Post-harvest Drying Treatment Effects on Amtimalarial Constituents of *Artemiasia annua* L.

J.C. Laughlin
Agricultural Consultant - Medicinal Crops
1/14A Sherburd Street, Kingston
Tasmania 7050, Australia

Keywords: qinghao, annual wormwood, malaria, artemisinin (qinghaosu), artemisinic (qinghao) acid, essential oil, sun drying, shade drying, dark drying.

Abstract

Two field experiments were carried out in cool temperate maritime latitudes in NW Tasmania (41ºS) to assess whether wilting and drying *Artemisia annua* plants in the field after harvest had any detrimental effects on artemisinin (the source of important antimalarial drugs) or its precursor artemisinic acid. A third field experiment studied the effect of steam distillation of *A. annua* for its essential oil, prior to oven drying, on artemisinin and artemisinic acid. In the first two experiments whole plants were cut off at the base and left in situ for 1, 3 and 7 days (Experiment 1) and for 7, 14 and 21 days (Experiment 2). Experiment 2 included two additional treatments: (i) shade drying whole plants under ambient conditions in the field for 21 days and (ii) drying leaves, detached at harvest, for 21 days under ambient conditions inside in the dark. The effects of all of these treatments were compared with oven drying (35ºC) leaves which had been detached immediately after harvest.

Field drying for 1, 3 or 7 days had no adverse effect on either artemisinin or artemisinic acid in Experiment 1 and all leaf concentrations were similar to oven drying. Field drying for 7 days in Experiment 2 also gave artemisinin and artemisinic acid levels similar to oven drying. However there was a trend for sun-, shade- and dark drying for 21 days to give higher artemisinin than oven drying although artemisinic acid was unaffected. Distillation of *A. annua* plants for oil extraction, prior to oven drying at 35ºC, resulted in nil to negligible leaf concentration of artemisinin but artemisinic acid was unaffected. Field drying may be a way of reducing the cost of antimalarial drugs and the dual production of oil and artemisinic acid is a possibility.

INTRODUCTION

Annual or sweet wormwood *(Artemisia annua* L., family *Asteraceae)* has been used in traditional Chinese medicine (where it is called *qinghao)* for centuries as a herbal treatment for malaria and other fevers (Ferreira et al., 1997). Artemisinin (*qinghaosu)*- the specific compound in *A. annua* with marked antimalarial activity against the single celled parasitic, protozoan *Plasmodium* species: *falciparum vivax, malariae* and *ovale* - was first isolated and structurally defined in China in 1972 (Klayman, 1985). Artemisinin is a rare sesquiterpene lactone endoperoxide of the cadinane series and with the possible exception of *Artemisia apeacea* L. (Liersch et al., 1986) it has only been detected in *A. annua*. Malaria is a devastating disease in tropical, developing countries with up to 2.7 million deaths annually (Miller, 1992). The World Health Organization (WHO) has concluded that antimalarial drugs derived from artemisinin have a vital key role in all countries where the disease is an endemic problem (WHO, 1998).

Oven drying plant material in large scale gas or oil fired kilns is an expensive operation but the less expensive alternative of field drying in the sun is reputed to severely reduce artemisinin (A. Singh, personal communication). In USA shade drying of *A. annua* under ambient conditions inside or outside gave higher artemisinin than sun drying (Charles et al, 1993) and shade drying inside gave 30 % greater artemisinin than oven drying at 40 ºC (Ferreira et al., 1992). Other than these studies very little work has

been published on post-harvest drying effects on the artemisinin content of *A. annua* and none on the effect of drying method on artemisinic acid (Laughlin et al., 2001). This paper will describe the comparative effects of: (i) sun and shade drying of whole plants in the field with oven and dark drying leaves immediately after harvest and (ii) oil distillation of plants prior to oven drying on artemisinin and artemisinic acid concentration in *A. annua.*

MATERIALS AND METHOD

Field and Oven Drying Comparisons (Experiment 1)

An initial explanatory field trial was carried out in 1990 at Forthside Research Station in N W Tasmania (Lat. 41º12'S. Elevation 150m.) This experiment on krasnozem soil was set out in randomized blocks with four replications in plots of 35 m^2 with six rows of 30 cm spacing. Weeds were controlled by hand and the plots were irrigated throughout the growing season. Plants of Chinese selection were cut off at the base at the late vegetative stage (early March, 1990) and left in situ in the field for 1, 3 and 7 days. Leaves from a random sample of twelve plants were removed from all branches, oven dried for 24 hours at 35ºC, ground to pass a 0.1 mm sieve and assayed for artemisinin and artemisinic by an HPLC method. These assays were compared with assays of leaves which had been removed from plants immediately after harvest and oven dried in a fan-forced oven for 24 hours at 35ºC.

Field, Shade, Dark and Oven Drying Comparisons (Experiment 2)

A field experiment was carried out at Wesley Vale in N W Tasmania (Lat. 41º10'S. Elevation 100m). Seedlings of a Chinese selection of *A. annua* were transplanted in spring (October 1994) into plots of 35 m^2 with six rows of 30 cm spacing. The plots were set out in randomized blocks with four replications, weeds were controlled by hand and the plots were irrigated throughout the growing season. At harvest (early March 1995) at the late vegetative stage whole plants were cut off at the base to compare four drying methods: (i) field drying in situ for 7, 14 and 21 days, (ii) shade drying outside under ambient conditions for 21 days, (iii) leaves detached at harvest and dried in the dark inside under ambient conditions for 21 days, (iv) leaves detached immediately after harvest and oven dried for 24 hours at 35ºC. After the completion of treatments (i), (ii) and (iii) their leaves were also oven dried for 24 hours at 35ºC and all treatments were ground to pass a 0.1 mm sieve and assayed for artemisinin and artemisinic acid by an HPLC method.

Steam Distillation and Oven Drying (Experiment 3)

The same Chinese selection of *A. annua* used in Experiments 1 and 2 together with selections from Yugoslavia and USA were used to assess the effect of steam distillation for their essential oils, prior to oven drying, on artemisinin and artemisinic acid in leaves. Single unreplicated plots of the three selections were transplanted in spring (October) 1991 into plots of 27 m^2 with six rows at 30 cm spacing. Weeds were controlled by hand and the trial was irrigated throughout the growing season. A random sample of twelve plants was harvested at the late vegetative stage (February 1992) by cutting the top three quarters and a random sub-sample of twelve kilograms was machine cut into small (1 cm) chips. Two kilograms of the sub-sample were dried immediately in a fan-forced oven for 24 hours at 35 ºC and the leaves separated by sieving. Ten kilograms of the sub-sample were steam-distilled for one hour in a scaled-down industrial type still. The oil was collected and a random 2 kg sample of the distilled plant material was oven dried in a fan-forced oven for 24 hours at 35 ºC and the leaves were separated by sieving. The leaves from both pre- and post-distilled samples were ground to pass a 1.0 mm sieve and assayed by an HPLC method for artemisinin and artemisinic acid. The oils from the three selections were also assayed for artemisinin and artemisinic acid.

RESULTS

Experiment 1

There were no differences in either artemisinin or artemisinic acid concentration between leaves of *A. annua* which had been oven dried immediately after harvest and those from whole plants which had been left in the field for 1, 3 or 7 days (Table 2).

Experiment 2

There were no differences in the artemisinin concentration of leaves which had been oven dried immediately after harvest and those from whole plants left to dry in the field for 7 days (Table 3). However there was a trend for leaves from whole plants which had been either left in the field for 21 days, shade dried outside for 21 days, or dried in the dark inside for 21 days to have higher artemisinin concentration than leaves oven dried immediately after harvest (Table 3). There were no differences in artemisinic acid content of leaves from any of the four drying treatments (Table 3).

Experiment 3

The artemisinin concentration of leaves from the Chinese selection of *A. annua* used in Experiments 1 and 2 and distilled prior to oven drying was negligible (0.1%) and the concentrations was about one tenth of that of the leaves which had only been oven dried (Table 4). The artemisinin in the American selection was similar to the Chinese selection and nil in the Yugoslavian selection. The artemisinic acid concentration in the leaves of all three seed selections appeared to be unaffected by steam distillation (Table 4). Assay of the essential oils detected no artemisinin in any of the three selections and only small traces (0.001-0.004 %) of artemisinic acid.

DISCUSSION

The most important conclusion from both Experiments 1 and 2 is that, under Tasmanian conditions (Table 1), harvesting whole plants by cutting them off at the base and leaving them to wilt in the field for 7 days had no detrimental effect on artemisinin concentration. The trend in Experiment 2 for the artemisinin content of leaves from whole plants which had been dried in situ, in either the sun or shade for 21 days, to be higher than that in leaves which had been detached and dried immediately after harvest and oven dried was quite unexpected. This experiment needs to be repeated to confirm the consistency of this pattern. Periods of drying as long as 7, 14 or 21 days may be unnecessary in intertropical regions which have high temperature conditions. Currently small scale commercial crops of *A. annua* in the highlands of Tanzania are left in the field for 2-3 days prior to a further period of shade drying where necessary (A. Ellman, personal communication). The main reason for the inclusion of 14 and 21 day drying periods was to assess the stability of artemisinin and artemisinic acid. After 14 days the leaves of *A. annua* turned quite brown but with no detrimental effect on either of the antimalarial constituents. However in areas of high tropical humidity extended field drying may precipitate fungal contamination of leaves and may possibly deplete artemisinin (P.M. Magalhaes, personal communication).

The results of the Tasmanian post-harvest drying studies differ from those of Charles et al. (1993). However detached branches were used by Charles et al. in contrast to whole plants, their field temperatures were 5-8 ºC higher and drying was only continued for 48 hours. It may well be useful to reassess the role of field drying in some intertropical regions where *A. annua* is currently grown, particularly with the newer high artemisinin selections which have recently been developed (Laughlin et al., 2001).

The artemisinic acid content of the *A. annua* plant selection used in this Tasmanian study was very stable and resistant to any of the drying methods or times used in Experiments 1 or 2. The contrast between artemisinin and artemisinic acid was particularly evident when *A. annua* plants were steam-distilled for essential oil extraction prior to oven drying. Distillation virtually destroyed artemisinin while artemisinic acid

was unaffected (Table 4). Artemisinic acid has been shown to be an effective precursor source of artemisinin (Vonwiller et al., 1993) and hence a dual purpose use of *A. annua* for oil and artemisinin would be a commercial possibility if the economics were appropriate. Another incentive to maintain an interest in artemisinic acid is that, in the search to develop a new range of antimalarial drugs from *A. annua*, artemisinic acid may often be a more appropriate source than artemisinin (Ziffer et al., 1997). However the high artemisinin (ca., 1.0 %) strains of *A. annua* which have been developed in Vietnam (Woerdenbag et al., 1993) or specially bred and selected for intertropical regions (Magalhaes et al., 1996) are typically very low in artemisinic acid (ca., 0.1-0.2 %). It may be appropriate to maintain selections of *A. annua* which are reasonably high in both artemisinin and artemisinic acid to cater for a range of antimalarial drugs which could still be developed from *A. annua*.

Literature Cited

Charles, D.J., Simon, J.E., Shock, C.C., Feibert, E.B. and Smith, R.M. 1993. Effect of water-stress and post-harvest handling on artemisinin content in the leaves of *Artemisia annua* L. In: J. Janick and J.E. Simon (eds.). New Crops. John Wiley, New York 640-642.

Ferreira, J.F., Charles, D., Simon, J.E. and Janick, J. 1992. Effect of drying methods on the recovery and yield of artemisinin from *Artemisia annua* L. Hort. Science (Abstr. 565) 27:63.

Ferreira, J.F., Simon, J.E. and Janick, J. 1997. *Artemisia annua*: Botany, Horticulture, Pharmacology. In: J. Janick (ed.) Horticultural Reviews (Vol. 19). John Wiley, New York 319-371.

Klayman, D.L., 1985. *Qinghaosu* (Artemisinin): An antimalarial drug from China. Science 228:1049-1055.

Laughlin, J.C. 1993. Effect of agronomic practices on plant yield and antimalarial constituents of *Artemisia annua* L. Acta Hort. 331:54-61.

Laughlin, J.C., Heazlewood, G.N. and Beattie, B.M. 2001. Cultivation of *Artemisia annua* L. In: C.W. Wright (ed.) *Artemisia.* Taylor & Francis, London. 159-195.

Liersch, R.H., Soicke, C., Stehr, C. and Tullner, H.V. 1986. Formation of artemisinin in *Artemisia annua* during one vegetation period. Planta Med. 52:387-390.

Magalhaes, P.M. and Delabays, N. 1996. The selection of *Artemisia annua* L. for cultivation in intertropical regions. In F. Pank (ed.), Proceedings of an International Symposium on Breeding Research on Medicinal and Aromatic Plants. Quedlinburg, Germany. 185-188.

Miller, L.H. 1992. The challenge of malaria. Science 257:36.

Vonwiller, S.C., Haynes, R.K., King, S. and Wang, H. 1993. An improved method for the isolation of *qinghao* (artemisinic) acid from *Artemisia annua.* Planta Med. 59:562-563.

WHO (World Health Organisation) 1998. The use of artemisinin and its derivatives as anti-malarial drugs. Report, Malaria Unit, Division of Control of Tropical Diseases World Health Organisation, Geneva, Switzerland

Woerdenbag, H.J., Bos, R., Salomons, M.C., Hendriks, H., Pras, N. and Malingre, Th. M. 1993. Volatile constituents of *Artemisia annua* L. (*Asteraceae*). Flav. and Frag. J. 8:131-137.

Ziffer, H., Highet, R.J. and Klayman, D.L. 1997. Artemisinin: An endoperoxidic antimalarial from *Artemisia annua.* In: W. Herz, G,W, Kirby, R.E. Moore, W. Steglich and Ch. Tamm (eds.). Progress in the Chemistry of Organic Natural Products, Springer, Wien. 72:123-214.

Tables

Table 1A. Mean meteorological data at Forthside Research Station during Experiment 1.

Drying Time (days)	Rain (mm)	Temperature (C°) max	mean	min	Bright Sun (h./day)	Wind (km/day)
1	0	21	18	15	6	107
3	0	21	18	14	6	116
7	6	21	17	12	7	135

Table 1B. Mean meteorological data at Wesley Vale during Experiment 2.

Drying Time (days)	Rain (mm)	Temperature (C°) max	mean	min	Bright Sun (h./day)	Wind (km/day)
7	0	22	18	14	8	95
14	14	21	17	13	7	134
21	15	20	15	11	7	149

Table 2. Effect of method and time of drying on the artemisin and artemisinic acid content in the leaves of *Artemisia annua* (% of dry matter).

Drying method	Artemisinin (%)	Artemisinic acid (%)
Oven	0.12	1.01
Field drying		
1 Day	0.11	1.00
3 Days	0.11	1.12
7 Days	0.13	1.08
LSD (P<0.05)	NS	NS

Table 3. Effect of method and time of drying on the artemisinin and artemisinic acid content in the leaves of *Artemisia annua* (% of dry matter).

Drying method	Artemisinin (%)	Artemisinic acid (%)
Oven	0.045	0.85
Field drying		
7 Days	0.033	0.90
14 Days	0.053	0.88
21 Days	0.087	0.78
Shade 21 days	0.078	0.75
Dark 21 days	0.070	0.76
LSD (P<0.05)	0.023	NS

Table 4. The effect of steam distillation and subsequent oven drying on artemisinin and artemisinic acid content in the leaves of *Artemisia annua* (% of dry matter).

Origin of seed	Distilled (%)			Non-distilled (%)	
	Oil	Artemisinin	Artemisinic acid	Artemisinin	Artemisinic acid
Chinese	0.42	0.01	1.04	0.11	0.84
Yugoslv*	0.76	0.00	0.90	0.06	0.85
USA	0.70	0.01	0.98	0.06	0.98

* Yugoslv = Yugoslavian