] Tamás Emri[d] and Gábor Vasas[a] *

Phytochemical Analysis
Volume 23, Issue 1
January/February 2012
Pages 88–93

* Correspondence to: S. Gonda and G. Vasas, University of Debrecen, Department of Botany, Division of Pharmacognosy; H-4010 Debrecen, Egyetem tér 1, Hungary. E-mail: gondasandor@gmail.com; vasasg@tigris.unideb.hu

[a] University of Debrecen, Department of Botany, Division of Pharmacognosy; H-4010 Debrecen, Egyetem tér 1, Hungary

[b] University of Szeged, Faculty of Pharmacy, Department of Pharmacognosy; H-6720 Szeged, Eötvös u. 6, Hungary

[c] University of Debrecen, Department of Inorganic and Analytical Chemistry; H-4010 Debrecen, Egyetem tér 1, Hungary

[d] University of Debrecen, Department of Microbial Biotechnology and Cell Biology; H-4010 Debrecen, Egyetem tér 1, Hungary

ABSTRACT

ABSTRACT:
Introduction – Modern phytotherapy and quality assurance requires stability data on bioactive metabolites to identify and minimise decomposing factors during processing and storage. A compound's stability in a complex matrix can be different from the stability of the purified compound.

Objective – To test the stability of iridoids and acteoside and quantify changes in colour and microbiological quality in a common herbal tea, dried *P. lanceolata* leaves during exposure to high-humidity air. To test the contribution of fungi to metabolite decomposition.

Methodology – Dried *P. lanceolata* leaves were exposed to atmospheres of different relative humidity (75, 45 and 0%) for 24 weeks. Changes in aucubin and catalpol concentration were determined by CE-MEKC, and those in acteoside on TLC. Colour and chlorophyll-like pigments were measured by different spectrophotometric methods. The number of fungi was monitored; 10 strains were isolated from the plant drug, and their ability to decompose the analytes of interest was tested.

Results – During incubation at 75% relative humidity (RH), aucubin, catalpol and acteoside concentrations decreased by 95.7, 97.0 and 70.5%, respectively. Strong shifts were detected in CIELAB parameters a* and b* (browning) as a result of conversion of chlorophyll to pheophytin. Intensive microbial proliferation was also observed. Changes at 45 or 0% RH were typically insignificant. Seven of the 10 isolated fungal strains could decompose both iridoids, and five could decompose acteoside in vitro.

Conclusion – It was shown that exposure to water results in loss of bioactive molecules of *P. lanceolata* dried leaves, and that colonising fungi are the key contributors to this loss.

Keywords
Plantago lanceolata L.; verbascoside; iridoid stability; long-term storage; microbiological contamination

Introduction

More and more medicinal plant products are consumed every year by people living in the developed countries. A considerable proportion of the US population (18.9%) consumed some herbal medicine in the last 12 months according to survey data (Bardia et al., 2007). Modern evidence-based phytotherapy requires stability data on these preparations, as availability of the bioactive products must be ensured from the site of production to the customer. More precisely, factors leading to instability of these matrices have to be identified and minimised during storage.

Stability studies on medicinal plants or medicinal plant extracts are rare. As there is a virtually infinite number of possible metabolite–metabolite interactions in a medicinal plant extract, the stability of a compound in the original plant matrix can be unpredictable from the stability data of the compound alone. The situation becomes even more complex in the case of dried plant materials. As there is no extraction step, the reduction of plant enzyme activity and inactivation of microorganisms is only achieved by drying (Kolb, 1999). Rehydration can thus lead to the decomposition of the bioactive metabolites by enzymes from microorganisms or the plant itself. The plant tissues often contain the activating enzyme and the metabolite compartmentalised separately, as these metabolites are frequently parts of chemical defence systems against herbivores and pathogens. This role of iridoid glycosides was described by Biere et al. (2004) in *P. lanceolata* leaves. The phenomenon of enzymatic activation has been described for the iridoids oleuropein and also aucubin, the decomposing enzyme being β-glucosidase (Konno et al., 1999). Being a once living organism, and containing huge amounts of organic carbon and nitrogen sources, a dried plant drug can be an excellent medium for microbial proliferation, when adsorbing sufficient water. As it is harvested from field cultivation, contamination is to some extent unavoidable (Kolb, 1999). Many common fungal species detected in herbal samples (for example Aspergillus sp.) were found to secrete an array of enzymes (Janda-Ulfig et al., 2009), and are thus possible contributors to bioactive molecule loss.

The role of reactivated plant enzymes, microbiological contaminants, water and oxygen in metabolite decomposition is to date less understood, mainly because studies on long-term stability of dried herbal teas and preparations are rare. Complete metabolite classes need to be examined in the future. Some example studies include a study on flavonoid-containing plant drugs (Heigl and Franz, 2003) and a study on tea polyphenols (Ortiz et al., 2008). Stability studies in complex matrices are not available for aucubin and catalpol, in particular, although aucubin has been shown to be sensitive to low pH (Suh et al., 1991). The available studies on iridoid stability in complex matrices deal with the post-harvest stage of plant materials, where the roles of plant enzymes and thermal stability are much greater than during rehydration of an already dried material. The concentration of iridoids in the end-product was found to depend on the method of drying in devil's claw (Harpagophytum procumbens; Joubert et al., 2005). The only available stability data for acteoside were assayed in solution in a complex matrix, an extract of Olea europaea leaves (Malik and Bradford, 2008). The authors detected that acteoside decomposed in the water extract, but not in methanol. Unfortunately there was no mention of possible background mechanisms, nor information on microbiological proliferation (or its absence) in the water extract.

Ribwort plantain (Plantago lanceolata L.) contains secondary metabolites of different biosynthetical origins, such as iridoids (aucubin and catalpol) and caffeoyl phenylethanoid esters (acteoside; Fig. 1), as well as mucilage and flavonoids (Ronsted et al., 2003). These metabolites are responsible for the therapeutic effects that are useful in plantain leaves' common indications, including common cold, cough, bronchitis, fever and inflammation of the mouth and pharynx (Awang, 2009). While iridoids are rather associated with the plant's antibacterial or anti-inflammatory activity (Awang, 2009), the chief polyphenolic substances, acteoside and flavonoids are responsible for the antioxidant activity, as well as anti-inflammatory and spasmolytic effects (Fleer and Verspohl, 2007).

The aim of this study was to achieve better understanding of the quality parameter changes in a commonly used medicinal plant drug, Plantago lanceolata L. dried leaves, during exposure to high-

humidity air. Therefore, easily perceptible changes, like colour and microbiological quality, were quantitatively assayed. More importantly, changes in concentrations of chief bioactive molecules were quantified during improper storage, and experiments were also run to show the contribution of proliferating fungi to the decomposition.

Experimental

Chemicals

Aucubin and catalpol were purchased from Sigma-Aldrich. Acteoside was isolated from the 1-butanol fraction of Plantago lanceolata L. leaves, on preparative silica gel TLC plates followed by column chromatography on a Toyo-Pearl HWE-40. The spectroscopic data found (UV–vis, MS) were identical to those described by Kurkin (2003). For microbiological purposes, agar from Merck and peptone (for general microbiological use) from Oxoid was used. Other mentioned common solvents and inorganic salts were purchased from Reanal (Budapest, Hungary). All reagents used were of analytical purity. For all applications, bi-distilled water was used.

Plant material

Dried Plantago lanceolata L. leaves were obtained from a local market, and were stated to be of pharmacopoeial grade. The samples were placed over adsorbent silica (0% relative humidity, RH) to remove residual moisture, and were stored there until the experiment started. A specimen is stored from the sample in our laboratory, as “2006C20”. The material was subjected to tests described in the Ph. Eur. 6, Monograph 1884. It passed all chemical and microbiological tests, including acteoside content, macroscopic examination and qualitative TLC.

Experimental design and general procedures

Plant material was exposed to two atmospheres of different RHs, 45 ± 1% and 75 ± 1% (written 45 and 75% throughout the paper, respectively), 0% being control. All treatments were run at 23 ± 1°C, in darkness, in desiccators. Constant RHs in incubating atmospheres were set and maintained by glycerin–water mixtures; 0% was maintained by adsorbent silica. RH was monitored throughout the study using commercial hygrometers. The experiment was run for 24 weeks. From each system, three randomised samples with weights of about 1 g were taken on every sampling day. The three sample replicates were processed separately. Removal of adsorbed water from the samples was accomplished by heating the plant drugs at 50°C, followed immediately by reduction to powder in commercial grinders. After sampling and subsequent drying, all samples were stored at −24°C in airtight containers until further processing.

Colour measurement

The completely dry plant drug powders were used directly for colour measurements. Colour was assayed using an Avantes-2048 fibre optic spectrophotometer, with an Avalight-DHS (combined halogen and deuterium) lamp and a 2048 pixel CCD detector. Detector distance from the sample was 2 mm. Samples were put in a special interface to exclude background illumination. Barium sulphate was used as a white reference, turned off lamp (zero signal) as black. Measurements were done at 140 ms integration time. Reflectance spectra from 400 to 700 nm were converted to CIELAB colour parameters according to Westland and Ripamonti (2004) in GNU Octave 3.0.1. Colour values are given for the CIE standard illuminant D65, CIE 1964 standard colorimetric observer. Determination of chlorophylls and pheophytins For chlorophyll (Chl) and pheophytin (Pheo) determination, the plant material was extracted overnight with acetone, at 4°C, in darkness. For quantification of the four main pigments, Chl a, Chl b, Pheo a and Pheo b, the method of Kupper et al. (2000) was used for the study. Spectra were recorded on a Shimadzu UV-1601 spectrophotometer in quartz cuvettes from 550 to 750 nm, at 0.2 nm intervals. Using the curve-fitting functions as described by Kupper et al. (2000), the Chl

and Pheo concentrations were calculated from the spectra. The procedure was implemented in fityk 0.8.8-1 (freeware, URL: www.unipress.waw.pl/fityk/) using the standard Marquardt least-square algorithm to fit functions to the data. R2 values over 0.98 were typical.

Quantification of fungal contaminants

An accurately weighed 10 mg of dried sample powder was suspended in 1000 μL of buffered sodium chloride peptone solution (BSCPS; peptone 1 g, sodium chloride 4.3 g, disodium hydrogen phosphate 7.2 g and dipotassium hydrogen phosphate 3.6 g to 1 L, pH set 7.2 ± 0.2), and allowed to stand for 10 min. After resuspension, sufficient number of 10-fold dilutions were carried out with BSCPS so that the resulting colony count would be below 100 per plate. Sterile Petri dishes 9 cm in diameter were used for the study, poured with 10 mL Saboraud's glucose agar (casein peptone 10 g, meat peptone 10 g, glucose monohydrate 40 g to 1 L, pH 5.6 ± 0.2, solidified with 2% agar) supplemented with 50 mg/L chloramphenicol. The final dilution (500 μL) was spread on the surface of the plates, allowed to dry and, after incubation at room temperature for 48–72 h, the colonies were counted. Each measurement was accomplished in three replicates.

Determination of iridoids

The method used was a modified version of Suomi et al. (2000). An accurately weighed 50 mg of *P. lanceolata* leaf powder was extracted with 1000 μL of 10% 1-propanol, for 60 min at 100°C, after being allowed to stand at room temperature for 20 min. The mixture was cooled and centrifuged at 13000 rpm and the supernatant was used for capillary electrophoresis (CE) analysis directly. This extraction procedure extracted the same amount ($p > 0.05$) of iridoids as the hot water extraction used by Suomi et al. (2000), but allowed three times more sample-to-extractant ratio within a linear extraction range for aucubin and catalpol. Sample solution storability (over a year) at −24°C was also maintained. For the quantification of iridoids, a PrinCE-C 700 capillary electrophoresis instrument with a diode array detector was used. Five-point standard calibration curves were used from both aucubin and catalpol, dissolved in 10% 1-propanol. The background electrolyte contained 25 mM sodium tetraborate and 100 mM sodium dodecyl sulphate, the pH was set at 9.35 and the applied voltage was +30 kV.

Determination of acteoside

Accurately weighed 50 mg of *P. lanceolata* leaf powder was extracted with 1000 μL of 50% ethanol, for 60 min at 100°C, after being allowed to stand at room temperature for 20 min. After cooling and centrifugation at 13000 rpm, the supernatant was subjected to quantitative TLC according to Biringanine et al. (2006). A five-point calibration curve was used; serial dilutions of acteoside dissolved in methanol were applied to the plates. After heating at 105°C for 10 min, spots were visualised by spraying with 1% natural product reagent A in ethanol. Quantitative analysis was performed by CP Atlas 2.0 freeware (URL: http://lazarsoftware.com/). R2 values were above 0.98.

Isolation of fungal strains from dried *P. lanceolata* leaves

Ten, apparently different single filamentous fungi-colonies were isolated from CFU (colony forming unit) counting plates, from both early stage (week 0 samples) and stages of intensive microbial proliferation (week 12 samples). Isolates were maintained on Czapek-Dox plates or liquid media [sodium nitrate 3 g, dipotassium phosphate 1 g, magnesium sulphate 0.5 g, potassium chloride 0.5 g, iron (II) sulphate 0.01 g, sucrose 20.0 g to 1 L, pH 6.8 ± 0.1, solidified with 2% agar] at 24°C.

Metabolite stability in sterile model solutions

To test the vulnerability of iridoids and acteoside against possible decomposition factors, model solutions were made. Briefly, a *P. lanceolata* water extract containing the analytes of interest was infested with fungal strains isolated from the dried leaves. Sterile distilled water was added to controls.

Solutions of 1000 μL volume were incubated with the strains in sterile culture plates, at 23 ± 1°C, for 9 days.

The substrate solution, containing the mixture of the chief metabolites of *P. lanceolata*, was created in the following procedure: a zero time sample of the leaves (33.0 g) was extracted with 350 mL water, and centrifuged at 20000 rpm. This water–plant drug ratio approximately represents the original concentration of the metabolites in the fresh plant. The solution was autoclaved (121°C for 20 min). Incubation was done in six replicates – three for iridoid, three for acteoside determination.

During incubation, samples 50 μL in volume were taken for chemical analysis. Iridoid and acteoside quantification was done as described earlier for the plant drug. For CE measurement, 10 μL 1-propanol, and for TLC analysis, 50 μL ethanol, were added to the aliquots, which were mixed and centrifuged at 13000 rpm before subjection to analysis.

Statistical analyses

To test our hypotheses, one-way and multi-way ANOVA models were used. ANOVA analyses were followed by Tukey's HSD test or Dunnett's test (overall level of significance was set at $p < 0.05$). The p-values for hypotheses are given in the Results and Discussion section. Differences were considered insignificant at $p > 0.05$. Statistical calculations were done with R 2.10.1 (R Foundation for Statistical Computing, Vienna, Austria) statistical software.

Results and Discussion

Changes in colour and pigment contents of dried *P. lanceolata* leaves

To quantify the easily recognizable browning at high RH, CIELAB parameters were determined for the *P. lanceolata* leaves. CIELAB parameters showed marked changes during incubation at 75% RH ($p < 0.001$), most of which took place in the first 8 weeks (Fig. 2). At 45 and 0% RH, no change was visually perceptible. Although the reflectance spectrophotometry detected minor changes at 45% RH as well, these were nevertheless negligible compared with those occurring at 75% RH (Fig. 2). Most prominent shifts were detected in coordinate a*. The changes in the parameters a*, b* and L* were 17.89, 5.07 and 2.61 times greater (respectively) at 75% RH than in controls. All colour parameters remained relatively constant at 45% RH and in controls. Parameters a* and b* can be used to describe the browning of the plant material accurately, while parameter L* gives quantitative data on the darkening of the plant drug (Fig. 2).

To identify the cause of the colour change, Chls and Pheos were quantified. The zero time pigment concentrations found are typical for dry plant organs. RH affected the stability of Chls in Plantago lanceolata L. dried leaves ($p < 0.001$). Chls and Pheos were virtually unchanged and showed similar trends at 45% RH and in controls; the changes were typically insignificant. At 75% RH, however, exponential decay of the Chl a and b content could be observed with the concurrent rise of Pheo a and Pheo b, virtually the same exponential curves as in the case of colour parameter changes. Thus we can state that Chls and Pheos were key contributors to colour change itself, and possibly also darkening, but iridoid and polyphenolic decomposition products and microbial proliferation may also add to the colour change – many of the strains isolated from the plant material showed intensively colourful appearance in our in vitro culture. Conversion of Chls to Pheos requires no enzymatic activity in plant matrices (Kohata et al., 2004), thus the browning can possibly be the consequence of an increased reaction rate between H+ and Chls, supported by the increased amount of water acting as the reaction medium. This hypothesis is also reinforced by the exponential slope of the curves.

Pigments themselves are not considered important to achieve therapeutic benefits with *P. lanceolata* leaves, making colour a secondary parameter from the therapeutic point of view. However, as the response of Chl a to water exposure was the most rapid among all the tested parameters, colour itself can be a useful indicator of water exposure during storage in the case of *P. lanceolata* L. dried leaves.

Growth of fungal contaminants in dried *P. lanceolata* leaves

Another phenomenon that could be easily recognised was the growth of moulds at 75% RH. A characteristic, foul odour also could be detected. The control system and the plant drug at 45% RH did not show major changes in CFU count and no observable growth was seen. At 75% RH (different at $p < 0.001$ from 0 and 45% RH), the incubation started with a lag phase at the beginning, followed by a rapid growth of contaminants; 75% RH caused an increase in fungal CFU by 3–4 orders of magnitude (Fig. 3). The absorbed amount of water was 2.68 ± 0.23 and 25.94 ± 0.94% at 45 and 75% RH, respectively, on a dry weight basis. This relatively low level of adsorbed water was sufficient for xerophilic species to grow at 75% RH (Gock et al., 2003) and is enough to provide water media for some enzymes to operate (Müller and Heindl, 2006). The fact, that approximately 50% RH is insufficient for the growth of moulds is described in the former works of Müller and Heindl (2006) and Gock et al. (2003), and was supported by our observations.

The maximum acceptable level of contamination is generally 105 fungal CFU/g DW (dry weight) for dried plant material to be used in therapy as a decoction (Kolb, 1999). This was exceeded during storage at 75% RH. Once the growth began, the plant material became unusable for therapeutic purposes within a single week (Fig. 3), making the storage at high RH risky. High (75%) RH is not unusual in houses, especially in the winter (Zhang and Yoshino, 2010). To avoid microbial proliferation, it is important to inform customers that dried plant material should be stored under dry conditions, or in airtight containers, once the original packaging has been opened or removed. As browning was found to be a good indicator of the exposure to water, it is also important to draw the customer's attention to the fact that products that have altered in colour should be discarded.

Changes in bioactive metabolite content of dried *P. lanceolata* leaves and contribution of fungi

Besides visible changes (colour and mould growth), high RH also caused severe changes in the chemical pattern of the leaves (Fig. 4). RH caused decomposition of iridoids in the *P. lanceolata* L. leaves ($p < 0.001$) (Fig. 5a and b). In the case of aucubin, 95.7% of the initial content was destroyed during the 24 weeks at 75% RH ($p < 0.05$), while the 45% RH system lost only 4.5% of starting concentration by the end of the experiment, the control showing no significant changes. In the case of catalpol, 97.0 and 33.5% of the initial content was lost at 75 and 45% RH, respectively. High RH also decreased the amount of acteoside ($p < 0.01$) in the plant drug. At 75% RH, 70.5% of acteoside decomposed by the end of the experiment (Fig. 5c). In the case of all three determined metabolites, very similar trends were observed at 45% RH and in controls (Fig. 5).

To test the contribution of fungal contaminants in the decomposition of the metabolites, model mixtures were incubated with 10 fungal strains isolated from the plant material. All filamentous fungi grew well on the liquid media prepared solely from the *P. lanceolata* leaf extract. Seven of 10 strains were capable of decomposing both aucubin and catalpol in solution ($p < 0.05$; see Fig. 6). Most isolates decomposed both iridoids with a similar efficiency, while strains 4 and 7 degraded aucubin more effectively than catalpol. Five of 10 strains were shown to be able to decompose acteoside in solution ($p < 0.05$); three of these degraded all acteoside during the short incubation time ($p < 0.001$). Three strains (1, 9, 10) were, however, unable to reduce the amount of acteoside in the model solutions. As no significant reduction of metabolites was detected in the control model solution ($p > 0.05$), we can state that water does not initiate metabolite decomposition by itself, or this effect is minimal. It rather acts as a medium, by supporting favourable conditions for microbial contaminants to grow in number and by allowing decomposing fungal enzymes to operate via rehydration (Janda-Ulfig et al., 2009). As the tested organisms have quite different decomposing activities for the metabolites of interest (Fig. 6), the composition of the microbial community must have a great impact on the decomposition rates of metabolites in the plant materials. The central role of fungi is also supported by the following coincidence detected during the incubation of the plant material: the speed of metabolite decomposition greatly increases after the beginning of the rapid fungal proliferation (weeks 8–12).

This can be easily recognised by comparing Figs 5(a) and 3, especially in the case of aucubin. The fact that iridoids were susceptible to more strains than acteoside might explain the phenomenon that practically all of the iridoids were destroyed at 75% RH, while acteoside showed more stability under the same conditions (Fig. 5).

The minimal acteoside amount required for pharmacopoeial grade is 1.5% DW. As acteoside decreased below 1.5% at 75% RH, and detectability of aucubin on TLC also failed from end-time samples, the quality of the plant drug became unacceptable.

The detected chemical pattern changes make RH a parameter to be controlled during storage and processing, because the bioactive metabolites are unable to tolerate long-term exposure to high RH (Fig. 5), but are stable in a low-RH atmosphere. This general phenomenon was also shown for different metabolite classes by Ortiz et al. (2008) and Heigl and Franz (2003) in plant drugs, but there was no mention of the role of microbiological contaminants. We have shown that colonising fungi have a central role in the reduction of bioactive molecules (iridoids and acteoside), which has several important consequences. First, fungi not only present a problem because of the well recognised health hazard associated with microbiological contamination (see for example Kneifel et al., 2002; Kosalec et al., 2009). Second, even if the plant material is stored for nontherapeutical (e.g. preparative) purposes, prevention of fungal activation is necessary to avoid loss of chief bioactive constituents. Third, reduction of the microbial contaminant number (e.g. by irradiation, as showed by Kumari et al., 2009) can theoretically also add to bioactive metabolite stability in the plant drug system. In order to avoid metabolite decomposition and thus maintain therapeutic and commercial value of the plant drug, it is necessary to prevent activation and proliferation of the fungi, e.g. by storing the plant drug at low ($\leq$ 45%) RH.

Acknowledgements

The authors would like to acknowledge the financial support of the following programmes: Cro_tech, OMFB-00571/2009 (Baross_EA07_EA_ONKFI_07-2008-0002), Hungarian National Research Foundation Grants OTKA K 81370 and GVOP-3.2.1.- 2004-04-0110/3.0.

References

Awang DV. 2009. Tyler's Herbs of Choice: the Therapeutic Use of Phytomedicinals, 3rd edn. CRC Press: Boca Raton, FL; 84.

Bardia A, Nisly NL, Zimmerman MB, Gryzlak BM, Wallace RB. 2007. Use of herbs among adults based on evidence-based indications: findings from the National Health Interview Survey. Mayo Clin Proc 82: 561–566.

Biere A, Marak H, van Damme J. 2004. Plant chemical defense against herbivores and pathogens: generalized defense or trade-offs? Oecologia 140: 430– 441.

Biringanine G, Chiarelli M, Faes M, Duez P. 2006. A validation protocol for the HPTLC standardization of herbal products: Application to the determination of acteoside in leaves of Plantago palmata Hook. f.s. Talanta 69: 418–424.

Fleer H, Verspohl E. 2007. Antispasmodic activity of an extract from Plantago lanceolata L. and some isolated compounds. Phytomedicine 14: 409–415.

Gock MA, Hocking AD, Pitt JI, Poulos PG. 2003. Influence of temperature, water activity and pH on growth of some xerophilic fungi. Int J Food Microbiol 81: 11–19.

Heigl D, Franz G. 2003. Stability testing on typical flavonoid containing herbal drugs. Pharmazie 58: 881–885.

Janda-Ulfig K, Ulfig K, Markowska A. 2009. Extracellular enzyme profiles of xerophilic fungi isolated from dried materials of medicinal plants. Pol J Environ Stud 18: 391–397.

Joubert E, Manley M, Gray B, Schulz H. 2005. Rapid measurement and evaluation of the effect of drying conditions on harpagoside content in Harpagophytum procumbens (devil's claw) root. J Agric Food Chem 53: 3493–3502.

Kneifel W, Czech E, Kopp B. 2002. Microbial contamination of medicinal plants – a review. Planta Med 68: 5–15.

Kohata K, Jyonoshita M, Takashima K, Ujihara T, Horie H. 2004. A relationship and pH in tea between leaves. conversion J Jpn Soc rate Food of Sci chlorophyll Technol Nippon into pheophytin Shokuhin Kagaku Kogaku Kaishi 51: 177–180.

Kolb N. 1999. Microbiological status of untreated herbal materials. Deutsch Lebensm-Rundsch 95: 263–269.

Konno K, Hirayama C, Yasui H, Nakamura M. 1999. Enzymatic activation of oleuropein: A protein crosslinker used as a chemical defense in the privet tree. Proc Natl Acad Sci USA 96: 9159–9164.

Kosalec I, Cvek J, Tomic S. 2009. Contaminants of medicinal herbs and herbal products. Arh Hig Rada Toksikol 60: 485–501.

Kumari N, Kumar P, Mitra D, Prasad B, Tiwary B, Varshney L. 2009. Effects of ionizing radiation on microbial decontamination, phenolic contents, and antioxidant properties of triphala. J Food Sci 74: M109–M113.

Kupper H, Spiller M, Kupper F. 2000. Photometric method for the quantification of chlorophylls and their derivatives in complex mixtures: fitting with Gauss-peak spectra. Anal Biochem 286: 247–256.

Kurkin V. 2003. Phenylpropanoids from medicinal plants: distribution, classification, structural analysis, and biological activity. Chem Nat Compd 39: 123–153.

Malik N, Bradford J. 2008. Recovery and stability of oleuropein and other phenolic compounds during extraction and processing of olive (Olea europaea L.) leaves. J Food Agric Environ 6: 8–13.

Müller J, Heindl A. 2006. Drying of medicinal plants. In Medicinal and Aromatic Plants. Agricultural, Commercial, Ecological, Legal, Pharmacological and Social Aspects. Springer: Berlin; 237–252.

Ortiz J, Ferruzzi M, Taylor L, Mauer L. 2008. Interaction of environmental moisture with powdered green tea formulations: effect on catechin chemical stability. J Agric Food Chem 56: 4068–4077.

Ronsted N, Franzyk H, Molgaard P, Jaroszewski J, Jensen S. 2003. Chemotaxonomy and evolution of Plantago L. Plant Syst Evol 242: 63–82. Suh N, Shim C, Lee M, Kim S, Chang I. 1991. Pharmacokinetic study of an iridoid glucoside – aucubin. Pharm Res 8: 1059–1063.

Suomi J, Siren H, Hartonen K, Riekkola M. 2000. Extraction of iridoid glycosides and their determination by micellar electrokinetic capillary chromatography. J Chromatogr A 868: 73–83.

Westland S, Ripamonti C. 2004. Computational Colour Science using MATLAB. John Wiley and Sons: Chichester; 41– 44.

Zhang H, Yoshino H. 2010. Analysis of indoor humidity environment in Chinese residential buildings. Build Environ 45: 2132–2140.

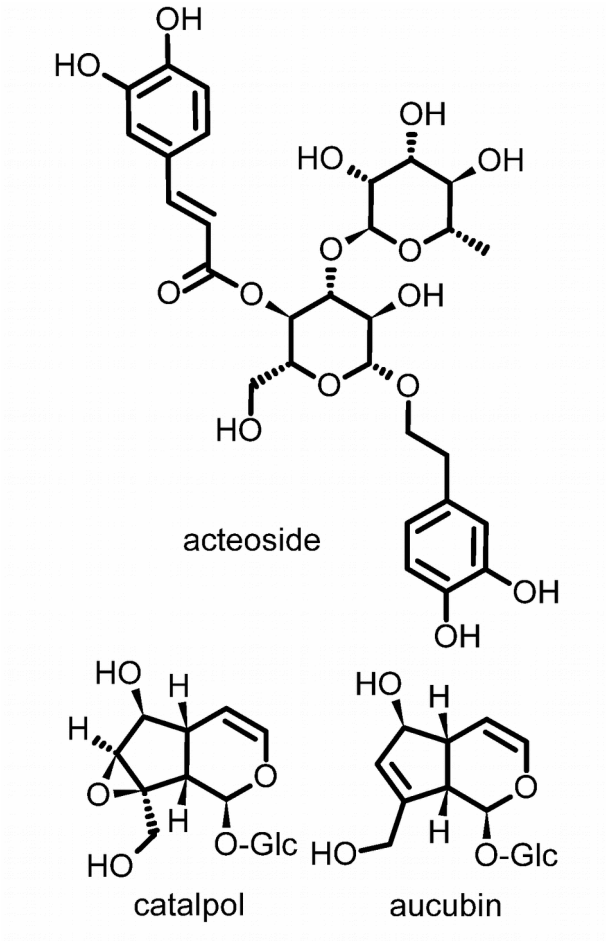

Fig. 1. Chemical structures of the studied metabolites of *Plantago lanceolata* L. Glc, O-glucosyl.

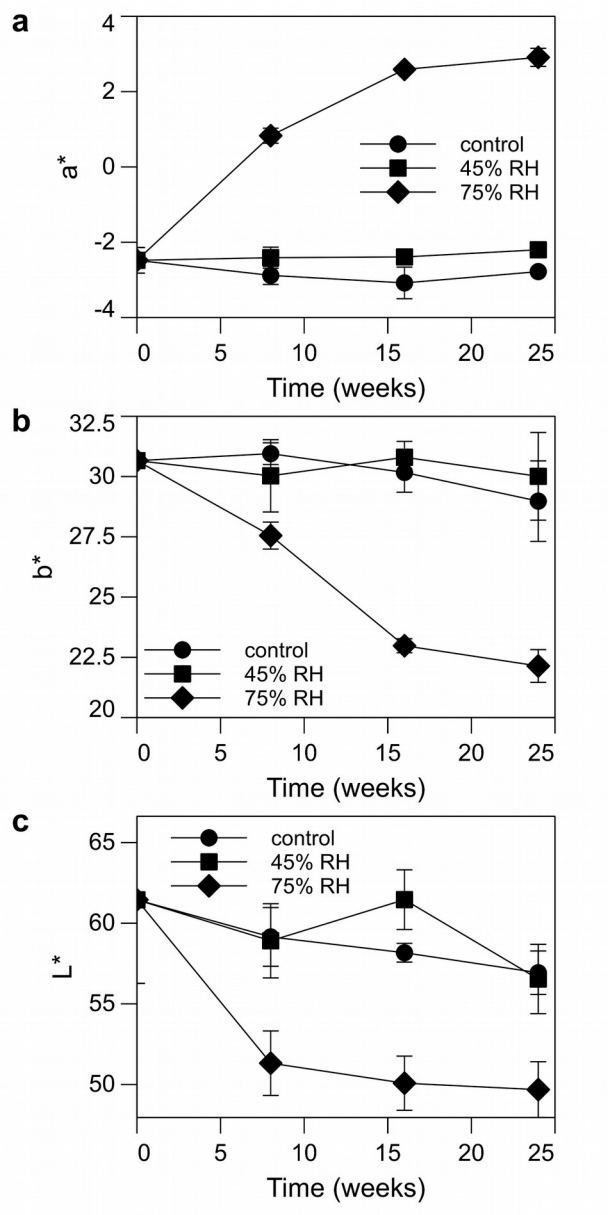

Fig. 2. Changes in CIELAB color parameters of dried *P. lanceolata* leaves during 24 weeks of storage at different relative humidities. Each data point shows the mean + SD from three measurements.

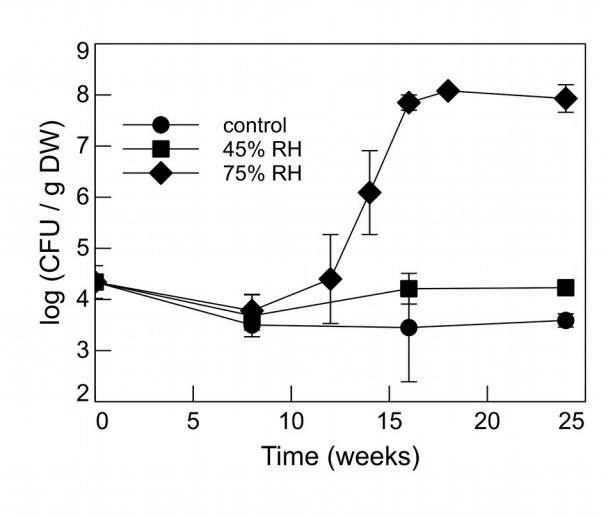

Fig. 3. Growth of fungi (total CFU count) in dried plantain leaves during 24 weeks of storage at different relative humidities. Each point shows a mean + SD from three measurements.

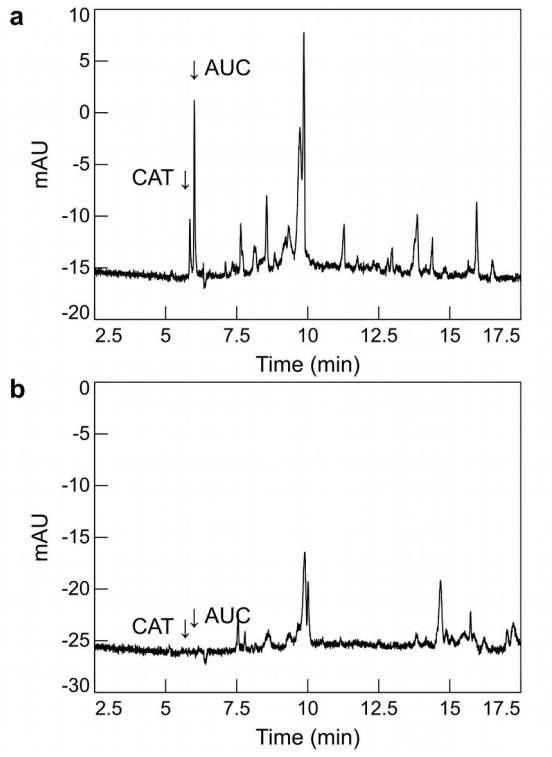

Fig. 4. Typical electropherograms from 10% 1-propanol extracts of two *P. lanceolata* leaf samples stored at 75% RH. Peaks of aucubin (AUC) and catalpol (CAT) are indicated by arrows. **a**: Zero time sample; **b**: Sample after incubation at 75% for 24 weeks.

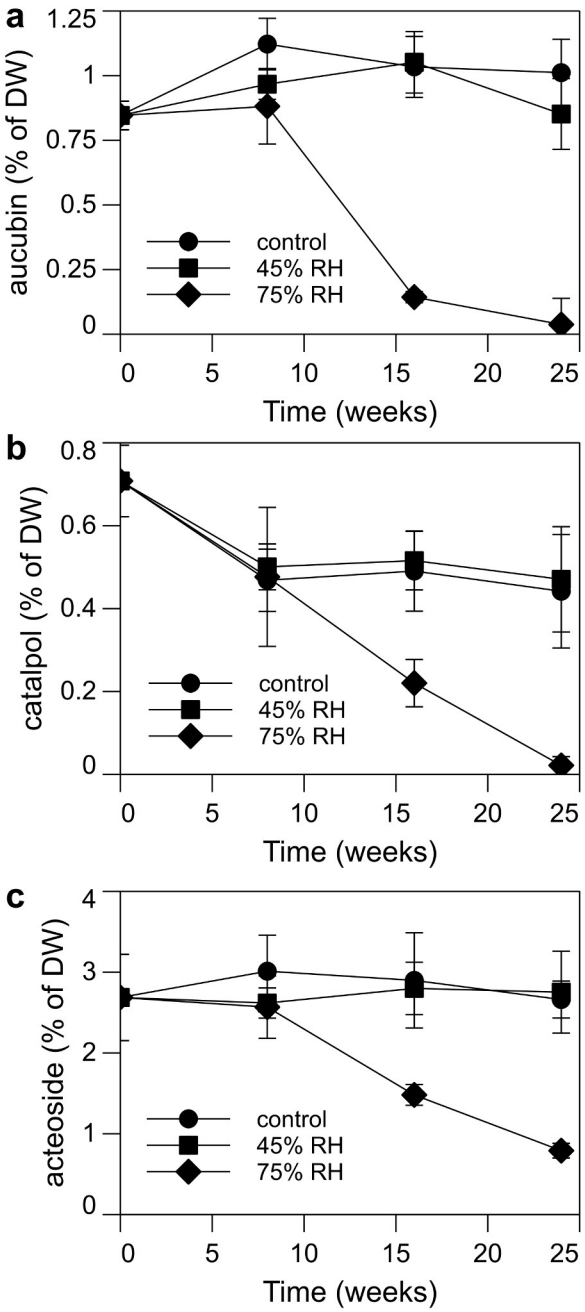

Fig. 5. Bioactive metabolite content changes in dried *P. lanceolata* leaves during 24 weeks of storage at different relative humidities. Each data point shows the mean + SD of three measurements.

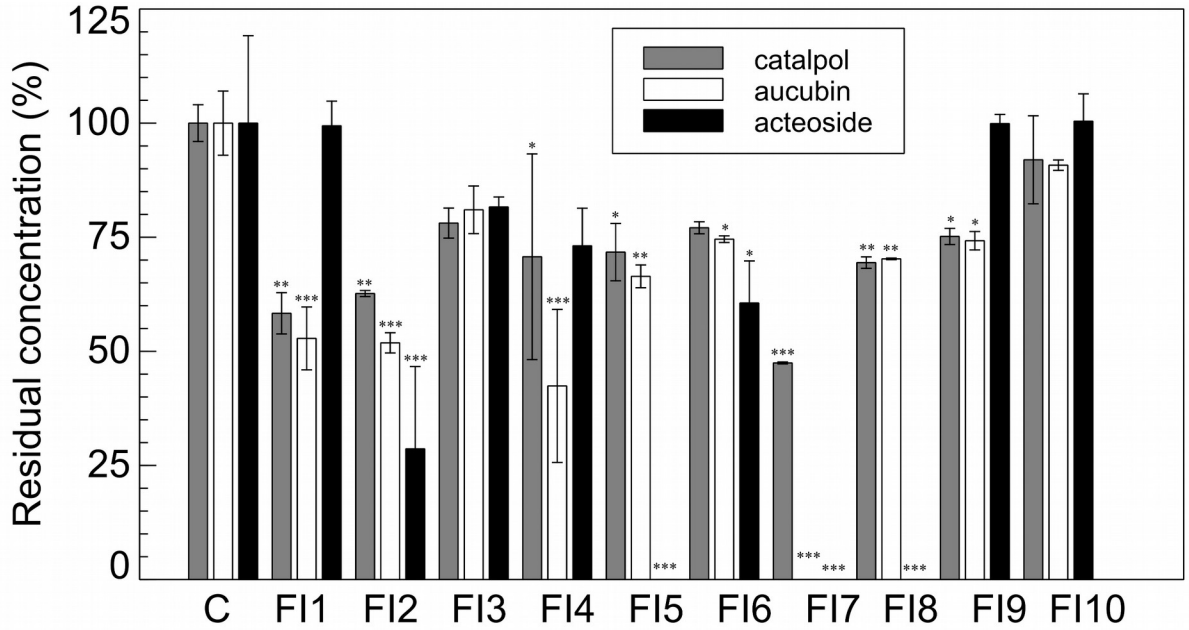

Fig. 6.
Residual concentrations of bioactive metabolites in sterile *P. lanceolata* water extracts infested with isolated fungal strains. Data plotted are after concentrations after 9 days of incubation. Each data point shows the mean + SD of three measurements. C, control, FI1-10, Fungal isolate no. 1-10.