Chemical changes in rhubarb heated after processed with liquor

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Abstract

In China, rhubarb (da-huang in Chinese; daiou in Japanese) has been processed with liquor, e.g., liquor-dipping (LDi), liquor-soaking (LSo), and heated after being processed with liquor (LH) since ancient times. We previously found that LH rhubarb was used as a substitute for LDi rhubarb, a purgative, or LSo rhubarb, a remedy against blood stasis described in the ancient Chinese medical literature. Now, liquor-sprayed and heated (LSpH) rhubarb, called ‘liquor-rhubarb’ is used due to its expected effects against blood stasis. However, the characteristics of LH rhubarb have not been elucidated scientifically. In this report, we prepared LDi, LSo, liquor-sprayed (LSp), LDi and heated (LDiH), LSo and heated (LSoH), and LSpH rhubarb using 16% ethanol (as a substitute for huangjiu) and compared the contents of their principal compounds. The sennoside (a purgative) contents of LDiH, LSoH, and LSpH were significantly decreased, while the anthraquinone (an anti-inflammatory) contents of LSoH and LSpH increased to the same level as LSo, and the lindleyin contents of LSoH and LSpH were greater than that of LSo. Therefore, we found that LH rhubarb does not have an increased purgative effect and that the LSoH and LSpH processes may produce greater anti-inflammatory effects than LSo.

Key words rhubarb, processing with liquor, heating, sennoside, anthraquinone, lindleyin.

Abbreviations C40, control dried at 40°C; C180, control dried at 180°C; LDi, liquor-dipping; LH, heating after processing with liquor; LSo, liquor-soaking; LSp, liquor-spraying; LDiH, liquor-dipping and heating; LSoH, liquor-soaking and heating; LSpH, liquor-spraying and heating.

Introduction

In China, crude drugs are sometime processed to enhance particular desirable effects and reduce side effects. Rhubarb is a crude drug treated various processes. Since ancient times, rhubarb has been processed with liquor, e.g., by liquor-dipping (LDi), liquor-soaking (LSo), and processing it with liquor before heating it (LH).1,2) In modern times, LH rhubarb named ‘liquor-rhubarb’, rhubarb sprayed with huangjiu and then heated, is used to treat blood stasis in China.3) Although there are many kinds of liquor-processed rhubarb, their medicinal effects have not been elucidated thoroughly. The composition of principal compounds in rhubarb may change.

Therefore, we previously determined the contents of the principal compounds in liquor processed rhubarb and found that LDi rhubarb maintained its sennoside (a purgative4,5) content and that LSo rhubarb had an increased anthraquinone (an antibacterial6) and an anti-inflammatory7,8,9) content. We also performed a herbal study and found that LH rhubarb was described as a substitute for LDi rhubarb and LSo rhubarb in the Chinese medical literature published since the Jin and Yuan Dynasties.1) However, the properties of LH
rhubarb have not been scientifically elucidated. In this report, we prepared LH rhubarb with 16% ethanol (as a substitute for huangjiu) and investigated the change in its principal compound contents i.e., sennoside A, sennoside B, aloe-emodin, rhein, emodin, chrysophanol, physcion, lindleyin, isolindleyin, and total tannins.

Materials and Methods

Crude drug material: JP15-rhubarb, belonging to section Palmata, was purchased from Tochimoto Tenkaido Co., Ltd. (Lot 007008001, 2008).

Reagents: Sennoside A and sennoside B were purchased from Wako Pure Chemical Industries, Ltd.; aloe-emodin, rhein, emodin, and chrysophanol were from Funakoshi, Ltd.; physcion and gallic acid were from Nakalai Tesque, Ltd.; and Folin-Ciocalteu’s reagent was from Merck, Ltd. Lindleyin and isolindleyin were provided by Tumura Co., Ltd. All chemicals were of an analytical grade, and the chromatographic solvents were of an HPLC grade.

Method of preparation: In China, the process of heating means roasting at low heat. However, because heat of fire is nonconstant, we used oven to heat rhubarb. And 16% ethanol was used as a substitute for huangjiu. We previously found that rhubarb processed with ethanol showed hardly any differences from that processed with huangjiu.

Ten grams of rhubarb chips were used in each examination. The approximate number of rhubarb chip was 690 /5 g. We processed the rhubarb by the following methods: LDi, LDiH, LSo, LSoH, LSp, and LSpH.

LDi: Rhubarb chips were dipped in 40 mL of 16% ethanol, stirred to ensure thorough saturation with alcohol, and left for 30 minutes, before being removed from the alcohol and then the excess fluid was absorbed with paper. The chips were dried overnight in an oven set to 40°C.

LDiH: Rhubarb chips were processed with 16% ethanol in the same way as LDi and then dried in an oven set to 180°C for 40 minutes before being dried at 40°C overnight.

LSo: Rhubarb chips were soaked in 40 mL of 16% ethanol and stirred to ensure thorough saturation with alcohol, left for 24 hours, before being removed from the alcohol, and then having the excess fluid was absorbed with paper. The chips were dried overnight in an oven set to 40°C.

LSoH: Rhubarb chips were processed with 16% ethanol in the same way as LSo and then dried in an oven set to 180°C for 40 minutes before being dried at 40°C overnight.

LSp: 16% ethanol was sprayed onto rhubarb chips six times (approximate amount was 4 mL) and left for 1 hour. The chips were dried overnight in an oven set to 40°C.

LSpH: Rhubarb chips were processed with 16% ethanol in the same way as LSp and dried in an oven set to 180°C for 40 minutes.

Rhubarb chips that were dried overnight in an oven set to 40°C (C40) or dried at 180°C for 40 minutes (C180) were used as controls.

Analysis of principal compound contents: We used HPLC to determine the contents of sennoside A, sennoside B, 5 kinds of anthraquinones, lindleyin, and isolindleyin and used the Folin-Ciocalteu method to determine total tannin content. The HPLC and Folin-Ciocalteu methods were performed as described in a previous paper.

Statistical analysis: The significance of differences was determined by the Dunnett method. All data are expressed as mean ± S.D.

Results

Henceforth, we refer to sennoside A and sennoside B; the 5 kinds of anthraquinone measured (aloe-emodin, rhein, emodin, chrysophanol, and physcion); and lindleyin and isolindleyin as sennoside, anthraquinone, and lindleyin, respectively.

1. Change in sennoside content (Fig. 1)

The sennoside contents of LDiH, LSoH and LSpH were decreased compared to that of C40, and those of LDiH and LSoH were significantly greater than that of C180, while that of LSpH was the same as that of C180.
The difference in sennoside content can be summarized as follows: C40, LDi, LSp > LSo, LSoH, LDiH > C180, LSpH.

2. Change in anthraquinone content (Fig. 2)

The anthraquinone contents of LSpH and LSoH were increased significantly compared to that of C40, while that of LDiH was the same as that of C40. The contents of LDiH and LSoH were lower than that of C180, while that of LSpH was the same as that of C180. The difference in anthraquinone content can be summarized as follows: C180, LSpH, LSo > LSoH, LDiH, C40, LDi, LSp.

3. Change in lindleyin content (Fig. 3)

The lindleyin contents of LSoH and LSpH were significantly decreased compared to that of C40. The content of LDiH was significantly greater than that of C180, that of LSoH was significantly lower than that of C180, and that of LSpH was the same as that of C180. The difference in lindleyin content can be summarized as follows: C40, LDiH, LDi, LSp > C180, LSpH > LSoH, LSo.

4. Change in total tannin contents (Fig. 4)

The tannin contents of LDiH, LSoH, and LSpH had a tendency to be lower than that of C40. However, there was no significant change in tannin content.

5. Differences in the relative amounts of various components between liquor-dipped (LDi) rhubarb, liquor-soaked (LSo) rhubarb, and 3 types of LH (LDiH, LSoH, and LSpH) rhubarb (Fig. 5)

(1) Comparison of LDi rhubarb and 3 types of LH rhubarb
The sennoside contents of LDiH, LSoH, and LSpH were lower than that of LDi. The lindleyin and total tannin contents of LDi were the same as those of LDiH.

2. Comparison of LSo rhubarb and 3 types of LH rhubarb

The anthraquinone contents of LSoH and LSpH were the same as those of LSo. The sennoside contents of LSoH and LSpH were lower than those of LSo. The lindleyin contents of LDiH, LSoH, and LSpH were greater than that of LSo. The total tannin contents of these processed rhubarbs were all similar.

Discussion

We determined the characteristics of rhubarb heated after being processed with liquor by chemical analysis and revealed the following:

1. We found that the process of heating decreased the sennoside content and increased the anthraquinone content of the rhubarb. Yoshida et al.\(^\text{12}\) previously reported that sennoside were broken down and anthraquinone content was increased by heating rhubarb at 180°C. Our results agreed with their report; however, the changes seen in LDiH and LSoH were less drastic than those seen in C180 and LSpH.

Furthermore, we also found that the process of heating decreased lindleyin and total tannin contents, however those contents of LH (LDiH and LSoH) rhubarb was similar or higher than unheated rhubarb (LDi) and LSo, respectively. Thus, we think that applying alcohol to the surface of rhubarb decreases heat conduction.

2. We previously searched the medicinal literature published since the Jin and Yuan Dynasties.\(^\text{1}\) We found that LH rhubarb was used as a substitute for LDi rhubarb, as a purgative, and LSo rhubarb, as a remedy for blood stasis.\(^\text{1,12}\) However, in this experiment, the liquor-processed rhubarb dried at 180°C (LDiH, LSoH, LSpH) had a decreased sennoside content; therefore, we found that no LH rhubarb had the enhanced purgative\(^\text{4,5}\) effect required for it to act as a suitable substitute for LDi rhubarb.

3. The LSoH and LSpH processes increased the anthraquinone content of the rhubarb to the same level as LSo, and the lindleyin contents of the LSoH and LSpH rhubarb were greater than that of LSo. The medicinal effect of anthraquinone is anti-bacterial\(^\text{10}\) and anti-inflammatory\(^\text{7,10}\) and that of lindleyin is anti-inflammatory.\(^\text{13}\) Therefore, we think that LSoH and LSpH of LH rhubarb will have a greater anti-inflammatory effect than LSo. These effects may be linked to their ability to improve blood stasis.\(^\text{2,11}\) However we need to confirm those effects of processed rhubarb by pharmacological test.

4. In modern times, LH rhubarb named ‘liquor-rhubarb’, rhubarb sprayed with huangjiu and heated, is used to treat blood stasis in China.\(^\text{3}\) Our experiment shows that the increase in anthraquinone content produced by LSpH was more drastic than that produced by LSoH and that the lindleyin content of LSpH was higher than that of LSoH. Therefore, LSpH is expected to have a greater anti-inflammatory effect than LSoH. Thus, we think that the method used to process LH rhubarb was changed from LSoH to LSpH, and that LH rhubarb is similar to ‘liquor-rhubarb’.

5. The total tannin contents of LDiH, LSoH, and LSpH had a tendency to be lower than those of C40. The Folin-Ciocaltceu method, which we used, is able to analyze condensed tannin, gallic acid, catechin, \((\pm)\)-epicatechin 3-O-gallate, etc. However, Yoshida et al.\(^\text{12}\) reported that the gallic acid content of rhubarb was increased while the catechin and \((\pm)\)-epicatechin 3-O-gallate contents were decreased by heating it at 180°C, and it was reported that gallic acid is a strong radical scavenger,\(^\text{14}\) \((\pm)\)-epicatechin 3-O-gallate is an antioxidant of LDL,\(^\text{15}\) and catechin is an anticoagulant.\(^\text{16}\) Thus, LH rhubarb may show an increased radical scavenging effect, and these effects may affect blood stasis.

6. The changes observed in the principal compound contents of LSpH were the same as those seen in C180. Because the amount of liquor using LSpH process may be insufficient to evoke a response, its effect was not confirmed in this experiment, and so requires further exploration.

7. Matsuda et al.\(^\text{17}\) reported that stilbene compounds from rhubarb have inhibitory activity on NO, linking to the effect of anti-blood stasis. It is known that stilbene compound is mainly contained in the rhubarb belong to section Rhapontica. In this experiment, we used medicinal rhubarb that belongs to section Palmata. Thus we estimated only anthraquinone and lindleyin content that may be linked to the effect of anti-blood stasis.
However, recently Komatsu et al.\textsuperscript{15} reported that stilbene compound contents also contained in the rhubarb belong to section Palmata, and we think further exploration is needed.

**Conclusion**

The process of heating decreased sennoside, lindleyin, and total tannin content and increased anthraquinone content of the rhubarb. However the rhubarbs applied alcohol to the surface (LSoH, LDiH) reduced that heat conduction.

**References**


