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Drying Affects Artemisinin, Dihydroartemisinic Acid, Artemisinic Acid, and the Antioxidant Capacity of *Artemisia annua* L. Leaves

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There is limited information on how postharvest drying of *Artemisia annua* affects artemisinin (ART) biosynthesis and *A. annua* antioxidant capacity. Antioxidants may boost the bioactivity of ART and the crop commercial value. We evaluated the effect of freeze, oven, shade, and sun drying, time of drying, and light intensity on the leaf concentration of ART, dihydroartemisinic acid (DHAA), artemisinic acid (AA), and on the leaf antioxidant capacity. Freeze-dried samples had the lowest ART concentrations as compared to the other drying methods. However, the ferric reducing antioxidant power assay showed that freeze- and oven-dried samples had similarly high antioxidant activities, which declined significantly after plants were shade- and sun-dried. Shade drying for 1, 2, and 3 weeks, under ambient or low light, did not change the ART content but significantly decreased the leaf antioxidant activity, mainly if sun-dried. A significant decrease (82% average) in DHAA was observed for all drying procedures as compared to freeze drying, with a simultaneous, significant increase in ART (33% average). The average bioconversion of DHAA to ART was 43% for oven- and shade-dried plants and 94% for sun-dried plants, reiterating the hypothesis that DHAA, not AA, is the main biosynthetic precursor of ART and suggesting that sun drying improves the bioconversion from DHAA to ART. Data also indicate that oven drying for 24 h at 45 °C can provide good levels of both ART and antioxidants in leaves. These findings are valuable for the commercial production of ART and of bioactive antioxidants that might synergize with the antimalarial and anticancer effects of ART when combined in traditional preparations to improve human and animal health.

KEYWORDS: Postharvest metabolism; bioconversion; drying procedures; antioxidant capacity; *Artemisia annua*; artemisinin

INTRODUCTION

Artemisinin (ART) is the only natural compound after quinine to become vital in the fight against multidrug resistant *Plasmodium falciparum* malaria. *Artemisia annua* (Asteraceae) is the sole commercial source of ART, which is the raw material for ART-based combination therapies (ACT). The World Health Organization recommended ACT as the first-line treatment for malaria in 2001, leading to an increased cultivation of *A. annua* as a pharmaceutical crop. The world's cultivation of *A. annua* is dominated by East Asia, mainly China and Vietnam, with recent introduction of the crop to East and South Africa. China and Vietnam supply 70% and East Africa supplies 20% of the global supply of ART, but the current area planted with *A. annua* is still insufficient to meet the estimated 60% increase in ACT demand over the next few years (*1*). *A. annua* is also grown in Brazil, Ghana, India, Kenya, Madagascar, Mozambique, Nigeria, Tanzania, and Uganda, with an estimated (2009) cultivated area of 4000 ha for China, 500 ha for Vietnam, and 2000 ha total for Africa, with an estimated total of 143−180 tons of ART (including production from the wild, previously, and currently planted areas) but still short of the 200 tons required to meet the 2009/2010 demand and to replenish current stocks (*2*). The current prices for ART vary between U.S. $315−380/kg based on leaf biomass/ha, ART content in the leaves, and industrial extraction efficiency (Mark Blanchard, Artemisinin International, personal communication on July 27, 2009). In Vietnam, profits are estimated to be U.S. $770/ha of *A. annua* leaf biomass (estimated in 2.0 tons/ha), but the paying mechanism used for *A. annua* growers in China depends on the leaf ART content, with a proposed bonus of U.S. $40/ton for every additional 0.1% (g/100 g) ART above the expected content of 0.5% ART (*3*), with a clear profit to farmers who can deliver more ART in g/100 g of dried leaves.

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ART is a sesquiterpene lactone with a rare peroxide bridge, which is responsible for its effectiveness against malaria, cancer, and, possibly, a range of human and livestock diseases caused by protozoa, trematodes, trypanosomes, virus, and bacteria, as recently reviewed (*4*).

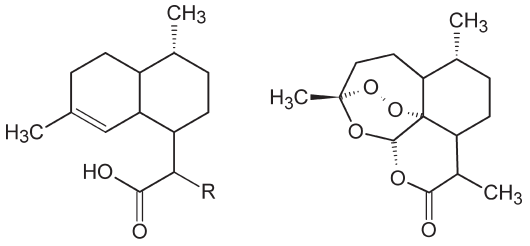
Besides ART, *A. annua* leaves (*5*) are also a great source of antioxidants, being one of the four Chinese medicinal plants with the highest ORAC (oxygen radical absorbance capacity) level (*6*), with the total ORAC averaging 1125 and 1234 μmol Trolox equiv/g for leaves and inflorescences, respectively (*5*), while oregano (with the highest reported ORAC values) ranges from 1230 (*7*) to 2800 μmol Trolox equiv/g (*5*), depending on cultivar and growth conditions. The *A. annua* antioxidant activity is probably due to its high flavonoid content and diversity (*8*), including the methoxylated flavones artemetin, casticin, crysoplenetin, chrysosplenol-D, and circilineol, reported to enhance the antiplasmodial activity of ART (*9*), to catalyze the reaction between ART and heme (*10*), or to suppress cytochrome P450 enzymes and *p*-glycoproteins, thus enhancing the bioavailability of several oral drug formulations including ART itself. Quercetin, a major flavonoid in *A. annua* (*8*), was recently reported to enhance the bioavailability of the anthelmintic moxidectin in lambs (*11*). These results support the synergistic effect between flavonoids and ART, previously reported for traditional ART preparations (tea) against malaria (*12*). These pharmacological and nutritional applications and the growing demand for ART stress the importance of understanding how postharvest methods affect both the ART biochemistry and the plant antioxidant capacity. The storage temperature and sample preparation are known to decrease phenolics in plants (*13*), but there is little information on how drying procedures affect leaf phenolics of herbs (*14*, *15*). Also, there are only a few reports on postharvest drying effects on ART (*16*−*18*). Although it has been stated that ART is fairly stable to both heat and light (*19*), no specific temperature or light intensity was reported. ART is fairly stable in neutral organic solvents up to 150 °C (*20*), but most ART degrades upon drying at 190 °C for 10 min (*20*). Although the antioxidant activity of *A. annua* is an important component of its traditional use as an antimalarial herb, as a new anticancer agent (*21*), and as an animal feed component (*5*), there is no report on the effect of drying procedures on the antioxidant capacity of *A. annua* or on the biosynthesis of ART.

The objective of this work was to determine the effect of postharvest drying (oven, shade, sun, and freeze drying) on the concentration of ART, dihydroartemisinic acid (DHAA), artemisinic acid (AA) (**Figure 1**), and on the total antioxidant activity of *A. annua*. This information is of vital importance for commercial producers to maximize extraction of bioactive phytochemicals from *A. annua* by optimizing postharvest drying.

MATERIALS AND METHODS

Chemicals. Trolox (6-hydroxy-2,5,7,8-tetramethyl-chroman-2-carboxylic acid), TPTZ [2,4,6-Tris(2-pyridyl)-*s*-triazine], and ferric chloride were purchased from Sigma-Aldrich (St. Louis, MO). Sodium acetate trihydrate was obtained from J. T. Baker (Phillipsburg, NJ). High-performance liquid chromatography (HPLC) grade methanol was obtained from Fisher Scientific (Fair Lawn, NJ). Polyvinylidene difluoride (PVDF) syringe filters with a pore size 0.45 μm were purchased from National Scientific Co. (Duluth, GA). Deionized water (18 MΩ) was prepared using a Millipore Milli-Q purification system (Millipore Corp., New Bedford, MA).

Plant Cultivation, Harvesting, and Drying. One *A. annua* plant from the cultivar Artemis (Mediplant, Conthey, Switzerland), representative of the germplasm currently in cultivation in Africa, was cloned by cuttings. Cuttings were 5 cm in size, dipped in Rhizopon AA#2 with 0.3% indole-butyric acid (Hortus USA Corp., New York, NY), placed in a tray



1 2

Figure 1. Structures of DHAA (**1**) (R, $-CH_3$), AA (R, $=CH_2$), and ART (**2**).

filled with Pro-Mix BX growing medium (Premium Horticulture, Inc., Quaquerstown, PA), and kept in a greenhouse under a 16 h photoperiod with additional light provided by 1000 W high-pressure sodium lamps (Voigt Light Industries, Philadelphia, PA). After approximately 30 days, rooted cuttings were transferred to individual 7.6 cm pots filled with Pro-Mix/Metro-Mix 510 (Hummert International, Earth City, MO). Once the plants were 20−25 cm in height, they were transplanted to a field plot in early June until harvest in September of 2005, 2006, and 2007. The cultivation field was in Beaver, WV (latitude 37° 46′ 30″ N and 81° 7′ 00″ W, altitude 738 m), and soil and fertilization schemes were the same as described elsewhere (*22*). Field plants were harvested and submitted to three drying treatments, with three replicates, as follows: (1) shade drying (roofed picnic shelter with open sides—indirect ambient light or in a covered and walled warehouse with incoming light only through the front—low light) from 1 to 3 weeks; (2) sun drying from 1 to 3 weeks; and (3) oven drying in a forced-air oven at 45 °C for 12−16 h. Treatments 1 and 2, after 1, 2, or 3 weeks, were also oven-dried to establish that oven drying at 45 °C had no effect on ART concentrations. In 2006, before the plants were harvested on September 19 and submitted to these three drying procedures, subsamples collected from the bottom, middle, and top of each plant were combined, immediately dipped in liquid nitrogen for 60 s in the field, placed into a cooler with dry ice for transportation, and freeze dried (Lyophilizer model 119875, The Virtis Co., Gardiner, NY) in our facilities. Freeze-dried subsamples were used as controls and compared with the remaining respective plant, submitted to each of the three drying procedures, for their concentrations of ART, DHAA, and AA and for the antioxidant leaf capacity. Freeze drying was meant to take a "snapshot" of the plant biochemistry before they were submitted to drying procedures. Minimum and maximum temperatures, relative humidity (RH), and precipitation data are provided for the 3 weeks of the experiment in 2006.

Light Intensity Measurements. Light intensity (illuminance, in Lux) was measured by HOBO (Onset Computer Corp., Buzzards Bay, MA) data loggers (RH, Temp, Light) placed on the outer edge of the plants that were dried in a roofed shelter (one HOBO on the outside of the plants and a second one placed inside the plant branches to obtain an average light intensity received by the plant). A single HOBO was used to collect data in a roofed/walled building where plants hung in the back third of the building, away from the door, and surrounded by several other plants drying the same way. Previous light measurements showed that there was almost no variation in light intensity in the inside of this building. The sunlight intensity was measured by two Li-Cor 190 (Li-Cor, Lincoln, NE) quantum sensors using the average of the two sensors. These sensors had been calibrated in November, 2005. Photosynthetically active radiation (PAR) was collected in $\mu moles \cdot m^{-2} \cdot sec^{-1}$ and transformed to Lux. Data for hourly measurements are provided from October 5 to 11, while average PAR measurements from September 19 to October 11 are provided for the 3 weeks of the experiment in 2006.

Extraction and Analysis of ART, DHAA, and AA. Dried ground (2 mm) leaves were weighed (500 mg) and refluxed in 70 mL of petroleum ether for 1 h. Extracts were evaporated to dryness in a fume hood overnight in the dark, reconstituted the following day in 20 mL (2 washes of 10 mL each) of acetonitrile, filtered through a 0.45 μm nylon or PTFE leuer-lock syringe filter (Millipore Corp., Billerica, MA), and immediately analyzed by HPLC with UV detection for ART, DHAA, and AA, as described previously (*23*).

Ferric Reducing Antioxidant Power (FRAP) Assay. For the FRAP assay, dried and ground leaf samples were weighed (100 mg) and placed into 15 mL plastic tubes, and 3 mL of methanol:water (6:4, v/v) was added to it. The mixture was vigorously shaken using a vortex mixer for 1 min and then left in an ultrasonic bath for 60 min. Sample tubes were centrifuged at 5000 rpm for 15 min, and the supernatant was removed. The extraction was repeated twice with 3 mL of methanol:water (6:4, v/v). The supernatant fractions were combined in a volumetric flask, and the volume was adjusted to 10 mL. Extracts were then filtered through a 0.45 μm PVDF syringe filter prior to the antioxidant activity assay by FRAP. Each sample was extracted in triplicate, and each extract was analyzed at least in duplicate.

The FRAP assay was performed using a method described by Benzie and Strain (*24*) with some modifications. The FRAP reagent was prepared fresh from 300 mmol/L acetate buffer, with the pH adjusted to 3.6 with glacial acetic acid. TPTZ (10 mmol/L), $FeCl_3 \cdot 6H_2O$ (20 mmol), and acetate buffer were mixed together in a ratio of 1:1:10 (v/v/v). Aliquots of 10 μL of sample extract, blank solvent mixture, or standard were mixed with 2.4 mL of freshly prepared FRAP reagent. The mixture was allowed to stand at room temperature in the dark for 10 min, before the absorption was measured at 593 nm using a Spectramax 384 Plus microplate reader (Molecular Devices, Sunnyvale, CA). The results were expressed in Trolox equivalents (μmol) per 100 mg of dried leaves sample weight.

Moisture Content Determination. Moisture was quantified using a halogen moisture analyzer balance model HG 63 (Mettler-Toledo, Inc., Columbus, OH). In short, approximately 125 ± 5 mg of the ground dried leaves sample was placed in a moisture analyzer balance, and the samples were heated at 105 °C until the mean weight loss was less than 1 mg per 50 s. Each sample was analyzed in duplicate, and three samples were analyzed per drying procedure.

Statistical Analysis. Each treatment for the quantification of ART, DHAA, and AA and for the determination of antioxidant capacity had three replicates. Treatment differences were established through the Kruskal−Wallis one-way analysis of variance on Ranks established by the Student−Newman−Keuls, a multiple comparison procedure (SigmaStat Version 3.11, Systat, www.systat.com). Significance levels of $P < 0.05$ were used to compare means, and standard error bars are presented to illustrate variation within replicates.

RESULTS AND DISCUSSION

Drying Effects on ART, DHAA, and AA. The moisture contents of ground leaves dried by sun, shade, oven, and freeze drying were determined by the thermogravimetric method and varied from 5.2 to 7.4%, which is below the minimum moisture content of 12.0% expected for the dry *A. annua* leaf (*25*). The mean moisture content in all dried sample was determined as 6.1%, and the standard deviation for the moisture content of all samples was 0.9%.

While sun drying was reported by some to increase ART (*18*), others stated that sun drying decreased ART (*26*). Data obtained in 2006 (**Figure 2**) indicate that sun drying is superior to shade and oven drying in increasing ART, although the cumulative data obtained with the same cloned Swiss cultivar (Artemis), field-grown in the same location for three consecutive years (**Figure 3**), indicate that there is no significant difference between shade and sun drying, while both drying treatments slightly, but significantly, enhanced ART as compared to oven drying, and all three drying methods significantly increased ART as compared to freeze drying (**Figure 3**), the latter being an average of nine freeze-dried subsamples taken in 2006. Results in **Figures 2** and **3** also agree with previous results (*16*) that reported the lowest content of ART for freeze-dried leaves as compared to shade- and oven-dried leaves. The significant increase in ART under sun drying in 2006 (**Figure 2**) will be discussed later according to the relevance of light to the process of ART biosynthesis in planta, but predrying *A. annua* plants (either under shade or sun) has been cited as a feasible way to reduce costs of the *A. annua* crop-drying process (*18*) and has been postulated as a possible way to

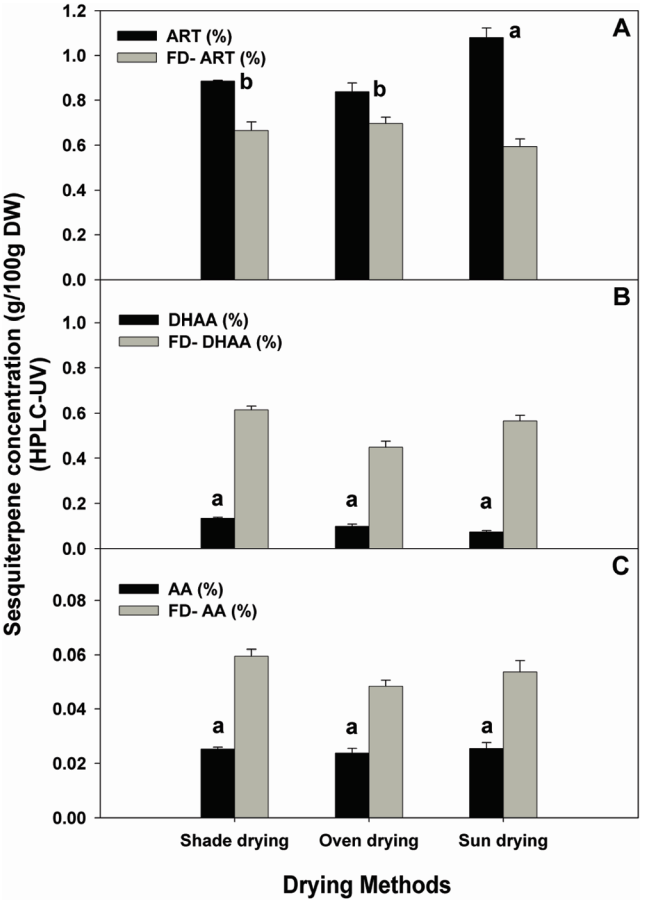


Figure 2. Effects of drying on (**A**) ART, (**B**) DHAA, and (**C**) AA in a cloned *A. annua* cultivar field-grown in 2006 [on a dry weight (DW) basis]. The effects of the three drying methods on each sesquiterpene concentration (black bar) were compared to freeze-dried (FD) subsamples of each plant immediately before harvesting (gray bar). Treatments with the same letters (across drying methods) do not differ statistically at $p < 0.05$.

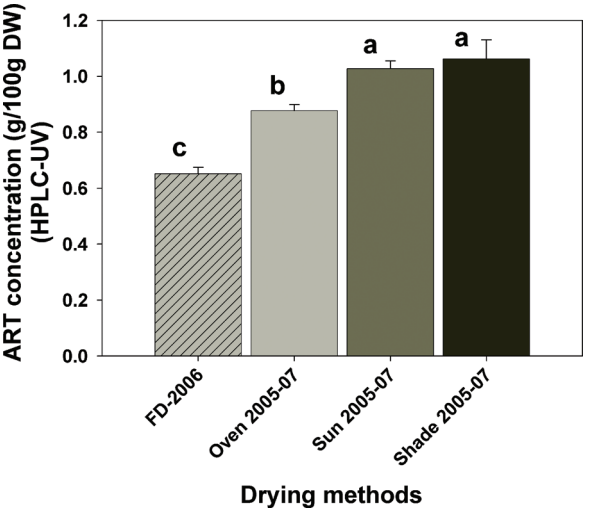


Figure 3. Average ART concentration (based on DW) in a cloned *A. annua* cultivar field-grown and submitted to three drying methods, from 2005 to 2007. Analysis was done in triplicate every year ($n = 9$ for oven, sun, and shade). Freeze-dried (FD) data obtained in 2006 ($n = 9$) are given for comparison. Treatments with the same letters (across drying methods) do not differ statistically at $p < 0.05$.

Table 1. Dissection of Average Data Presented in **Figure 2A**,**B** for the Loss in Leaf DHAA from Freeze-Dried Subsamples ($DHAA_{FD}$) as Compared to Leaf DHAA of Field-Grown Cloned *A. annua* after Oven, Shade, and Sun Drying ($DHAA_{FD}$−DHAA Postdrying or A) and the Corresponding Gain in ART from Freeze-Dried Subsamples after Each Postdrying Procedure (B) Is Presented as an Estimate Percentage of Lost DHAA from FD Subsamples That Was Converted into Leaf ART in Response to Each Drying Procedure [(B/A) × 100]

drying method	$DHAA_{FD}$ (g/100 g)	lost DHAA (A) in g/100 g (%)	ART gain (B) in g/100 g (%)	lost DHAA converted to ART [(B/A) × 100] (%)	lost AA in g/100 g (%)
oven	0.45	−0.35 (−80.0)	+0.14 (+20)	40.0	−0.024 (−50)
shade	0.61	−0.48 (−78.7)	+0.22 (+33)	46.0	−0.035 (−58)
sun	0.58	−0.52 (−87.5%)	+0.49 (+45)	94.0	−0.029 (−54)
average	0.55	−0.45 (−82)	+0.28 (+33)	60.0	−0.03 (−54)

increase ART yield by improving the conversion of DHAA to ART (*27*).

Although most of the few past studies reported an ART increase caused by sun and shade drying, none evaluated the biochemical transformation of bioprecursors into ART triggered by postharvest drying. This is the first time that postharvest drying is shown to increase ART with simultaneous, and significant, decreases in the concentrations of DHAA and AA (**Table 1** and **Figure 2**). Considering their concentration in leaves and the greater reduction in DHAA (average 82.0% or 0.45 g/100 g) as compared to AA (average 54% or 0.03 g/100 g) in dried plants (**Table 1** and **Figure 2B**,**C**), we can say that DHAA, not AA, might be the main precursor of ART. According to the current line of thought (*28*, *29*), ART biosynthesis starts with the conversion of DHAA into dihydroartemisinic acid hydroperoxide (DHAAHP) under exposure to oxygen and light; then, DHAAHP converts into ART by oxidation, without the need for light or enzymes (*28*, *30*). Although AA also decreased significantly (average 54% or 0.03 g/100 g) during drying, the decrease was not as sharp as DHAA (average 82% or 0.45 g/100 g) or in a proportion that could justify the pertinent increase in ART concentration (33% average, 0.28 g/100 g) (**Table 1** and **Figure 2**). Recently, evidence favors DHAA (not AA) as being the precursor of ART (*31*), with stem-fed DHAA decreasing in 80% just 80 h (3.3 days) after *A. annua* plants were allowed to desiccate (*30*). Data summarized in **Table 1** support the better conversion of DHAA to ART under light than under shade or oven (no light). As compared to freeze-dried subsamples, the DHAA decrease varied from 78.7% for shade drying to 87.5% for sun drying (**Table 1**), which agrees with results mentioned above (*30*). According to those authors, DHAA is metabolized in dying plants the same as in live plants. They also reported that DHAA was metabolized to other compounds, besides ART, which also explains why ART increases of 0.22 g/100 g (shade drying) and 0.14 g/100 g (oven drying) did not correspond to the same loss in DHAA (**Table 1**). However, the gain in 0.49 g/100 g in ART is similar to the loss in DHAA of 0.52 g/100 g in sun-dried plants (**Table 1** and **Figure 2**). The DHAA converted into ART was only 40−46% in oven- and shade-dried plants but 94.0% in sun-dried plants (**Table 1**), indicating that the postharvest conversion of DHAA into ART is more efficient under the more intense sunlight (**Figure 6**) than under shade (ambient or low light) and oven (**Table 1**). The conversion of DHAA into DHAAHP would not be favored in a dark environment (oven drying), and any conversion of DHAAHP into ART would be of the DHAAHP already present in the tissue (Geoff Brown, personal communication). Brown and Sy (*30*) stated that there might be an increased rate of autoxidation of DHAA during the hours of daylight, consistent with the higher sunlight intensity, as compared to shade drying (**Figure 6**), leading to a more efficient conversion of DHAA into ART (**Table 1**). The sundried plant extracts were visually devoid of chlorophyll, indicating that sunlight induced a higher photosynthetic activity in desiccating

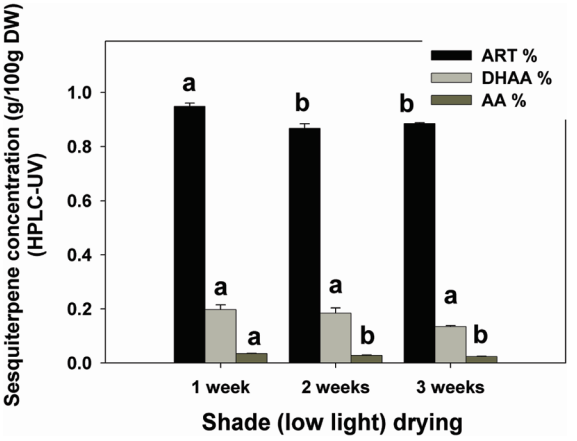


Figure 4. Effects of shade drying over 3 weeks, at low light (n = 3), on ART, DHAA, and AA of a cloned field-grown (2006) *A. annua* cultivar. Treatments with the same letters (across weeks) do not differ statistically at $p < 0.05$.

plants than dark (oven drying) or indirect (ambient and low) light. This is consistent with the hypothesis that DHAA photooxidizes into DHAAHP, and this, in turn, oxidizes into ART as a protection mechanism against reactive oxygen species (ROS) triggered by stress (*32*). The buildup of ROS in the chloroplasts and peroxysomes is a response to several abiotic stresses including high light (*33*). Although our data report changes in DHAA, AA, and ART after 1 week, Brown and Sy (*30*) demonstrated that DHAA decreased 80% in desiccating plants in 3.3 days, and that is probably the longest that plants need to be exposed to sun or shade drying to achieve a significant increase in ART before oven drying and commercial extraction.

Regarding the lower ART concentration for a fast oven drying (45 °C) as compared to both slower shade and sun drying for three consecutive years (**Figure 3**), it could arguably be a dilution effect caused by longer photosynthetic activity and solids degradation caused by the slower shade and sun drying. Although possible, that assumption would not explain the simultaneous increase in ART and decrease of DHAA and AA (**Table 1** and **Figure 2**). Although drying *A. annua* for 7, 14, and 21 days in the dark showed a trend to increase ART (*17*), our results showed no trend for the concentration of ART, DHAA, and AA after 1−3 weeks of shade drying (**Figure 4**). However, our results agree with those (*17*) that indicated that shade and sun drying significantly increased ART as compared to oven drying. Ferreira and collaborators (*16*) reported that ART from shade-dried detached leaves was 30% higher than in oven-dried (40 °C) leaves. Our first drying experiment in 2005 also resulted in a significant increase (55%) in ART from whole plants dried in the shade (ambient light) and a 15% increase for sun-dried plants as compared to whole plants oven-dried at 45 °C (data not shown). However, sun-dried plants in 2005 were only exposed to outdoor light for

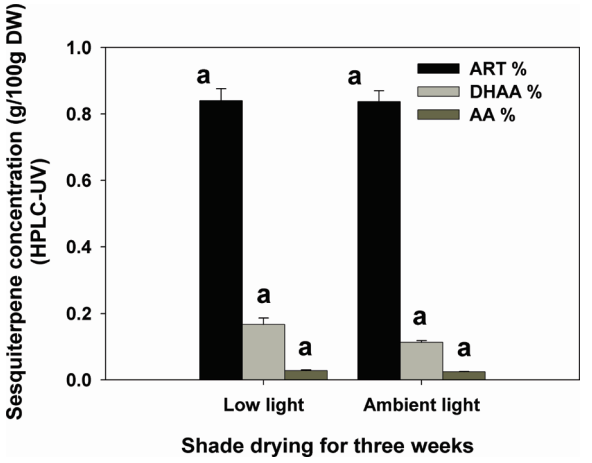
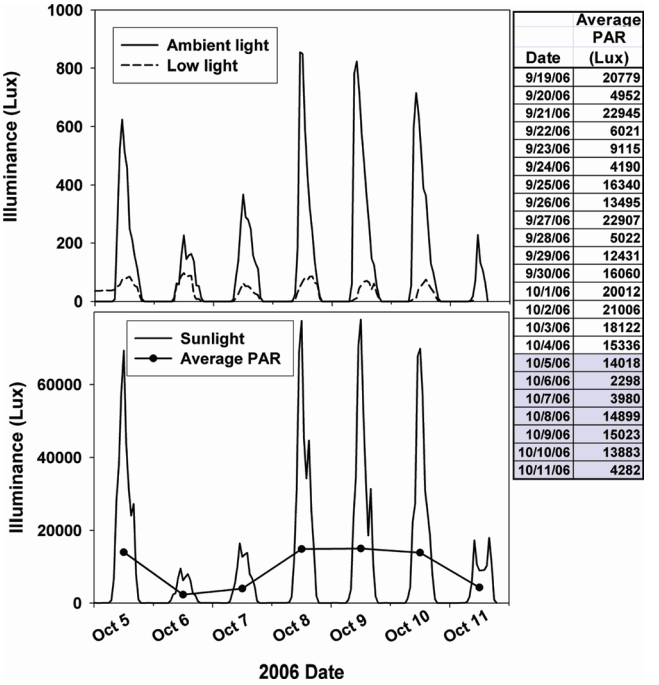


Figure 5. Effect of low light vs ambient light for 3 weeks of shade drying on ART, DHAA, and AA concentration (based on DW) of a field-grown (2006) cloned *A. annua* cultivar. Treatments with the same letters (across treatments) do not differ statistically at $p < 0.05$.



Date	Average PAR (Lux)
9/19/06	20779
9/20/06	4952
9/21/06	22945
9/22/06	6021
9/23/06	9115
9/24/06	4190
9/25/06	16340
9/26/06	13495
9/27/06	22907
9/28/06	5022
9/29/06	12431
9/30/06	16060
10/1/06	20012
10/2/06	21006
10/3/06	18122
10/4/06	15336
10/5/06	14018
10/6/06	2298
10/7/06	3980
10/8/06	14899
10/9/06	15023
10/10/06	13883
10/11/06	4282

Figure 6. Hourly light intensity in a roofed shelter (ambient light), in a roofed/walled building (low light), and in the open (sunlight). Light meters were placed on the outer edge of canopies of plants dried under low and ambient light for 3 weeks, while sunlight was from an on-site weather station. The average sunlight PAR is in the side table with darkened dates representing the last week of data collection. Low peaks correspond to cloudy days, which did not affect low light intensity (dotted line). Illuminance peaked from 11:00 a.m. to 12:00 p.m. on sunny days.

8 h/day (working hours) and were indoors overnight. In 2006 and 2007, the same clone cultivated in the same field was exposed to external light throughout the day, remaining outdoors for the duration of the drying treatment (1−3 weeks). Although the ART increase varied somewhat from year to year, shade- and sun-dried plants were consistently higher in ART than oven-dried plants (**Figures 2** and **3**). Our results also agreed with those (*17*) who reported that AA did not change between oven-, shade-, and sun-dried plants (**Figure 2C**). However, both DHAA and AA decreased significantly between the freeze-dried subsamples and the pertinent plants submitted to all conventional drying treatments, indicating that both DHAA and AA were bioconverted in drying plants (**Figure 2**).

Although plants were cloned, differences in freeze-dried subsamples for sesquiterpene lactone concentration (%RSD ≤ 15%) among the nine freeze-dried subsamples ($n = 3$/treatment) occurred (**Figure 2**). These changes are attributed to plant-to-plant and analytical variations. Because DHAA metabolizes to 16 compounds, including ART (*30*), we only evaluated treatment differences that resulted in significant increases in leaf ART concentration. Differences within treatments between both DHAA and AA versus ART were discussed according to their relative contributions to the increase in ART concentration in leaves, based on data from **Table 1**.

Although there was no difference in the ART concentration between whole plants shade-dried for 3 weeks at low light versus ambient light (**Figure 5**), their ART contents (0.85 g/100 g) were lower than those of sun-dried plants (1.08 g/100 g) in 2006 (**Figure 2A**). The light intensities of the three environments are shown in **Figure 6**. Glandular trichomes are accepted as the site of ART production and sequestration in *A. annua* (*34*−*36*). The existence of functional chloroplasts in *A. annua* glandular trichomes (*36*) indicates that these chloroplasts could be involved in ART production. Adding this information to reports that ART increases in sun-dried plants (*18*) and to our own results indicates that light intensity during drying could affect ART concentration if its biosynthesis is directly linked to trichome chloroplasts. Although there was a large difference in light intensity between the low light and the ambient light environment (**Figure 6**), that difference, when applied postharvest, did not affect the concentration of ART or AA, and the decrease in DHAA in plants shade-dried under ambient light did not affect the final ART concentration (**Figure 5**). Brown and Sy (*30*) reported that radiolabeled DHAA bioconverted into 16 metabolites during plant desiccation, the most abundant ones being compounds **5**, **7**, and **14**, while labeled ART and compound **21** were found in smaller amounts than the other 14 compounds. Those authors postulated that the conversion of DHAA into the 14 main compounds is favored by a hydrophilic environment such as the cytoplasm, but the conversion of DHAA into ART would be favored in a predominantly lipophilic environment such as the glandular trichomes but not reached by the radiolabeled DHAA fed through the stems or roots of the plants. This lipophilic environment of the glandular trichomes and the presence of active chloroplasts could be the ideal environment for the chemical conversion of pre-existing DHAA into ART, catalyzed by sunlight, accounting for the more efficient conversion of DHAA into ART in sundried plants (**Table 1** and **Figure 2**).

Effect of Drying and Light on Antioxidant Activity of *A. annua* Leaves. In 2006, a subsample of each plant was frozen in liquid nitrogen immediately before harvesting, freeze-dried, and compared to oven, shade, and sun drying for antioxidant activity (FRAP). There was no significant difference among all three sets of freeze-dried samples from nine ($n = 3$/treatment) cloned plants (**Figure 7**). Small differences (%RSD < 5%) among the three sets of freeze-dried subsamples may be attributed to plant-to-plant and analytical variation. Oven-dried samples had similar antioxidant capacities to freeze-dried subsamples. These results contrast with those reported for the sea buckthorn leaves, where reduction in phenolics was observed in the leaves dried at temperatures between 50 and 100 °C (*37*). This may be attributed to either lower drying temperature (45 °C) used in the present study, due to greater stability of phenolics extracted from *A. annua* leaves or due to thermal degradation of complex polyphenols to simpler, more active polyphenols. Also, in a

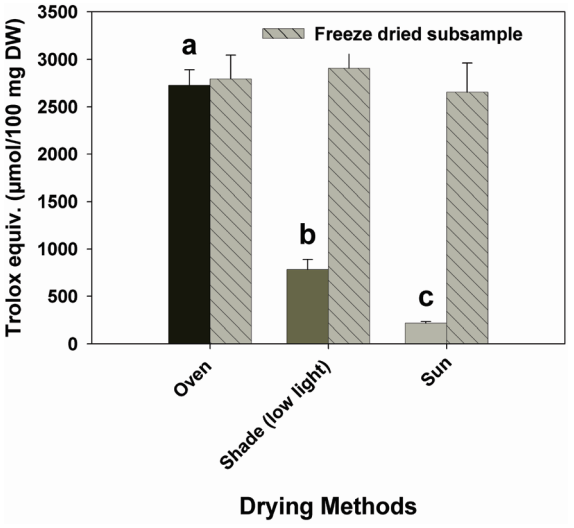


Figure 7. Effect of drying on the antioxidant capacity (based on DW) of *A. annua* leaves as compared to freeze-dried subsamples of plants submitted to each drying method. The cultivar was cloned and field-grown in 2006. Shade (at low light) and sun drying were applied for 3 weeks. Treatments with the different letters, across drying procedures, were statistically different at $p < 0.05$.

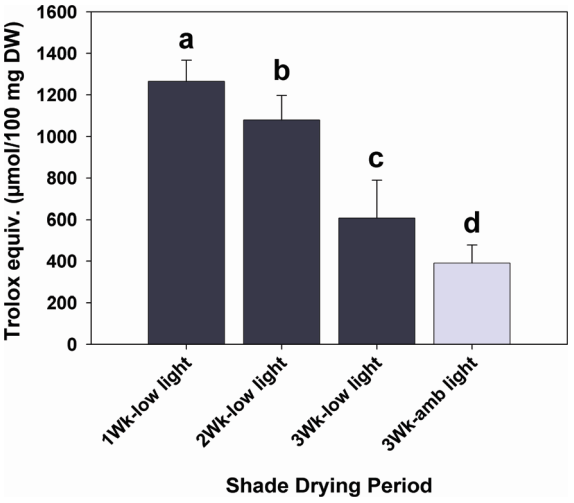


Figure 8. Effect of shade drying, at low light, as compared over time and as compared to ambient light (at 3 weeks) on the antioxidant capacity of *A. annua* leaves (based on DW). The cultivar was cloned and field-grown in 2006. Treatments with different letters were statistically different at $p < 0.05$.

forced-air oven at 45 °C, enzyme inactivation and/or degradation will occur faster than with shade and sun drying, and the samples are protected from light in the oven as opposed to continuous direct or indirect light exposure during drying from 1 to 3 weeks. A statistically significant difference in antioxidant activity occurred when samples were dried for 3 weeks under either shade or sun (**Figure 7**). The shade-dried and sun-dried samples showed 73.0 and over 90.0% reduction in antioxidant activity, respectively, as compared to freeze-dried samples. These results suggest that antioxidants extracted from *A. annua* are light sensitive, even when plants are exposed to indirect light under shade drying.

To further explore the influence of environmental light on the reduction in antioxidant activity, whole cloned plants were dried for 3 weeks under shade with low and ambient light. The results (**Figure 8**) indicated that drying under shade for a period of 1 week under low light intensity reduced the antioxidant activity by 54.6% as compared to previous freeze-dried samples (**Figure 7**). Gradual reduction of antioxidant activity was observed when drying was extended to 2 and 3 weeks at low light intensity. The antioxidant activity was reduced by 78.2−86.0% when plants were dried for 3 weeks at low and ambient light intensity, respectively (**Figure 8**). Although ambient daily temperatures averaged from 8.0 to 17.5 °C during the drying period, the RH for the period varied between 35 and 98.5%, with the maximum RH being always higher than 94% (**Table 2**). Although we believe that temperature did not play a role on decreasing the *A. annua* antioxidant activity, the RH might have kept some of the tissue oxidative enzymes active during shade and sun drying, while these enzymes might have been quickly deactivated by oven drying and freeze drying. A similar marginal reduction in olive oil phenols from 323 to 314 mg (gallic acid/kg of oil) was observed when olive oil was exposed to light, as compared to dark-stored oil (*38*).

Other than a high antioxidant activity, *A. annua* is a rich source of flavonoids that have the potential to increase the plant medicinal value. Flavonoids have antimalarial, antileishmanial, antitrypanosomal, and antiamoebial activities (*39*). The flavonoid quercetin (widely occurring in plants, including *A. annua*) was reported to increase the bioavailability of the anthelmintic drug moxidectin in sheep by suppressing the activity of both *p*-glycoproteins and enzymes of the cytochrome (CY) P450 family, responsible for the accelerated metabolism and degradation of moxidectin and other drugs (*11*). These inhibitory activities of quercetin might also be found with other related flavonoids present in *A. annua* such as quercetagenin, which might account for the antimalarial activity of *A. annua* tea, even when tea ART was present at a concentration equivalent to only 19.0% of the recommended antimalarial dose for ART monotherapy (*12*). Although this indicates the value of combining flavonoids and ART for the temporary relief of malaria, monotherapy with either ART or the tea alone is not recommended as a substitute for the ACT, currently supported by the World Health Organization. However, the antioxidant, antiprotozoal, and suppressive effects of flavonoids on CY P450 enzymes and *p*-glycoproteins indicate that they can be synergistically combined with currently used antiparasitic drugs, instead of in substitution to those drugs, to extend their life span, to increase their biovailability, and to delay the development of drug resistance by parasites and microorganisms.

In conclusion, our results support those of other researchers who indicated that predrying of harvested *A. annua* plants under both shade and sun leads to a significant increase in ART and that only a week (or less) of sun/shade drying is enough for a significant increase in ART. This could mean additional financial benefits to crop farmers who would receive bonus pay for the additional ART content of their crops. Because shade and sun drying, when followed by oven drying at 45 °C for up to 16 h, showed no decrease in ART associated with the oven temperature, oven drying at mild temperatures can be applied to the plant material, after sun or shade drying, with added economic benefits as compared to the direct oven drying of freshly harvested plants. The choice between shade drying and sun drying will rely on local environmental conditions at the time of harvest and on the final product intended as well as on the cost−benefit ratio of predrying plants (sun or shade) before commercial oven drying. If only ART production is the goal, either sun or shade drying for 3 days might suffice. However, if the final product is destined to be used as a traditional Chinese medicine (herbal teas or crude alcoholic extracts), oven drying is recommended over sun or shade drying to maintain the leaf antioxidant capacity. In the lack of oven drying, shade drying at low light (indoors) is preferred over sun drying, which reduced the antioxidant activity over 10-fold as

Table 2. Temperature, RH under Sun and Shade Conditions, and Total Daily Precipitation (pptn.) for the 3 Weeks That Plants Were Dried under Sun and Shade under Environmental Conditions in Beaver, West Virginia, in 2006[a]

2006 date	min (°C)	max (°C)	min RH sun (%)	max RH sun (%)	min RH shade (%)	max RH shade (%)	total pptn. (mm)
9/19	12.6	21.6	42.2	98.9	41.5	97.1	0.3
9/20	4.2	12.7	67.3	98.3	69.3	95.8	0.0
9/21	3.1	20.1	42.4	99.5	41.4	96.6	0.0
9/22	10.5	16.1	74.1	98.8	73.7	97.5	0.9
9/23	15.8	24.3	68.4	99.8	67.5	98.5	1.5
9/24	13.4	19.4	84.0	99.4	83.3	97.1	0.4
9/25	8.3	18.9	45.9	98.0	46.1	94.7	0.0
9/26	8.0	19.2	49.0	99.8	49.7	97.8	0.0
9/27	6.0	22.3	42.2	100.0	42.4	97.0	0.0
9/28	7.6	17.3	70.6	99.7	69.8	97.9	2.0
9/29	3.9	13.4	46.5	99.4	45.8	97.8	0.5
9/30	4.7	19.7	49.3	99.3	47.8	97.7	0.8
10/1	9.0	20.2	48.5	100.0	46.9	98.5	0.0
10/2	7.6	26.1	32.7	100.0	35.2	97.6	0.0
10/3	9.4	25.1	53.1	97.5	50.7	93.9	0.0
10/4	13.6	25.1	60.1	98.8	57.2	96.1	0.0
10/5	12.9	23.0	65.8	100.0	64.0	98.3	2.0
10/6	7.1	13.1	98.9	100.0	96.9	98.5	0.5
10/7	5.4	11.0	92.0	100.0	93.9	98.4	0.3
10/8	10.7	21.6	65.2	100.0	63.9	98.3	0.0
10/9	9.1	23.7	55.8	100.0	53.2	97.9	0.0
10/10	9.8	24.1	47.9	100.0	43.2	98.2	0.0
10/11	12.9	17.4	77.2	99.6	74.9	97.4	1.3

[a] Minimal (min) and maximum (max) temperatures were from a weather station exposed to 100% sun (thermometers were protected from solar radiation). Temperatures recorded by a shaded weather station were within 0.1−0.5 °C and within 0.1−1.5 °C of sun min and max and are not shown in this table.

compared to oven and freeze drying. Our results should allow producers to choose a postharvest drying method to fit their production goals, to optimize recoveries of ART while reducing energy costs related to direct commercial oven drying, or to achieve quasi-optimal ART production (oven drying only), while maintaining optimal antioxidant capacity for human or animal consumption. Finally, the production of ART from *A. annua* would certainly benefit from research investments on the preharvest conversion of DHAA into ART by stress (e.g., drought during the harvest week) and on the postharvest chemical conversion of DHAA into ART.

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The mention of brand names is solely for the convenience of the reader and does not mean that the authors, or the U.S. Department of Agriculture, endorse those products over other similar ones. We have no conflict of interest, financially or otherwise.

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