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

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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.

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

* 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 occurs 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. If 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...
ties.

the tea infusion and on its subsequent pharmacokinetic proper-
dardize. Many factors including reaction temperature, contact
time, ratio of material to solvent, chemotype of plant material,
do not aid in increasing the solubility of ART but
in the extract do not aid in increasing the solubility of ART but
closely related to the [ART] and it appears that other components
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 re-
maining unfiltered samples (pure ART standard) which were left
uncovered for 24 h did return to the expected ART aqueous solu-
ibility of 49.2 ± 4.7 mg/L. This finding indicates that a supersatu-
rated solution of ART possibly forms in the tea infusion as well
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 re-
maining unfiltered samples (pure ART standard) which were left
uncovered for 24 h did return to the expected ART aqueous solu-
ability of 49.2 ± 4.7 mg/L. This finding indicates that a supersatu-
rated 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 emul-
sion.

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 ex-
pected [ART] can be achieved in the tea infusion. Finally, it can be
noted that the A. annua tea infusion will be very difficult to stan-
dardize. 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 proper-
ties.

Materials and Methods

Artemisia annua L. (Asteraceae) (anan A–3) samples were ob-
tained from Anamed and identified by Dr. Hans-Martin Hirt. The
plants were grown near Mainz (Germany), harvested in Septem-
ber 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 deion-
ized 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 con-
struct 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 investi-
gated 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 sam-
ple were analyzed for [ART] at three time points to test the sta-
ibility 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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