Comparative Study of Seventeen *Salvia* Plants: Aldose Reductase Inhibitory Activity of Water and MeOH Extracts and Liquid Chromatography–Mass Spectrometry (LC-MS) Analysis of Water Extracts

Rena Kasimu, Ken Tanaka, Yasuhiro Tezuka, Zhu-Nan Gong, Jian-Xin Li, Purusotam Basnet, Tsuneo Namba, and Shigetoshi Kadota

Research Institute for Yokohama (Traditional Sino-Japanese Medicines), Toyama Medical and Pharmaceutical University, Sagamihara 252-0034, Japan, National Research Institute of Police Science, 6 Sanban-cho, Chiyoda-ku, Tokyo 102, Japan, and Department of Medicinal Botany, China Pharmaceutical University, 24 Tong Jia Xiang, Nanjing 210009, China. Received November 4, 1997; accepted December 18, 1997

The dry root and rhizome of *Salvia miltiorrhiza* (Lamiaceae) are used as a crude drug Danshen, while those of *S. deserta* (Xinjiang-Danshen) are mixed in Danshen at Xinjiang province when the former is in short supply. The water and MeOH extracts of *S. deserta* showed strong aldose reductase (AR) inhibitory activity, and their active constituents were determined to be polar compounds different from "tanshinones" of *S. miltiorrhiza*, i.e., lithospermic acid B (1), salvinanolic acid K (2), salviaflavone (3), and rosmarinic acid (4) (IC\textsubscript{50} 2.63–3.91 μM).

We also examined the AR inhibitory activity of water and MeOH extracts of seventeen *Salvia* plants, including ten species of Danshen resources (*S. bowleyana, S. deserta, S. miltiorrhiza, S. miltiorrhiza var. alba, S. paramiltiorrhiza, S. paramiltiorrhiza f. purpureo-rubra, S. przewalskii, S. przewalskii var. mandarinorum, S. sinica f. purpurea, S. trijuga*), and their water extracts were also analyzed by liquid chromatography–mass spectrometry (LC-MS). The results indicated that there were four types with regard to the AR inhibitory activity and three types with regard to the amount of 1. Ten species used as Danshen resources showed good correlation between the AR inhibitory activity and the morphological classification. However, the intensities of their AR inhibitory activity varied, and they contained 1 in varying amounts. These facts suggested that the ten species were not the same, and thus their use as a Danshen resource should be based on their activity and/or active constituents.

Key words *Salvia;* aldose reductase inhibitory activity; LC-MS; *Salvia deserta;* lithospermic acid B; tanshinone

Danshen (丹参, Radix Salviae miltiorrhizae) is the dry root and rhizome of *Salvia miltiorrhiza* Bunge (Lamiaceae). It is officially listed in the Chinese Pharmacopoeia and used for treatment of menstrual disorder, menostasis, menorrhagia, insomnia, blood circulation diseases, and angina pectoris as well as against inflammation. Moreover, Danshen was reported to strongly inhibit aldose reductase (AR) and salvinolic acid A, one of the Danshen constituents, was reported to have AR inhibitory activity. In the course of our study on *Salvia* plants, we examined the components of roots of *S. miltiorrhiza* (Danshen) and *S. deserta* (Xinjiang-Danshen). *Salvia miltiorrhiza* contained "tanshinones" [tanshinone I, tanshinone IIA, dihydrotanshinone I, and cryptotanshinone] as abietane-type diterpenoids and a tetramer [magnesium lithospermate B (1a)] as the main caffeic acid derivative, while *S. deserta* contained "royleanones" [6,7-dehydroroyelenone, royleanone, 7-O-methyllithospermate, 7-O-acetylroyleanone, horminone] as abietane-type diterpenoids and a trimer [salvinolic acid K (2)] as the main caffeic acid derivative. The "tanshinones" in *S. miltiorrhiza* showed inhibitory activity against AR from rat eye lens, and their activity was considered to be due to the o- or p-naphthoquinone group. We thus examined the activity of the extracts of *S. deserta* containing very different constituents from *S. miltiorrhiza*.

In China one hundred and ten *Salvia* species are grown, and twelve of them (*S. bowleyana, S. deserta, S. miltiorrhiza, S. miltiorrhiza var. miltiorrhiza f. alba, S. paramiltiorrhiza, S. paramiltiorrhiza f. purpureo-rubra, S. przewalskii, S. przewalskii var. mandarinorum, S. sinica, S. sinica f. purpurea, S. trijuga, S. yunnanensis*) are used as resources of Danshen. Few comparative studies have been made of their composition and activities, however. Thus, we...
also examined AR inhibitory activity of seventeen *Salvia* plants (Table 1), including *S. miltiorrhiza* and *S. desert*, among which ten species are used as Danshens resources. In addition, their water extracts were examined by liquid chromatography–mass spectrometry (LC-MS). This paper deals with the AR inhibitory activity of the water and MeOH extracts and LC-MS analysis of the water extracts.

**Results and Discussion**

**AR Inhibitory Activity of *Salvia desert*** The MeOH and water extracts of *S. desert* (No. 4) inhibited AR from rat eye lens concentration-dependently, and their activities (IC$_{50}$: 78.5 and 84.3 μg/ml, respectively) were almost the same as the MeOH extract of *S. miltiorrhiza* (No. 9; IC$_{50}$: 93.0 μg/ml). Then, the activity of the MeOH extract was transferred into the AcOEt-insoluble fraction (IC$_{50}$: 7.2 μg/ml), and that of *S. miltiorrhiza* (No. 9) into the AcOEt-soluble fraction (IC$_{50}$: 9.9 μg/ml). This suggested that the active constituent of *S. desert* would be a polar compound(s), different from that of *S. miltiorrhiza* of which the active constituents were less-polar "tanshinones".

We measured the AR inhibitory activity of the eleven compounds isolated from *S. desert*. The less-polar diterpenoids (mainly "royleanones"), having no o- or p-naphthoquinone group, showed only weak activity (IC$_{50}$ > 10 μM; inhibition rate at 10 μM was 8.6—36.1%), while the polar constituents [lithospermic acid B (1), salvianolic acid K (2), salviflavase (3), and rosmarinic acid (4)] showed inhibitory activity (IC$_{50}$: 2.63, 2.81, 3.15, and 3.91 μM, respectively), weaker than epalrestat (IC$_{50}$: 0.04 μM), a strong AR inhibitor in clinical use, but stronger than quercetin (IC$_{50}$: 5.20 μM), a natural AR inhibitor. This result would support the previous consideration that the activity of "tanshinones" was due to the o- or p-naphthoquinone group. In addition, the results also explained the difference in the activities of...
the water extracts, because the constituents of the water extract were almost the same as those of the AcOEt-insoluble fraction by TLC and LC-MS analyses.

**AR Inhibitory Activity of Seventeen Salvia Plants** The results mentioned above suggested that there are at least two types in the genus *Salvia*. We then measured the AR inhibitory activity of seventeen *Salvia* plants, including *S. miltiorhiza* (No. 9) and *S. deserta* (No. 4) (Table 2). The MeOH extracts inhibited AR more strongly (IC$_{50}$, 27.9—99.8 µg/ml) than the water extracts, and the MeOH extracts of *S. przewalskii* (No. 13) and *S. przewalskii var. mandarinorum* (Nos. 14, 15) showed very strong activity (IC$_{50}$, 27.9—33.1 µg/ml). In addition, AcOEt-soluble fractions of *S. miltiorhiza* (Nos. 7—9), *S. miltiorhiza var. miltiorhiza f. alba* (No. 10), *S. przewalskii* (No. 13), *S. przewalskii var. mandarinorum* (Nos. 14, 15), and *S. trijuga* (No. 17) and AcOEt-insoluble fractions of *S. deserta* (No. 4), *S. przewalskii* (No. 13), and *S. przewalskii var. mandarinorum* (Nos. 14, 15) showed strong activity (IC$_{50}$, 7.2—19.5 µg/ml). Thus, as active constituents, *S. miltiorhiza* var. *miltiorhiza f. alba* and *S. trijuga* would contain less-polar compounds (e.g., "tanshinones")$^{13}$ as *S. miltiorhiza*, while *S. przewalskii* and *S. przewalskii var. mandarinorum* would contain both the less-polar and polar compounds.

Though the activity of water extracts was weaker than that of MeOH extracts, water extracts of five species [*S. bulleyana* (No. 3), *S. deserta* (No. 4), *S. przewalskii* (No. 13), *S. przewalskii var. mandarinorum* (Nos. 14, 15), *S. trijuga* (No. 17)] showed AR inhibitory activity comparable to that of MeOH extracts. Among the five, three (S. deserta, S. przewalskii, S. przewalskii var. mandarinorum, S. trijuga) showed AR inhibitory activity comparable to that of MeOH extracts.

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**Fig. 1. Total Ion Chromatogram of Water Extracts of Seventeen Salvia Plants**

*a* A mixture of 1—4.  b—r) Sample No. 1—No. 17.  * This is a solvent-derived peak, because an injection of solvent also revealed only this peak.
norum) were the species in which AcOEt-insoluble fraction showed stronger AR inhibitory activity than the corresponding AcOEt-soluble fraction.

These results suggest that, with regard to the AR inhibitory activity, there are three types in the genus *Salvia*: the first type containing less-polar active compounds (*S. miltiorrhiza, S. miltiorrhiza var. miltiorrhiza f. alba*), the next type containing polar active compounds (*S. bulleyana, S. desert*a), and the third type containing both active compounds (*S. przewalskii, S. przewalskii var. mandarinorum, S. trijuga*). It is noteworthy that in the yields and activities of the same species [*S. bowleyana* (Nos. 1, 2), *S. miltiorrhiza* (Nos. 7–9), *S. przewalskii var. mandarinorum* (Nos. 14, 15)] were almost the same and that *S. miltiorrhiza* var. *miltiorrhiza f. alba* (No. 10), *S. paramiltiorrhiza f. purpureo-rubra* (No. 12), and *S. przewalskii var. mandarinorum* (Nos. 14, 15) showed similar inhibitory activities to their corresponding species [*S. miltiorrhiza* (Nos. 7–9), *S. paramiltiorrhiza* (No. 11), and *S. przewalskii* (No. 13), respectively].

**LC-MS Analysis of Water Extracts of Seventeen *Salvia* Plants**

The polar compounds 1–4 obtained from *S. desert*a showed a slightly overlapped total ion chromatogram (TIC) but they were well separated on a mass chromatogram at the respective protonated molecular ion (1, m/z 719.2, *t* _g_ 9.20 min; 2, m/z 557.3, *t* _g_ 8.82 min; 3, m/z 533.3, *t* _g_ 7.48 min; 4, m/z 361.3, *t* _g_ 8.01 min). We thus calculated their amounts from the mass chromatogram except for that of 4 (Table 3), because the ion strength of 4 did not show linearity against the amount. The amount of 1 was large (100–260 µg/mg) as usual, but it was small in *S. bulleyana* (No. 3; 15.9 µg/mg), *S. desert*a (No. 4; 0.3 µg/mg), *S. flav*a (No. 5; 7.3 µg/mg), *S. przewalskii* (No. 13; 39.0 µg/mg), *S. przewalskii var. mandarinorum* (Nos. 14, 15; 19.0 and 6.2 µg/mg), and *S. trijuga* (No. 17; 77.5 µg/mg). On the other hand, the ratio of 1 against the total amount of 1–3 was high (>90%) as usual, but that of *S. desert*a, *S. flav*a, *S. przewalskii*, and *S. przewalskii var. mandarinorum* was low (0.94–80.4%).

Thus, with regard to the amount of 1, there were three types in the genus *Salvia*: the first containing a large amount of 1 (*S. bowleyana, S. melleinis*, *S. miltiorrhiza, S. miltiorrhiza var. miltiorrhiza f. alba, S. paramiltiorrhiza, S. paramiltiorrhiza f. purpureo-rubra, S. sinica var. purpurea*), the second containing a small amount but a high ratio of 1 (*S. bulleyana, S. trijuga*), and a third containing a small amount and a low ratio of 1 (*S. desert*a, *S. flav*a, *S. przewalskii, S. przewalskii var. mandarinorum*). Although the precise relationship between the amount of 1 and the AR inhibitory activity is not clear and more study is needed, the fact that five of the six species of the second and the last types showed relatively strong inhibitory activity could suggest that there is a negative correlation between the amount of 1 and the AR inhibitory activity. It should be noted that the plants of the same species [*S. bowleyana* (Nos. 1, 2), *S. miltiorrhiza* (Nos. 7–9), *S. przewalskii var. mandarinorum* (Nos. 14, 15)] were not same in their content of 1–3, though they belong to the same group.

**Conclusion**

We examined the AR inhibitory activity of water and MeOH extracts of seventeen *Salvia* plants, including ten species of Danshen resources, and their water extracts were also analyzed by LC-MS. The results indicated that there were four types with regard to the AR inhibitory activity and three types with regard to the amount of lithospermic acid B (1). Ten species used as Danshen resources showed the AR inhibitory activity with varied intensity, and contained 1 in varying amounts. These facts suggested that the ten species were not the same, although their AR inhibitory activity was well correlated with the morphological classification. Thus, their use as a Danshen resource should be based on their activity and/or active constituents.

**Experimental**

**Plant Materials** The roots of fifteen *Salvia* plants (sample Nos. 1–3, 5–8, 10–17), which had been identified by an expert and were preserved, were supplied from the Department of Medicinal Botany, China Pharmaceutical University. Those of *S. desert*a (sample No. 4) and *S. miltiorrhiza* (sample No. 9) were supplied by Alps Pharmaceutical Co.,

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Plant name</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>1/(1+2+3) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>S. bowleyana</em></td>
<td>210.3</td>
<td>0.40</td>
<td>1.7</td>
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<td>2</td>
<td><em>S. bowleyana</em></td>
<td>141.1</td>
<td>0.10</td>
<td>0.6</td>
<td>99.5</td>
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<td>3</td>
<td><em>S. bulleyana</em></td>
<td>15.9</td>
<td>0.36</td>
<td>0.5</td>
<td>94.9</td>
</tr>
<tr>
<td>4</td>
<td><em>S. desert</em>a</td>
<td>0.3</td>
<td>29.28</td>
<td>2.5</td>
<td>0.94</td>
</tr>
<tr>
<td>5</td>
<td><em>S. flav</em>a</td>
<td>7.3</td>
<td>0.03</td>
<td>9.7</td>
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<td>6</td>
<td><em>S. melleinis</em></td>
<td>118.1</td>
<td>0.18</td>
<td>0.8</td>
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<td>7</td>
<td><em>S. miltiorrhiza</em></td>
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<tr>
<td>8</td>
<td><em>S. miltiorrhiza</em> (cultivated)</td>
<td>127.1</td>
<td>0.05</td>
<td>0.1</td>
<td>99.9</td>
</tr>
<tr>
<td>9</td>
<td><em>S. miltiorrhiza</em></td>
<td>39.0</td>
<td>0.06</td>
<td>0.1</td>
<td>99.6</td>
</tr>
<tr>
<td>10</td>
<td><em>S. miltiorrhiza</em> var. <em>miltiorrhiza f. alba</em> (cultivated)</td>
<td>116.5</td>
<td>0.14</td>
<td>0.3</td>
<td>99.6</td>
</tr>
<tr>
<td>11</td>
<td><em>S. paramiltiorrhiza</em></td>
<td>130.0</td>
<td>0.02</td>
<td>0.4</td>
<td>99.7</td>
</tr>
<tr>
<td>12</td>
<td><em>S. paramiltiorrhiza f. purpureo-rubra</em></td>
<td>258.3</td>
<td>0.04</td>
<td>0.6</td>
<td>99.8</td>
</tr>
<tr>
<td>13</td>
<td><em>S. przewalskii</em></td>
<td>6.9</td>
<td>0.02</td>
<td>3.0</td>
<td>57.9</td>
</tr>
<tr>
<td>14</td>
<td><em>S. przewalskii var. mandarinorum</em></td>
<td>19.0</td>
<td>0.19</td>
<td>5.6</td>
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</tr>
<tr>
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<td><em>S. przewalskii var. mandarinorum</em></td>
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<td>0.01</td>
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<tr>
<td>16</td>
<td><em>S. sinica f. purpurea</em></td>
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<td>0.4</td>
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<td><em>S. trijuga</em></td>
<td>77.5</td>
<td>0.04</td>
<td>1.2</td>
<td>98.4</td>
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</tbody>
</table>
References and Notes


8) “Royaleanones” are characterized by $\mu$-benzoquinone C-ring within the abietane skeleton, while “tanzhimones” are by o- or p-naphthoquinone group within nor- or bisnor-abietane skeleton.


10) The LC-MS analyses of the MeOH extracts will be reported in our next paper.


