The Content of Artemisinin in the *Artemisia annua* Tea Infusion

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Abstract

The traditional use of the medicinal plant *Artemisia annua* for the treatment of malaria entails the preparation of a tea infusion. In the scientific literature there have been some discrepancies on the quantity of the active principle, artemisinin, in the tea infusion. Due to these discrepancies, we decided to quantify artemisinin in tea infusions prepared according to different methods. We also studied the water solubility of pure artemisinin at room temperature and at 100 °C and compared it to the solubility of artemisinin from the plant material. We found that the extraction efficiency is very sensitive to temperature and that efficiencies of above 90% can be reached. We also showed that the solubility of artemisinin is not improved by other components in the extract but that a supersaturated solution of artemisinin might be formed, which is stable for at least 24 hours.

Key words
Artemisia annua L. · Asteraceae · artemisinin · supersaturation · tea infusion

Supporting information available online at
http://www.thieme-connect.de/ejournals/toc/plantamedica

Table 1 The [ART] obtained by different preparation methods. Exp. 1: All the parameters were kept constant while only the temperature was varied. Exp. 2: The temperature was increased to above boiling point (b. p.). Exp. 3: The material: solvent ratio varied. All the results are expressed as [ART] in mg/L and percentage extraction efficiency.

Sample	Temperature (°C)	Contact time (min)	Material: solvent ratio (g/L)	[ART] mg/L	Extraction efficiency (%)
Experiment 1					
1*	RT	10	9	14.4 ± 3.6	15.8
2	40	10	9	3.7 ± 0.6	4.0
3	50	10	9	3.6 ± 0.4	3.9
4	60	10	9	4.1 ± 0.5	4.5
5	70	10	9	3.6 ± 0.8	3.9
6	80	10	9	8.4 ± 1.7	9.2
7	90	10	9	12.9 ± 5.6	14.1
8	100	10	9	23.9 ± 5.4	26.1
9	100	10 at b. p.	9	71.7 ± 0.1	78.4
10	100	5 at b. p.	9	74.9 ± 2.2	81.9
11	100	2 at b. p.	9	75.1 ± 0.5	82.0
12	100	1 at b. p.	9	47.3 ± 6.8	51.7
Experiment 2					
13	100	5 at b. p.	9	85.1 ± 6.9	92.7
14	115	1 at 115	9	71.8 ± 3.6	78.2
15	115	5 at 115	9	60.5 ± 2.4	65.9
16	115	10 at 115	9	39.4 ± 3.0	42.9
Experiment 3					
17	100	5	20	113.8 ± 2.3	62.2
18	100	5	40	174.8 ± 10.3	43.0
19	100	5	50	111.2 ± 5.8	21.9

* Sample 1 was prepared by crushing the plant material with a mortar and pestle at room temperature

The medicinal plant *Artemisia annua* L. (Asteraceae), which has been used in traditional Chinese medicine to treat fevers, contains the antiplasmodial compound artemisinin (ART). Derivatives of ART, in combination with other classes of antimalarials, are now being prescribed by the WHO as a first-line treatment for uncomplicated malaria. The main traditional medicinal use of the plant has been to prepare a tea infusion [1]. Here we focus on the quantification of ART in the tea infusion which has been a point of discussion for some time. Following similar preparation methods, concentrations of artemisinin – [ART] – of 24.5 mg/L [3], 94.5 mg/L [4], 94.0 mg/L [5], and 24.2 mg/L [6], respectively, were reported. These reports clearly show a large variation in [ART], and although only very few studies have been conducted, the traditional way of use still widely occurrs in Africa and Asia. These discrepancies reported in the scientific literature and also the renewed interest in this subject, not only for the treatment of malaria but also for certain types of cancer, were our main motivation for this study [2,7]. In this paper we focus on the following questions. What is the relationship between temperature and extraction efficiency of ART? (Can this explain the discrepancies found in scientific literature?) Does the aqueous solubility of ART increase due to the presence of other compounds when extracted from the plant material?

Table 1 gives the results for [ART] in mg/L and the extraction efficiency at different temperatures and with the use of different ratios of solvent: plant material. Crushing the plant material with mortar and pestle at room temperature (r. t.) gave a similar extraction efficiency as extracting at 90 °C. Although the contact time was only 10 min, it is clear that with an increase in temperature, the extraction efficiency of ART also increases. At the boiling point (b. p.), the concentration was found to be 23.9 ± 5.4 mg/L ([3] reported a concentration of 24.5 mg/L and [6] of 24.2 mg/L). If the water was kept at b. p. for as little as 2 min, the extraction efficiency reached around 90% (on separate occasions close to a 100% was even reached), giving us similar results as described in [4,5]. We also increased the temperature to 115 °C (autoclave) and kept it at this temperature for three different time points. From **Table 1** it is clear that ART breaks down at this temperature. Our third experiment was to test if it would be possible to produce an enriched tea preparation by increasing the ratio of plant material to water. The maximum concentration we found was 171 mg/L at a ratio of 40 g/L.

To investigate the question if other components in the tea infusion improve the solubility of ART, we compared the solubility of pure ART to the solubility of ART extracted from the plant material. The aqueous solubility of pure ART was found to be 51.8 ± 2.9 mg/L at room temperature. Extracting an equivalent amount of ART in the plant material (50 mg plant material containing 0.51 mg of ART [1.02% ART content]) with 10 mL of water should theoretically yield 51 mg/L. **Fig. 1** presents our results after extracting the plant material for 10 min, 1 h, and 12 h, with and without abrasive material (5 mg of kieselguhr silica crystals). We could not find any evidence that other components increase the solubility of ART but instead found that other components appear to decrease the solubility. This effect was clearly visible at r. t., at which ART could only be extracted at 30% from the plant material (around 15 mg/L) as compared to the pure standard

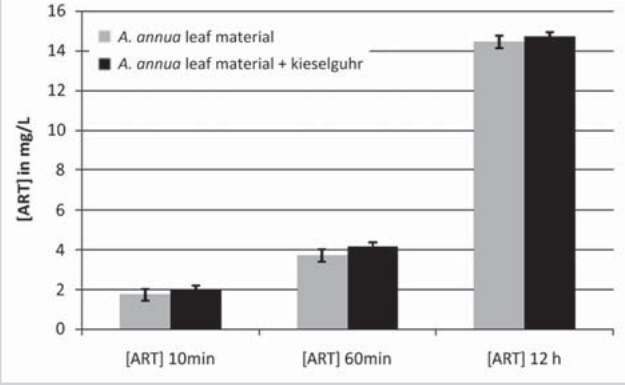

Fig. 1 The [ART] in mg/L measured after extraction of the leaf material for a specific time period, with and without an abrasive additive. *A. annua* leaf material (gray), *A. annua* leaf material + kieselguhr (black). The aqueous solubility of pure ART was determined to be around 50 mg/L after the same period of time.

(around 50 mg/L) under the same conditions. The addition of abrasive material to rupture the cellular components did not have any effect on the [ART]. The aqueous solubility of pure ART at b. p. was determined to be 216.5 ± 39.1 mg/L (using the same preparation method as for the tea infusion). We achieved the highest [ART] in sample 18 (**Table 1**), which contained 174.8 mg/L when 40 g/L of plant material were extracted. In this sample the total content of ART in the plant material can be calculated to be 408 mg (1.02 %). Theoretically, the water should have extracted at least 216.5 mg but only extracted 174.8 mg which translates into an extraction potential of 80.7 %.

To investigate the stability of ART in the tea infusion, separate tea samples were prepared and analyzed at three time points. The original content was found to be 85.1 ± 6.9 mg/L, decreasing slightly to 83.1 ± 6.4 and 82.4 ± 7.7 mg/L after 4 and 24 hours, respectively. The samples prepared to test the aqueous solubility of pure ART at 100 °C were also reanalyzed after 24 h (stored at r. t.) to determine if any other compounds in the tea infusion might play a role in stabilizing ART. We found that [ART] in the pure standard did not significantly decrease and that after 24 h the concentration remained remarkably stable. However, the remaining unfiltered samples (pure ART standard) which were left uncovered for 24 h did return to the expected ART aqueous solubility of 49.2 ± 4.7 mg/L. This finding indicates that a supersaturated solution of ART possibly forms in the tea infusion as well as in the pure ART standard. The transparent appearance of the pure ART sample will exclude the formation of a possible emulsion.

It can be concluded that the temperature and contact time is very closely related to the [ART] and it appears that other components in the extract do not aid in increasing the solubility of ART but instead decrease the solubility. The unexpected formation of a supersaturated solution possibly explains why a higher than expected [ART] can be achieved in the tea infusion. Finally, it can be noted that the *A. annua* tea infusion will be very difficult to standardize. Many factors including reaction temperature, contact time, ratio of material to solvent, chemotype of plant material, and even filter type will have an influence on the ART content of the tea infusion and on its subsequent pharmacokinetic properties.

Materials and Methods

Artemisia annua L. (Asteraceae) (anamed A-3) samples were obtained from Anamed and identified by Dr. Hans-Martin Hirt. The plants were grown near Mainz (Germany), harvested in September 2008 and consisted of dried leaf material. At the time of use the content of ART was determined according to [8] to be 1.02 %. The water used to prepare the tea infusions was Millipore deionized water. The tea samples were filtered with sterile 0.2 µm polyethersulfone syringe filters (VWR international) and HPLC – ELSD analysis were carried out according to [8]. Pure ART (> 98 % purity) was obtained from Sigma-Aldrich and was used to construct the standard curves and to study the aqueous solubility of ART. In the traditional preparation method, 1 L of boiling water is added to 5–9 g of dried leave material. During our experiments we used 90 mg in 10 mL of water. All samples were prepared in triplicate and were filtered before analysis. The plant material was however not squeezed to remove residual water. In the first experiment we tested the extraction efficiency of ART at different temperatures. Experiment 2 was carried out by increasing the temperature to above boiling point (autoclave), and experiment 3 tested different ratios of plant material to boiling water using the highest yielding method from experiment 1. We also investigated the solubility of pure ART at r. t. and b. p. and compared this to the solubility of ART from the plant material. This was done in order to investigate the hypothesis that other compounds in the extract improve the aqueous solubility of ART. A final set of samples were analyzed for [ART] at three time points to test the stability of ART in the tea infusion.

Supporting information

The detailed preparation methods for all of the tea infusions and analysis are available as Supporting Information.

Conflict of Interest

The authors declare no conflicts of interest.

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