Comparison of laxative and antioxidant activities of raw, processed and fermented Polygoni Multiflori Radix

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[ABSTRACT] AIM: To observe the anti-oxidative activity and adverse laxative effect of raw, traditional processed and fermented products of Polygoni Multiflori Radix (PMR), and furthermore, to evaluate the fermentation method used in the processing procedure of PMR. METHODS: In vitro ferric reducing antioxidant power (FRAP) assay was carried out to evaluate the anti-oxidative activity. Modulation of normal defecation and effect on gastrointestinal motility in mice were carried out to investigate their adverse laxative effect. RESULTS: Fermented PMR induced less severe laxative adverse effect than Polygoni Multiflori Radix Praeparata (PMRP). PMR fermented with Rhizopus sp. (FB) could modulate the defecation significantly. The gastrointestinal motility was inhibited by PMRP and PMR fermented with Rhizopus oryzae (FA). FA and FB showed better antioxidant activity than PMRP in 50% and 95% ethanol group. Contents of 2',3',5',4'-tetrahydroxy-stilbene-2-O-β-D-glucoside (TSG) were reduced significantly after traditional processing but maintained after fermentation. Emodin and physcion were increased after traditional processing and fermented with Rhizopus oryzae. CONCLUSION: All processing procedure, including fermentation, might reduce its anti-oxidative activity. However, most of the processed products could lessen the adverse effect on gastrointestinal tract compared to PMR. Fermentation with Rhizopus oryzae was considered as a promising processing method of PMR.

[KEY WORDS] Polygoni Multiflori Radix; Fermented; Laxative; Antioxidative

1 Introduction

Fermentation, one of the most frequently used processing procedures of traditional Chinese medicine, showed outstanding foreground in the research of traditional Chinese medicines, especially in researches aiming at improving pharmacological effect and reducing adverse effect. Nowadays, fermentation by pure fungus or other microorganisms has been investigated and applied more[1-2]. New application of fermentation mainly focused on improving bioavailability of crude drug and increasing yield of chemical constitu-

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Fermentations of PMR and Rhei Radix et Rhizoma have been investigated by our research group for several years[6-10]. Fermentation showed some advantages compared to traditional processing procedures. We found that the contents of conjugated anthraquiones, which were presumed to have laxative adverse effect inducing ingredients, could be reduced after fermentation. Importantly, the content of TSG, one of the most important active ingredients, could be maintained after fermentation, while the content of TSG might reduce significantly after traditional processing in PMRP.

Most researches showed that the laxative potency was correlated with the content of conjugated anthraquinone. The conjugated anthraquinone contents in Polygonum multiflorum were much lower than those in Rheum tanguticum Maxim. ex Reg, Reynoutria japonica Houtt, Rheum palmatum L., Rheum officinale Baill. and Cartsia tora Linn. The laxative activities of Polygonum multiflorum were correspondingly lower than the other crude drugs[11]. The laxative potency of PMR was significantly reduced after steamed under high pressure and
high temperature, with the content of conjugated anthraquinone being reduced from 3.31 mg·g⁻¹ to about 1.95 mg·g⁻¹ after 12 h[10].

Antioxidant activity was considered as the crucial pharmacological effect of PMR[12-13]. TSG[14-16], flavonoid glycosides[17] and polysaccharides[18-19] in PMR all exhibited outstanding antioxidant activity in vitro and in vivo. Researches indicated the anti-oxidative activity of PMR might be a cooperative effect of these constituents.

In this research, the anti-oxidative activity and adverse laxative effect of raw, traditional processed and fermented products of PMR were tested in order to estimate the effect of processing procedure on pharmacological effects and adverse effects. Furthermore, the evaluation of fermentation of PMR was carried out.

2 Materials and Methods

2.1 Plant material

Polygoni Multiflori Radix was collected in Luquan County of Yunnan Province by the authors. The plants were authenticated as root of Polygonum multiflorum Thunb. by Prof. Zhao, Yunnan University of Traditional Chinese Medicine.

2.2 Chemicals and microorganisms

TSG, emodin and physcion were purchased from National Institute for the Control of Pharmaceutical and Biological Products, China. Structures of TSG, emodin and physcion are listed in Fig. 1.

![Fig. 1 Structures of major constituents in PMR and PMRP: emodin, physcion and TSG](image)

2, 4, 6-Tris-(2-pyridyl)-s-triazine (TPTZ) was purchased from Sigma, USA. Other chemical substances in this research were of analytical grade.

Microorganisms used in the fermentation of PMR were isolated and identified by the authors as Rhizopus oryzae, Aspergillus niger, Penicillium sp., Fusarium sp. and Aspergillus fumigatus. Further PCR based identification was still under investigation.

2.3 Processing procedures of Ploygoni Multiflori Radix

PMRP was processed from PMR with black bean decoction according to the method recorded in the Pharmacopoeia of People’s Republic of China (2010 edition)[20].

Fermented products A, B, C, D, E were obtained from PMR by Rhizopus oryzae, Aspergillus niger, Penicillium sp., Fusarium sp. and Aspergillus fumigatus (FA, FB, FC, FD and FE), respectively. Microorganisms were inoculated in 250 mL sterilized potato liquid medium and incubated for 48 h (120 r·min⁻¹, 28 °C). 300 g PMR powder (0.3 mm) and 150 g bran were completely mixed and sterilized. After that, 10% microorganism solution was added with 360 mL sterilized water extracts of PMR, PMRP, FA, FB, FC, FD and FE separately in polythene cages with filter paper. The onset time of wet feces and the total counts of wet feces in 6 h were recorded.

2.4 Effect on gastrointestinal motility

2.4.1 Modulation of normal defecation in mice

80 Kunming mice of either sex were randomly divided into 8 groups of ten in each. The control group orally received physiological saline. The other groups orally received the water extracts of PMR, PMRP, FA, FB, FC, FD and FE (0.02 mL·g⁻¹). After administration, animals were placed separately in polythene cages with filter paper. The onset time of wet feces and the total counts of wet feces in 6 h were recorded.

2.4.2 Effect on gastrointestinal motility

80 Kunming mice of either sex were randomly divided into 8 groups of ten in each. The control group orally received physiological saline. The other groups orally received the test extracts of PMR, PMRP, FA, FB, FC, FD and FE (0.02 mL·g⁻¹). 30 min later, animals were given orally about 0.4 mL of charcoal meal (0.2 mL/10 g body weight). After another 20 min, animals were sacrificed and the movement of charcoal from pylorus to caecum was measured. The charcoal movement in the intestine was expressed in terms of percentage.

2.5 Antioxidant activity assay

1.5 g powder of PMR, PMRP, FA, FB, FC, FD and FE were immersed with 30 mL water, 50% ethanol and 95%...
ethanol under room temperature for 24 h. The extraction was collected after centrifuging at 7 000 rpm for 15 min. FRAP value was tested with the water extraction after 10 times dilution, 50% ethanol extraction after 20 times dilution and 95% ethanol extraction without dilution.

0.3 mL tested sample was added to 2.7 mL TPTZ work solution (0.3 mol·L⁻¹ sodium acetate: 10 mmol mol·L⁻¹ TPTZ: 20 mmol mol·L⁻¹ FeCl₃ = 10 : 1 : 1, V/V/V) preheated to 37 °C. Absorption in 593 nm was observed 10 min later for triplicate assays. Standard curve was calculated with FeSO₄ solution (25–400 μg L⁻¹). Corresponding FeSO₄ concentration (with the same absorption value) was recorded as the FRAP value of the tested sample (x ± s).

Fe³⁺-TPTZ in the working solution could be reduced to Fe²⁺, which showed the maximum absorption in 593 nm, by antioxidant constituents in the tested sample. The higher the FRAP value was, the higher antioxidant activity the sample showed.

2.6 Chemical analysis of PMR and its processed products.

Concentrations of TSG, emodin and physcion were analyzed with Dionex Ultimate 3000 HPLC system (Dionex Technologies, USA), which included a binary pump, an autosampler, a column oven and a diode array detector plus on-line degasser. Data were analyzed with Chromeleon 6.8.

The separation of PMR, PMRP and FA extractions was achieved on Zorbax SB-C18 analytical column (4.6 mm × 250 mm, I.D., 5 μm particle diameter, supplied by Agilent Technologies, USA).

Gradient elution with mobile phase consisting of (A) 0.1% H₃PO₄ and (B) methanol methanol was used. The nonlinear gradient elution program was utilized. Methanol percentage was 40% (in the initial time), 70% (5 min), 80% (10 min), 85% (15 min) and 90% (20-25 min). Detection wavelength was set at 254 nm. The oven temperature was set at 30 °C and the flow rate was set at 1.0 mL·min⁻¹.

10 mg of extraction of PMR, PMRP and FA mentioned in text 2.3 was accurately weighed and resolved in 10 mL methanol for analyses after filtering with 0.45 μm filter membrane. A 10 μL injection value was used in all analyses. The peaks of TSG, emodin and physcion were identified by comparing their retention time (tR) values and UV spectra with those of standards.

3 Results

3.1 Laxative adverse activities of PMR and its processed products

Raw PMR induced obvious adverse laxative effect compared to the control group. Most of the processed products showed significant inhibition effect on gastrointestinal motility compared to PMR in the wet feces assay (Table 1). The onset time of wet feces was shortened from 360 min to 116.9 min after taking raw PMR. The adverse laxative effect made it necessary to process PMR in clinic use. PMRP could not shorten the onset time of the first wet feces but could reduce the total counts of wet feces compared to PMR. All the fermented PMR showed better laxative inhibition activity than PMRP. FB could prolong the onset time of wet feces significantly (P < 0.001).

Table 1 Results of PMR and its processed products induced diarrhea. (x ± s, n = 10)

<table>
<thead>
<tr>
<th>Groups</th>
<th>Onset time of first wet faeces (min)</th>
<th>Total number of wet faeces (n/rat)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>360 ± 0</td>
<td>0</td>
</tr>
<tr>
<td>PMR</td>
<td>116.9 ± 57.9</td>
<td>3.8 ± 1.8</td>
</tr>
<tr>
<td>PMRP</td>
<td>181.8 ± 125.1 **</td>
<td>1.7 ± 1.6 **</td>
</tr>
<tr>
<td>FA</td>
<td>222.7 ± 136.5 **</td>
<td>1.5 ± 1.6 **</td>
</tr>
<tr>
<td>FB</td>
<td>307.4 ± 111.4 **</td>
<td>0.2 ± 0.4 **</td>
</tr>
<tr>
<td>FC</td>
<td>269.2 ± 146.2 **</td>
<td>1.0 ± 1.8 **</td>
</tr>
<tr>
<td>FD</td>
<td>269.4 ± 145.9 **</td>
<td>0.6 ± 1.0 **</td>
</tr>
<tr>
<td>FE</td>
<td>236.4 ± 159.7 **</td>
<td>0.7 ± 1.1 **</td>
</tr>
</tbody>
</table>

*P < 0.05, **P < 0.01, ***P < 0.001 vs PMR group

All the processed PMR could inhibit the gastrointestinal motility in mice (Table 2). PMRP and FA showed the best inhibition effect. The results displayed fine correspondence with previous reports, that the processing procedure could diminish its adverse laxative effect.

Judging from the above data, FA and FB showed great inhibition effect on activity of gastrointestinal motility. 3.2 Antioxidant activities of PMR and its processed products

The FRAP values of raw, traditional processed and fermented PMR extraction are listed in Table 3.

Table 2 Effects of PMR and its processed products on gastrointestinal motility (x ± s, n = 10)

<table>
<thead>
<tr>
<th>Groups</th>
<th>Distance traveled by charcoal meal (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>62.07 ± 5.03</td>
</tr>
<tr>
<td>PMR</td>
<td>76.44 ± 6.22 **</td>
</tr>
<tr>
<td>PMRP</td>
<td>62.06 ± 8.36 **</td>
</tr>
<tr>
<td>FA</td>
<td>64.68 ± 5.41 **</td>
</tr>
<tr>
<td>FB</td>
<td>66.91 ± 9.82 *</td>
</tr>
<tr>
<td>FC</td>
<td>65.63 ± 9.67 **</td>
</tr>
<tr>
<td>FD</td>
<td>62.52 ± 9.49 *</td>
</tr>
<tr>
<td>FE</td>
<td>65.28 ± 13.31 **</td>
</tr>
</tbody>
</table>

*P < 0.05, **P < 0.01, ***P < 0.001 vs PMR group

Raw PMR showed the strongest antioxidant activity compared to its traditional processed or fermented products. The results indicated all processing procedures might lead to the degradation of the reductive constituents.

FA and FB showed better antioxidant activity than PMRP in 50% ethanol and 95% ethanol group, which indicated fermentation by Rhizopus oryzae and Rhizopus sp. might be promising processing methods for PMR.
Table 3  FRAP values of various processed products of PMR (x ± s, n = 3)

<table>
<thead>
<tr>
<th>Sample</th>
<th>Water extraction (10 times dilution)</th>
<th>50% ethanol extraction (20 times dilution)</th>
<th>95% ethanol extraction (without dilution)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PMR</td>
<td>982.65 ± 22.07</td>
<td>886.82 ± 44.32</td>
<td>973.32 ± 23.01</td>
</tr>
<tr>
<td>PMRP</td>
<td>821.48 ± 69.41</td>
<td>556.32 ± 8.40</td>
<td>571.32 ± 23.75</td>
</tr>
<tr>
<td>FA</td>
<td>734.82 ± 21.18</td>
<td>733.32 ± 34.94</td>
<td>917.98 ± 10.97</td>
</tr>
<tr>
<td>FB</td>
<td>429.98 ± 5.46</td>
<td>643.65 ± 23.44</td>
<td>744.48 ± 13.73</td>
</tr>
<tr>
<td>FC</td>
<td>424.82 ± 16.06</td>
<td>312.32 ± 10.40</td>
<td>409.48 ± 23.53</td>
</tr>
<tr>
<td>FD</td>
<td>724.15 ± 35.19</td>
<td>457.65 ± 11.79</td>
<td>191.82 ± 6.53</td>
</tr>
<tr>
<td>FE</td>
<td>432.98 ± 10.79</td>
<td>424.15 ± 27.42</td>
<td>261.98 ± 7.52</td>
</tr>
</tbody>
</table>

*P < 0.05, **P < 0.01, ***P < 0.001 vs PMR group

3.3 Chemical constituents of PMR and its processed products

Concentrations of TSG, emodin and physcion in PMR, PMRP and FA were analyzed. Other fermentation products were not analyzed considering the similar chemical composition and relative indistinctive pharmacological effects. HPLC-DAD profiles are shown in Fig. 2 and Table 4.

The linear relationships between the injection quantities (mg/10 μL, x-axis) and peak area (y-axis) were y = 6 109.3 x + 0.821 20 for TSG (r² = 0.999 9), y = 62 930 x − 0.992 40 for emodin (r² = 0.999 9), and y = 10 758 x − 0.273 80 for physcion (r² = 0.999 9).

Contents of TSG were reduced significantly after traditional processing but maintained after fermentation. Emodin and physcion were increased after traditional processing and fermented with *Rhizopus oryzae*.

![Fig. 2](image)

**Fig. 2** HPLC profiles of PMR and its processed products
(A) Water extraction of PMR
(B) Water extraction of PMRP
(C) Water extraction of FA

Table 4 Concentrations of TSG, emodin and physcion in PMR and its processed products

<table>
<thead>
<tr>
<th>Sample</th>
<th>TSG (mg/g crude drug)</th>
<th>Emodin (mg/g crude drug)</th>
<th>Physcion (mg/g crude drug)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PMR</td>
<td>86.51</td>
<td>2.862</td>
<td>4.914</td>
</tr>
<tr>
<td>PMRP</td>
<td>62.91</td>
<td>6.072</td>
<td>11.33</td>
</tr>
<tr>
<td>FA</td>
<td>86.19</td>
<td>6.864</td>
<td>10.65</td>
</tr>
</tbody>
</table>

According to the results, the content of TSG was not the single factor affecting the antioxidant activity. The increases of free anthraquinones, hydrolyzed from conjunct anthraquinones, were directly related with the reduction of laxative activity.

4 Discussion

PMR and PMRP were used in China for centuries. PMR and PMRP had different pharmacological effects and were used for the treatment of different diseases. PMR was used for the treatment of constipation while PMRP for early graying and hyperlipemia because it was regarded as tonic of liver and kidney. Various methods were used in the PMR processing procedure. Steaming and steaming with black soybean decoction were most frequently used methods recorded by Pharmacopoeia of People’s Republic of China (2010 edition) [20]. However, the content of TSG, the active ingredient of PMR, was reduced after the traditional processing procedure.

In this research, we evaluated various kinds of processing methods including the traditional processing method and fermentation method in order to choose one that could reduce the potential adverse laxative effect risk while maintaining its antioxidant effect.

Antioxidant activity was significantly depressed after processing in the study. Similar results were obtained by other research group[21]. Normal processing methods could weaken the antioxidant activity of PMR possibly due to the destroying of active ingredients by high temperature in the processing. However, PMR fermented with *Rhizopus oryzae* showed better antioxidant activity than that of PMPP in 50% ethanol and 95% ethanol extraction group in the study.

In the research, the adverse laxative effect was lessened after processing, with both traditional and fermented processing methods were used. FB exhibited the most outstanding defecation modulation effect, which would not induce the wet feces at all. Although FA might still induce the adverse effect of diarrhea, but better meliorated than that of PMR and PMRP.

Fermentation with *Rhizopus oryzae* was considered as a promising processing method of PMR. The laxative adverse effect of PMR was conspicuously lessened after processing with *Rhizopus oryzae*. In the meantime, FA possessed notable antioxidant activity even better than that of PMRP in 50% ethanol and 95% ethanol extraction group.
References


何首乌、制何首乌及何首乌发酵炮制品致泻作用与抗氧化活性的比较研究

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【摘 要】 目的：比较何首乌生品、传统炮制品及发酵炮制品的抗氧化活性及致泻副作用的差异，评价发酵法制何首乌炮制中的应用方法。方法：利用铁离子还原抗氧化法（FRAP法）评价何首乌生品与不同炮制品的抗氧化活性。利用小鼠粪便色点法及粪未推进法评价何首乌生品与不同炮制品的泻下副作用。结果：发酵发酵的何首乌及用传统法炮制的何首乌及用Rhizopus oryzae发酵的何首乌的抗氧化活性都强于传统方法炮制的何首乌。发酵法在降低致泻副作用的同时也能显著降低何首乌的致泻副作用。该发酵法制何首乌可能成为何首乌炮制方法中新的研究方向。

【关键词】 何首乌；发酵；泻下；抗氧化

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