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Studies on the Ginseng Saponins(I)

On the Determination of the Ginseng Saponins in Ginseng Tea and Extract

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Abstract

A determination of the saponins in ginseng tea and extract was carried out by using the quantitative TLC autodetector equipped with a hydrogen flame ionization detector. In order to apply to the quality control of the ginseng tea and extract, the optimum condition and recovery percentage for the quantitative determination of saponins in those products were studied. The results obtained were as follows:

The method was adequate to estimate whether the ginseng extract used for the products and the raw ginseng extract were the same quality or not.

Most of the individual peak area was increased with the concentration of the total saponin. But some of the peak areas were not increased quantitatively in the case of the sample containing high concentration ginseng extract.

To determine the saponins in ginseng tea correctly high volume low concentration was better than the low volume high concentration.

Optimum concentration of ginseng extract in sample to determine the individual saponins was in the range of 0.5~1.5g.

The recovery percentage of the total saponin was 99.5% on the average.

Panax ginseng C.A. Mayer has been known to be an elixir in oriental medicine for a long time and especially, Korean ginseng, has been most widely used because of its eminent efficacy. For that reason, many reports on the clinical and pharmacological efficiency have been carried out recently, and now it is generally agreed that the main efficacy is due to the saponins, a series of dammarane, the special components in *Panax ginseng*. Some studies on the ginseng saponins carried out by Han,[1,2] Kim,[3,4] Woo,[5,6] Shibata,[7,8] and Tanaka *et al.*[9] Also, many different kinds of commercial ginseng products have been produced.

Of all those products, ginseng tea and ginseng extract are commercially produced

as instant type, and now are consumed in large quantity annually.

Nevertheless, a suitable evaluation method for those products has not been developed. Sakamoto *et al*[10] developed a new method to evaluate the ginseng tea and extract. They hydrolized ginseng saponins of dammarane series with sulfuric acid, and then quantitatively determined using Gas liquid chromatography. But the method of hydrolization and trimethylsilylation(TMS) of ginseng saponins was very complicated, and there was much apprehension concerning the possibility of errors.

It seemed to be an unsatisfactory method for the study on the variation of individual saponins because they estimated and evaluated the raw ginseng material according to the ratio of the 20 (s)-panaxadiol series saponins and 20 (s)-panaxatriol series.

Han *et al*[11] developed a radioactive isotope method in which the isotope C^{14} was introduced to the dammarane glycoside. That method is very accurate. But the method can not become a widely used method because it can be carried out only at the special institute and laboratory in which the radioactive isotope can be handled. Namba *et al*[12] studied on the saponin patterns of ginseng and related crude drugs by quantitative thin layer chromatopraph by using a new apparatus equipped with a flame ionization detector.

Hemolytic and its protective activities of those saponins were also determined in comparison with the saponin patterns. However they were not applied to the evaluation of the commercial ginseng products such as ginseng tea and extract. This paper describes the partitive determination of the main ginseng saponins in commercial ginseng tae and extract by quantitative thin layer chromatography and also the application to the evaluation of those products. The data of the analysis of hydrolized saponins of ginseng will be published elsewhere.

Material and Methods

1. Ginseng Roots

Dried lateral roots of ginseng produced at Keum San were washed with tap water, and dried at 50° in an air dryer for 6 hours.

2. Preparation of Ginseng Extract

Dried lateral roots of ginseng were submerged in about 5 times volume of 70% ehanol and extracted 5 times with a heating period of 3 hours each time. The alcoholic extract is then concentrated in a vacuum concentrator to become an extract containing 60% of total solid.

3. Preparation of Ginseng Tea Sample

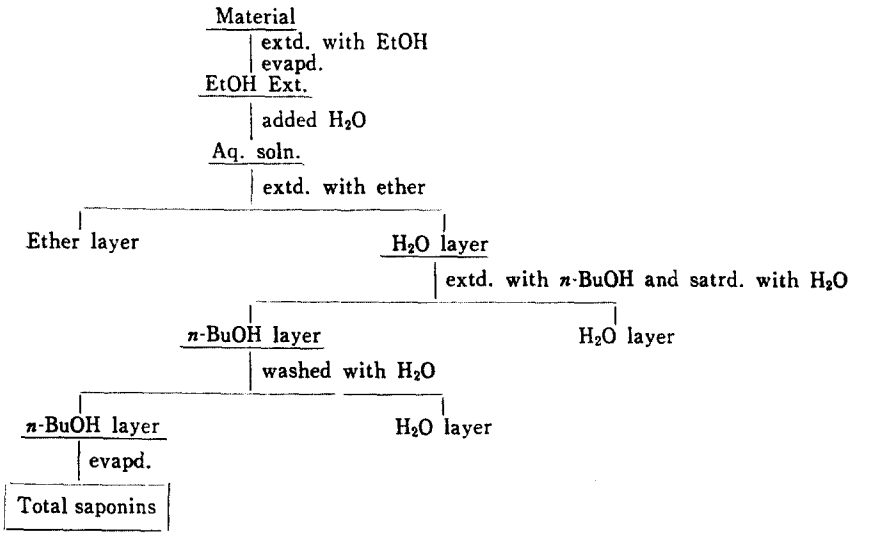

Chart 1. Extraction procedure of ginseng saponins

Each sample of ginseng tea which contained 5, 10, 15, or 20% of ginseng extract (total solid 60%) were prepared, and dried at 50° for 6 hours in an air dryer. Glucose and lactose were used for the excipient, the ratio of which was 8:2 by weight.

4. Preparation of Total Saponins from the Sample Ginseng Extract

Samples of 0.5g, 1g, 1.5g, and 2g of ginseng extract were dissolved individaually in 50m*l* of distilled water, poured into a 500m*l* volumetric flask and prepared total saponins according to the procedure of Shibata *et al.*[14]

5. Preparation of Total Saponins from the Sample Ginseng Tea

Samples of 5g and 10g of ginseng tea were dissolved individually in 50m*l* of distilled water, poured into a 500m*l* volumetric flask, and prepared total saponins as the above.

6. Partitive Determination of Individual Saponins

Prepared total saponins are dissolved in 5m*l* of methanol, taken exactly 1μl (10-20μg of total saponins/μl) using microsyringe which was washed with the saponin solution more than 10 times.

Then dropped on a glass-SiO2 rod, dried at 40° and develop in a developing chamder (solvents: $CHCl_3$: CH_3OH: H_2O=65:10, lower). After devloping, the solvent on the rod is removed with air current, and the saponin patterns are partitively and quantitatvely determined by the quantitative thin layer chromatography using apparatus (Iatron, Thinchrograph TFG-10) equipped with a flame ionization detector.

(Conditions: Apparatus; Iatron, Thinchrograph TFG-10,
Detector: H_2 -FID, Flow late; H_2, 167m*l*/min,
TLC: $CHCl_3$: CH_3OH: H_2O=65:35:10, Lower/SiO_2 rod.)

On the other hand, to compare with the standard method (TLC plate, ascending method) and to proof the individual saponins, the saponin solution is spotted on the preparative silcagel plate.

The plate was activated at 110° for an hour and cooled at room temperature before use.

After developing plate in the same solvent as the above the glass plate was dried and sprayed, then the R_f values of the individual saponins were measured(spray reagents, anisaldehyde: acetic acid: methanol: sulfuric acid=0.5:10:85:5).

Results and Discussion

First we determined the variation of the individual saponin peak areas. The total saponin was separated from 5g of each sample ginseng tea (which contains 5, 10, 15, 20% of ginseng extract). Also the total saponins of 0.5g and 1g of sample ginseng excract were separated. All of the individual saponins were separately dissolved in 5m*l* of methanol and 1μl of the saponin solutions were dropped individually on the glass-SiO_2 rod, and each peak was determined quantitatively.

The results were as shown in Fig. 2-7.

The peak area was calculated from the integrator and the ratio of each area was determined from the integrating curve. Individual peak areas are proportionally increased corresponding to total saponins increment as shown in Fig. 1. But some of the peak areas were not increased quantitatively according to the content of the raw ginseng extract.

This is true in the case of the sample tea which contained a higher concentration of ginseng extract more than that of the low concentration tea. To determine the individual saponins quantitatively, the optimum quantity of ginseng extract in the sample was in the range of 0.5-1.5g of ginseng extract. Table I shows that the most suitable data could be obtained when the quantity of sample tea was 10g and the concentration of ginseng extract was 5% by weight.

The sample tea which contained a high concentration of extract was more variable to the mean. It indicates that the greater volume of low concentration sample tea is better than that of the lesser volume, higher concentration tea.

The main cause of error in the case of ginseng tea was from the mixing procedure

of the ginseng extract and the excipient. Another cause of error was the weighing of sample tea.

Error is greater in the high concentration tea in saponin content than the low concentration tea in saponin content in spite of the same amount of error in weighing.

The partitive analytical method was adequate to estimate whether the ginseng extract used for the products (sample) and the raw ginseng extract were the same quality or not.

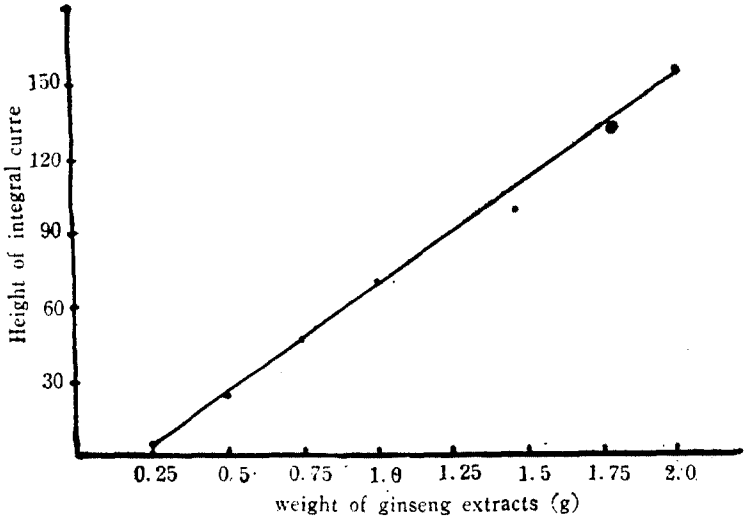

Fig. 1. Total saponin curve of ginseng tea and extract.

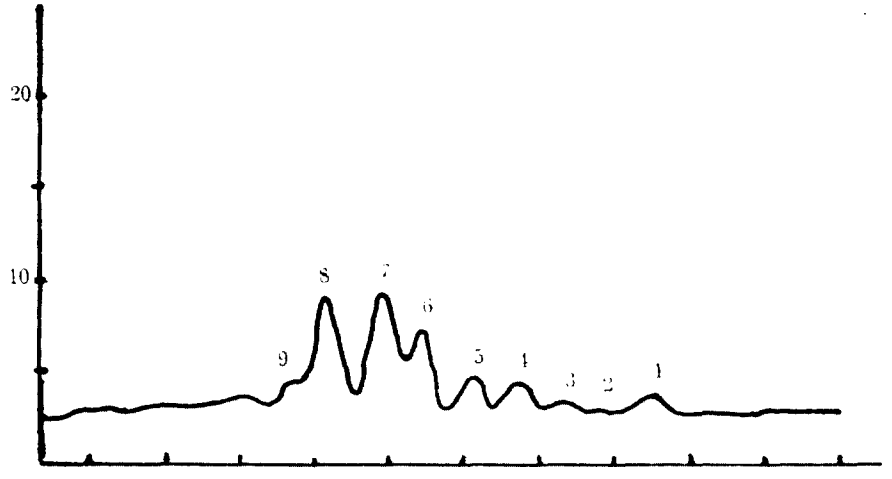

Fig. 2. Quantitative thin layer chromatogram of ginseng saponins extracted from 5g of ginseng tea containing 5% of ginseng extract.

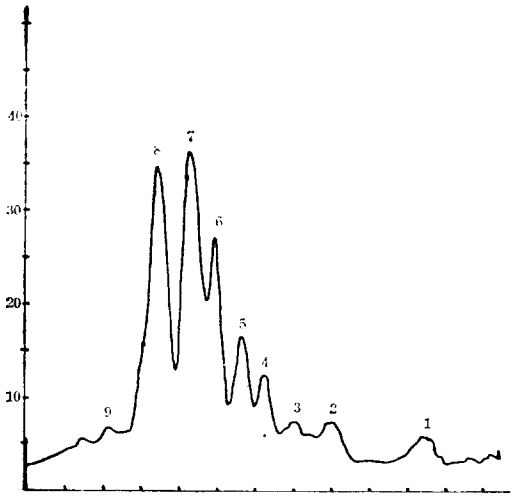

Fig. 3. Quantitative thin layer chromatogram of ginseng saponins extracted from 5% of ginseng tea containing 10% of ginseng extract.

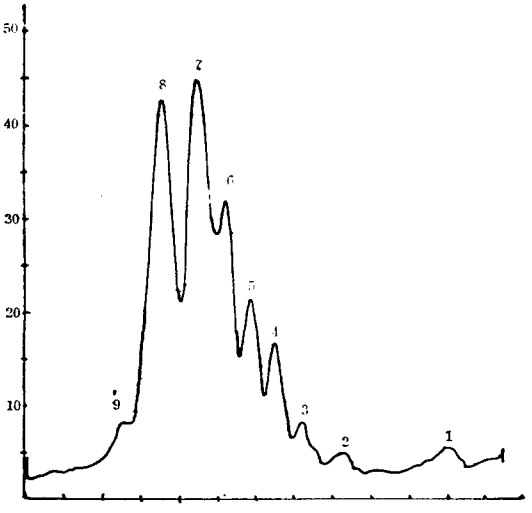

Fig. 4. Quantitative thin layer chromatogram of ginseng saponins extracted from 5g of ginseng tea containng 15% of ginseng extract.

Our method can be applied to quality control in the commercial plant. It can rule out at anytime whether other herb plant extract was added to the ginseng products or not.

Otherwise, to determine the accuracy of this method, the quantity of total saponin which was spotted on the glass-SiO_2 rod was compared with the amount recorded on the chart paper.

Table I shows that the average recovery percent was 99.5%. After comparing with the TLC method, the individual peak (peak No. 1—9) shown in Fig. 2-7 was idenifine as Rh, Rg_3, Rg_2, Rg_1 (F), Rd, Re Rb_2C, Rb_1, and Ra(O).

Table I. Recovery percentage of saponin in the partition determination of individual ginseng saponins.

Tea %×g \ peak No.	1	2	3	4	5	6	7	8	Total
10× 5	15	0	3.8	3.8	7.7	11.5	26.9	38.5	107.2
5×10	7	1.8	1.8	5.3	8.8	14.0	28.1	33.3	100.1
5× 5	5.5	2.8	2.8	5.5	5.5	13.9	25	38.9	99.9
10×10	9.5	1	1	9.5	9.5	11	26	30	97.5
15× 5	2.7	0.9	2.7	6.4	8.3	17	25	31	94.0
20× 5	1.2	2.4	3.6	9.7	13	20.7	24	24	98.6
Mean %	6.8	1.5	2.6	6.7	8.8	14.7	25.8	32.6	99.5

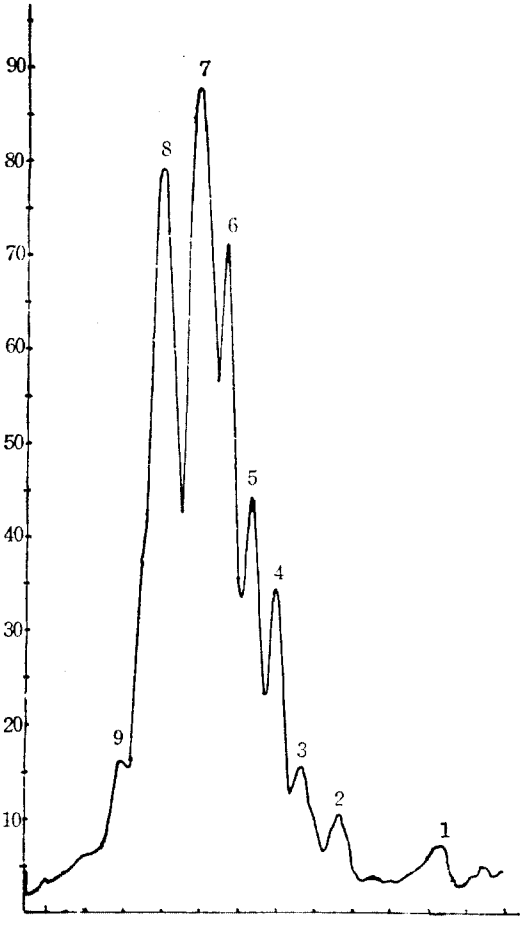

Fig. 5. Quantitative thin layer chromatogram of ginseng saponins extracted from 5g of ginseng tea containing 20% of ginseng extract.

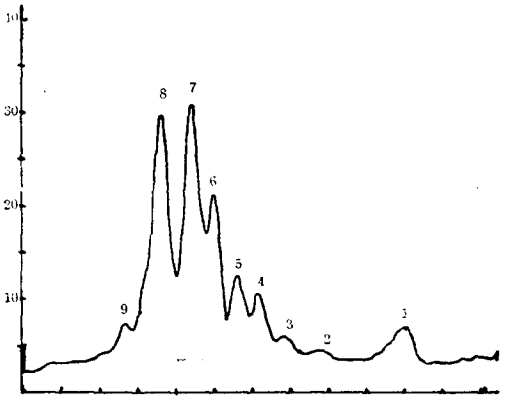

Fig. 6. Quantitative thin layer chromatogram of ginseng saponins extracted from 0.5g of ginseng extract.

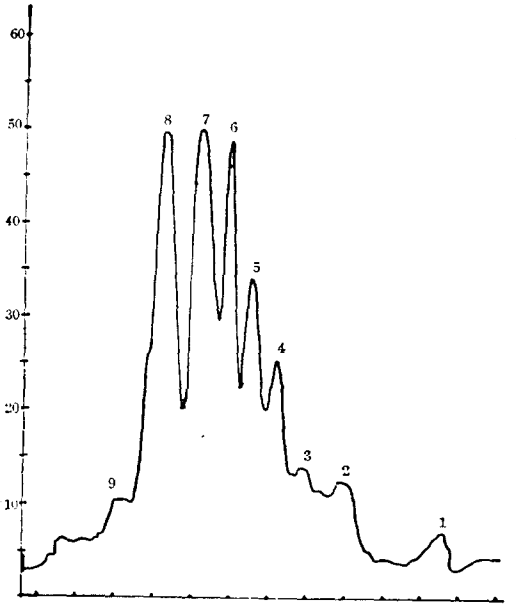

Fig. 7. Quantitative thin layer chromatogram of ginseng saponins extracted from 1g of ginseng extract.

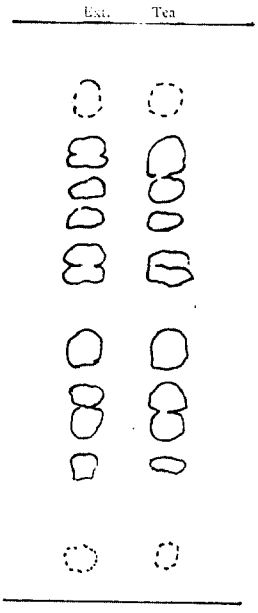

Fig. 8. Thin layer chromatogram of the saponins in ginseng extract (from lateral root) and ginseng tea.
plate: silica gel
solvent: $CHCl_3$: MeOH:H_2O=65 : 35 : 10, (lower).
Color reagent: anisaldehyde: acetic acid:methanol: sulfuric acid=0.5:10:85:5.

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인삼 사포닌에 관한 연구(I)

인삼 사포닌의 분별 정량에 대하여

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초 록

H_2-FID 박층자동검출장치를 사용하여 인삼차 및 인삼엑기스 중의 사포닌을 분별 정량하였다. 아울러 인삼차와 인삼엑기스의 품질관리에 응용하기 위하여 분석 최적조건과 정량시의 회수율에 관하여 조사하였으며 그 결과는 다음과 같았다.

1. 본 정량법은 품질관리에 있어서 제품생산에 사용하려고하는 인삼엑기스와 실제로 제품에 사용된 인삼엑기스와의 동일품질여부를 판별하는 데에 좋은 방법이 될 수 있었다.

2. 제품중의 총 사포닌 농도의 증가에 따라 거의 모든 각개 사포닌 peak면적도 정량적으로 증가되였다. 그러나 고농도로 인삼엑기스를 함유하는 인삼차를 시료로 사용했을 경우에는 몇몇 peak면적이 정량적으로 증가되지 않는 경우가 있었다.

3. 본 분석방법에 있어서 보다 정확한 분석을 위하여는 고농도시료를 소량 취하는 것보다 저농도시료를 다량 취하는 경우가 더 좋았다.

3. 분별정량에 있어서 제품중의 인삼엑기스함량은 0.5g~1.5g정도로 취하여 분석할 경우가 가장 좋았다.

4. 사용된 총 사포닌에 대한 각개 사포닌의 회수율은 99.5%였다.