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Selective removal of tannins from medicinal plant extracts using a collagen fiber adsorbent

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Abstract: A novel adsorbent, used for the selective removal of tannins from medicinal plant extracts, was prepared from bovine skin collagen fiber. Some typical active constituents of medicinal plants were selected as probe molecules to investigate the adsorption selectivity of the collagen fiber adsorbent to tannins. In batch adsorption experiments, the extent of adsorption of condensed tannins, including larch tannin, black wattle tannin and bayberry tannin, was 100%. The extent of adsorption of tannic acid and the hydrolyzable tannins was also 100%. In contrast, for the most active constituents of medicinal plants, their amounts adsorbed by collagen fiber adsorbent were limited. For procyanidin, the common active constituents in medicinal plant extracts, its extent of adsorption was also low, although it has a similar basic structure to condensed tannins. In comparison with traditionally used polyamide adsorbent, the collagen fiber adsorbent exhibited an obvious advantage in adsorption selectivity over tannins. Therefore, this study provides a novel and effective method for selective removal of tannins from medicinal plant extracts.

Keywords: collagen fiber adsorbent; larch tannin; black wattle tannin; bayberry tannin; tannic acid; medicinal plant extract; active constituent; procyanidins; adsorption selectivity; polyamide

INTRODUCTION

Herbal medicines have been used in medical practice for a long time and recognized especially as a valuable and readily available resource for healthcare.[1–3] There is a spectrum of herbal use from indigenous use of whole plants and extracts through the use of purified plant constituents. Extension of this trend eventually led to the use of isolated plant-derived pharmaceuticals and the development of modern medicine.[4] Therefore, the separation and purification of medicinal plant extracts are essentially important for the modernization of herbal medicines. In the processes of separation and purification, the removal of tannins is an essential procedure due to the fact that most medicinal plant extracts contain tannins.[5]

Tannins are widely distributed in the plant kingdom. They are found in approximately 80% of woody and 15% of herbaceous dicotyledonous species and can occur at high levels in some forages, feeds and foods.[6] Tannins comprise a group of phenolic compounds which has received much attention with respect to their possible nutritional and physiological actions.[7] On the basis of chemical structures, tannins are usually grouped into hydrolyzable tannins and condensed tannins.

It has been reported that some kinds of tannin constituents can reduce the mutagenicity of a number of mutagens and have anticarcinogenic, antimicrobial and anti-AIDS activities, which are beneficial effects to animals and humans.[8] However, there is growing evidence that tannins, especially for hydrolyzable tannins, produce adverse effects in certain animals and humans.[9] For example, inclusion of tannins in the diet can lead to perturbation of mineral adsorption from the intestinal canal,[10] a decrease in body-weight gain and growth retardation,[11] and inhibition of digestive enzymes.[12] Tannic acid had been shown to produce hepatic necrosis in humans and grazing animals.[8] Tannins were shown to bind epithelial proteins and cause precipitation. They then penetrate through the superficial cells and induce liver damage.[12] The patient will suffer loss of appetite, nausea, diarrhea and headache when taking medicines containing tannins.[13] Some kinds of tannins have antioxidant activity in small doses, but treatment with larger doses will possibly induce some cell toxicity.[14] A series of problems would be caused if tannins were not removed from injections made from herbal medicines. For example, tannins injected into the human body would cause jaundice and liver putrescence;[15] the

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injection site of the patient would be red and swollen;[16] repeated injection might cause local callosity and putrescence of tissue, and speed up agglomeration of erythrocyte to form precipitates.[17] More and more evidence indicates that hydrolyzable tannins are more toxic than condensed tannins.[18,19] Therefore, removal of tannins from medicinal plant extracts has received much attention, especially the removal of hydrolyzable tannins.

Many active constituents of herbal medicines contain phenolic structures, such as flavone, isoflavone, baicalin and chlorogenic acid, and these are similar to tannins. They have nearly the same solubility as tannins in water and organic agents. Therefore, selective removal of tannins from herbal medicines is usually very difficult.

Several methods have been tried to remove tannins from medicinal plant extracts, such as precipitation with gelatin, precipitation with acid–alcohol or alkali–alcohol, and adsorbtion by macroreticular resins such as polyamide.[17] Precipitation by gelatin impairs quality and storage stability of products due to the fact that gelatin is water-soluble and it is inevitable that some gelatin remains in treated solutions. Precipitation with acid–alcohol or alkali–alcohol has no specific selectivity for tannins and the loss of the active constituents is considerable. It has been reported that polyamide has high adsorption capacity for tannins, but its adsorption selectivity is doubtful.

The collagen molecule is composed of three polypeptide chains with triple helical structure, and they are aggregated through hydrogen bonding to form water-insoluble collagen fibers. The water-insoluble characteristic of collagen fibers implies that it can be used as an immobile phase for adsorption of tannins, which might avoid the disadvantage of gelatin. Tannins are traditionally used as tanning agents in leather manufacture. The studies indicate that tannins first approach the surface of the collagen fiber by hydrophobic bonding, and then combine with collagen fiber by multi-hydrogen bonding.[20,21] To achieve this stable association the molecules need to have sufficient molecular size, and phenolic hydroxyls are necessary. Most active constituents in medicinal plant extracts are not able to form multi-hydrogen bonding with collagen fiber due to the fact that they do not have enough phenolic hydroxyls or lack the structure of the adjacent phenolic hydroxyl. Indeed, they are 'non-tannins' in the tanning industry, meaning they have no ability to form stable associations with collagen fibers. This fact strongly suggests that collagen fiber could be used to remove tannins selectively from medicinal plant extracts. However raw bovine skin collagen fiber is easily attacked by chemicals and bacteria and possesses limited hydrothermal stability. Therefore, chemical modification of collagen fiber needs to be carried out.

In the present work, a novel adsorbent was prepared using bovine skin collagen and its adsorption selectivity to tannins was extensively investigated. As a comparison, the adsorption behaviors of polyamide to tannins and active constituents of medicinal plants are also presented.

EXPERIMENT

Materials

Baicalin (**1**), naringin (**2**), genistin (**3**), genistein (**4**) and resveratrol (**5**) (Fig 1), the typical active constituents of medicinal plants, were purchased from Hui Ke Botanical Research and Development Corp Ltd, China. The purity of the components was $\geq$98.0%.

Catechin (C) (**6**) and epicatechin (EC) (**7**) were purchased from Sigma Co Ltd. TrGG (β-1,2,3-tri-*O*-galloyl-D-glucose) and PGG (β-1,2,3,4,6-penta-*O*-galloyl-D-glucose) were provided by Unilever Co Ltd.

Procyanidins, extracted from grape seed, were purchased from Tianjing Jian Feng Co Ltd, China, the primary components of which are catechin, epicatechin, procyanidin B-1 (**8**) and procyanidin B-2 (**9**). The content of oligomer procyanidins was $\geqslant$85.0%.

Larch tannin (**10**) extract, black wattle tannin (**11**) extract and bayberry tannin (**12**) extract were obtained from the barks of *Larix gmelin, Acacia mearnsii* and *Myrica esculenta*, respectively, by extraction with acetone–water solution (1:1, v/v), and then spray drying. Their tannin contents were 72.7, 76.3 and 75.4%, respectively, determined by the hide powder method. Tannic acid (**13**) was an analytical regent and its average molecular weight was 1700.

Polyamide was purchased from Shanghai Medicinal Agents Co Ltd China, and prepared for column chromatography with a particle size of 0.25–0.5 mm.

Preparation of bovine skin collagen fiber

The bovine skin collagen fiber was prepared by a standard procedure.[22] Briefly, bovine skin was cleaned, limed, split and delimed according to the principle of leather processing, so that non-collagen components could be removed. Then the bovine skin was treated with acetic acid solution (16 g dm^{-3}) three times to remove mineral substances. The pH value was then adjusted to 4.8–5.0 with acetic acid–sodium acetate buffer solution and the bovine skin was subsequently dehydrated with absolute ethyl alcohol, dried in vacuum to moisture content $\leqslant$10%, ground and sieved to yield bovine skin collagen fiber with particle size 1.0–2.5 mm, moisture $\leqslant$12% and ash content $\leqslant$0.3%.

Preparation of collagen fiber adsorbent

Natural collagen fiber is easily attacked be bacteria and chemicals and has low hydrothermal stability. Therefore, a proper cross-linking reaction of collagen fibers should be carried out. Gutaraldehyde has been proved to be a suitable cross-linking agent due to its reaction activity with the -NH_2 group of collagen fibers.[20]

Samples of 15 g bovine skin collagen fiber and 1.5 g glutaraldehyde were add to 300 cm^3 distilled water

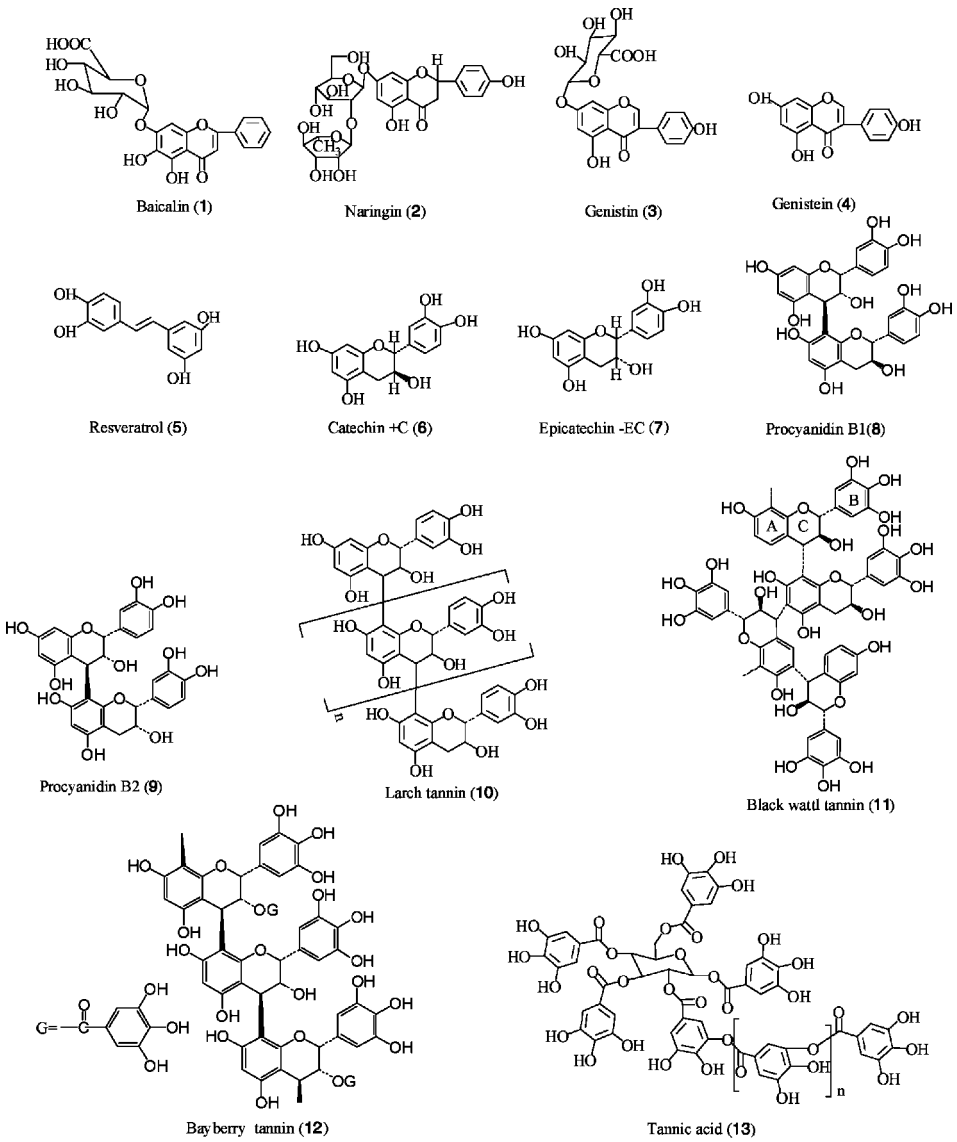

Figure 1. Molecular structures of active constituents of medicinal plants and the illustration of tannin structures.

and reacted at room temperature for 4 h, and then further reacted at 45 °C for 4 h. After washing with distilled water and drying at 50 °C in vacuum, the collagen fiber adsorbent with increased thermal and chemical stabilities was obtained. The decomposition temperature of collagen fiber adsorbent was 80–86 °C, detected by differential scanning calorimetry (DSC).

Adsorption procedures

Adsorption behavior of collagen fiber adsorbent in tannins–active constituents solution

A sample of 50 mg of each active constituent of medicinal plants was dissolved in ethanol–water solution (v/v = 50:50) and scaled to 50 cm^3. Samples of 200 mg larch tannin, black wattle tannin, bayberry tannin, tannic acid and procyanidins were dissolved in ethanol–water solution (v/v = 10:90), respectively, and scaled to 100 cm^3. Of each active constituent solution, 2 cm^3 were taken and mixed with 50 cm^3 tannin solution, and then the total volume was scaled to 100 cm^3 with distilled water. The content of tannin extract in the mixture solution was 1000 mg dm^{-3}, and the content of each active constituent was 20 mg dm^{-3}. In the mixture was suspended 0.5 g collagen fiber adsorbent and it was shaken at 25 °C for 24 h. The contents of the mixture before and after adsorption were analyzed by Agilent 1100 high performance liquid chromatography (HPLC), and the extent of adsorption of each component was calculated.

Adsorption behavior of polyamide in tannins–active constituents solution

The adsorption experiment was the same as in the previous section, but the adsorbent was polyamide instead of collagen fiber adsorbent.

RESULT AND DISCUSSION

Selective adsorption of collagen fiber adsorbent to condensed tannins

Figure 2 shows that the larch tannin in the mixture solution was completely adsorbed by the collagen fiber

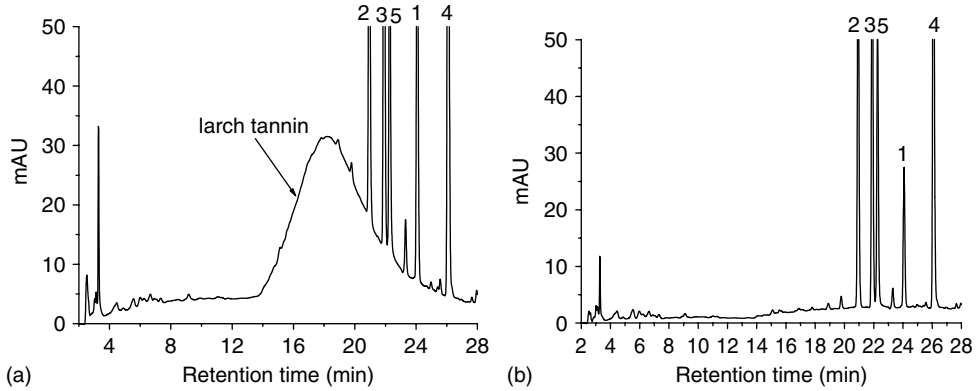

Figure 2. HPLC pattern of larch tannin–active constituents solution adsorbed by collagen fiber adsorbent. (a) Before adsorption; (b) after adsorption. Mobile phase: A = H_2O (0.5% H_3PO_4), B = CH_3OH; column, Hypersil ODS C_{18}, 4.0 × 250, 5 μm; flow rate, 0.8 cm^3 min^{-1}; column temperature, 30 °C; detector, DAD, 280 nm; gradient, 0–10 min with 18–24% B, 10–25 min with 24–70% B, 25–35 min with 70% B; injection volume, 20 × 10^{-6} dm^{-3}.

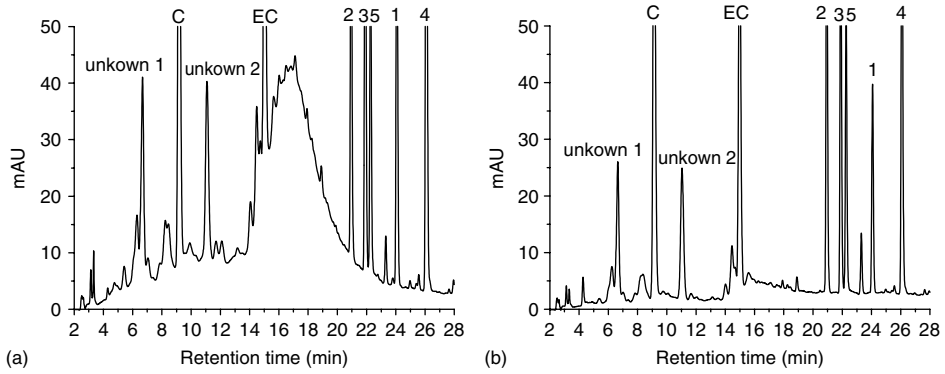

Figure 3. HPLC pattern of procyanidins–active constituents solution adsorbed by collagen fiber adsorbent (chromatographic conditions were as the same as Fig 2). (a) Before adsorption; (b) after adsorption.

Table 1. Extent of adsorption of condensed tannins and active constituents of medicinal plants in mixed solutions on collagen fiber adsorbent[a]

	Extent of adsorption, %					
System	Tannin	Naringin (**2**)	genistin (**3**)	Resveratrol (**5**)	Baicalin (**1**)	Genistein (**4**)
Larch tannin–active constituents	100	6.7	19.3	18.7	67.4	33.4
Black wattle tannin–active constituents	100	5.4	14.9	19.3	21.2	31.9
Bayberry tannin–active constituents	100	6.5	26.2	21.2	0.1	30.5

[a] Extent of adsorption = (peak area before adsorption − peak area after adsorption)/peak area before adsorption × 100%.

adsorbent. For the solution containing black wattle tannin and bayberry tannin, similar HPLC patterns were obtained. In general, collagen fiber adsorbent could effectively remove condensed tannins from the medicinal plant extracts, as shown in Table 1.

For most of the active constituents, their extents of adsorption on collagen fiber adsorbent were approximately the same when mixed with different tannins. However, baicalin is an exception. Its adsorption extent was 67.4% in the solution containing larch tannin, which dramatically decreased to 21.2 and 0.1% in the solutions including black wattle tannin or bayberry tannin. As shown in Fig 1, the B ring of larch tannin is of catechol structure, but those of black wattle tannin and bayberry tannin are of pyrogallol structure. In addition, part of the C ring of bayberry tannin is attached with a galloyl group. It may be these differences in tannins structures which remarkably affect the adsorption extent of some particular active constituents. The mechanism and the regularity of the effect should be further studied.

Adsorption behavior of collagen fiber adsorbent in procyanidins–active constituents solution

Commercial procyanidins, extracted from grape seed, are a mixture that contains oligomer procyanidins and polymeric procyanidins. The former include catechin (**6**), epicatechin (**7**) and their oligomer (polymerization degree <6) and are usually considered as active constituents of plant extracts, due to their antioxidative activity.[23–25] The latter are indeed the condensed tannins, having nearly the same structure as larch tannin. As shown in Fig 3, the peaks whose retention times were 2.0–16.0 min were the catechin,

Table 2. Extent of adsorption of procyanidins and active constituents on collagen fiber adsorbent[a]

Component	Before adsorption		After adsorption		Extent of adsorption (%)
	Retention time (min)	Peak area	Retention time (min)	Peak area	
Unknown 1	6.677	416.8	6.647	311.5	25.3
Catechin C	9.174	1135.4	9.131	1029.1	9.4
Unknown 2	11.085	453.3	11.043	334.5	26.2
Epcatechin EC	15.017	966.2	14.978	876.4	9.3
Naringin (**2**)	20.946	652.7	20.942	608.3	6.8
Genistin (**3**)	21.909	637.8	21.899	507.9	20.4
Resveratrol (**5**)	22.273	484.1	22.266	385.4	20.4
Baicalin (**1**)	24.075	629.4	24.072	280.4	55.4
Genistein (**4**)	26.087	1287.2	26.085	723.5	43.4

[a] Extent of adsorption = (peak area before adsorption − peak area after adsorption)/peak area before adsorption × 100%.

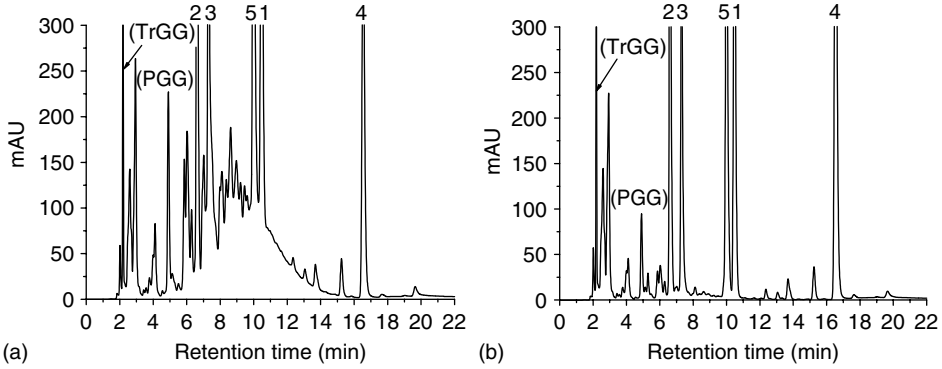

Figure 4. HPLC pattern of tannic acid–active constituents solution adsorbed by collagen fiber adsorbent. (a) Before adsorption; (b) after adsorption. Mobile phase: A = H_2O:CH_3CN:H_3PO_4 = (v/v/v = 850:150:5), B = H_2O:CH_3CN:H_3PO_4 = (v/v/v = 150:850:5); column, Hypersil ODS C_{18}, 4.0 × 250, 5 μm; flow rate, 1.0 cm^3 min^{-1}; column temperature, 40 °C; detector, DAD, 280 nm; gradient, 0–20 min with 6–30% B; injection volume, 20 × 10^{-6} dm^3.

epicatechin and oligomers of procyanidins, and the peaks whose retention times were 16.0–26.0 min were a characteristic pattern of polymeric procyanidins (condensed tannins). The interesting result is that the polymeric procyanidins in procyanidins were nearly completely removed, whilst most of the oligomer procyanidins were left in solution. Meanwhile, the adsorption extent of the typical active constituents of medicinal plants was also considerably lower, as shown in Fig 3 and Table 2. This characteristic of collagen fiber adsorbent would be very valuable in treating medicinal plants extracts containing procyanidins.

Selective adsorption of collagen fiber adsorbent to tannic acid

Tannic acid is also a mixture and the molecular size of its components changes over a large range, although its average molecular weight is 1700. The peaks with retention time 2.0–5.5 min in Fig 4 were the components with smaller molecular size in tannic acid, which had relatively lower adsorption capacity onto collagen fiber adsorbent. In fact, it was shown that the extent of adsorption of tannic acid components increased with the increase in their molecular size. For example, the adsorption extent of TrGG (1,2,3-tri-*O*-galloyl-β-D-glucose) was negligible, while that of PGG (1,2,3,4,6-penta-*O*-galloyl-β-D-glucose) was 71.3%. For the components which have more than five galloyl groups (retention time 5.5–16.0 min), they were completely adsorbed by collagen fiber adsorbent. Similarly, the extent of adsorption of the active constituents of medicinal plants was significantly lower than that of tannic acid.

Adsorption behavior of polyamide adsorbent towards condensed tannins and tannic acid

Polyamide has been considered as a useful adsorbent for the removal of tannins in practice. Its adsorption behavior in larch tannin–active constituents solution is shown in Fig 5. For black wattle tannin–active constituents solution and bayberry tannin–active constituents solution, similar HPLC patterns were obtained. It was found that the extent of removal of larch tannin, black wattle tannin and bayberry tannin was only 69.0, 82.7 and 74.6% respectively. Meanwhile, for most of the active constituents, their extents of adsorption on polyamide were obviously higher compared to those on collagen fiber adsorbent, as illustrated in Table 3. Therefore, the adsorption selectivity and adsorption capacity of polyamide for condensed tannins are poor in comparison with collagen fiber adsorbent.

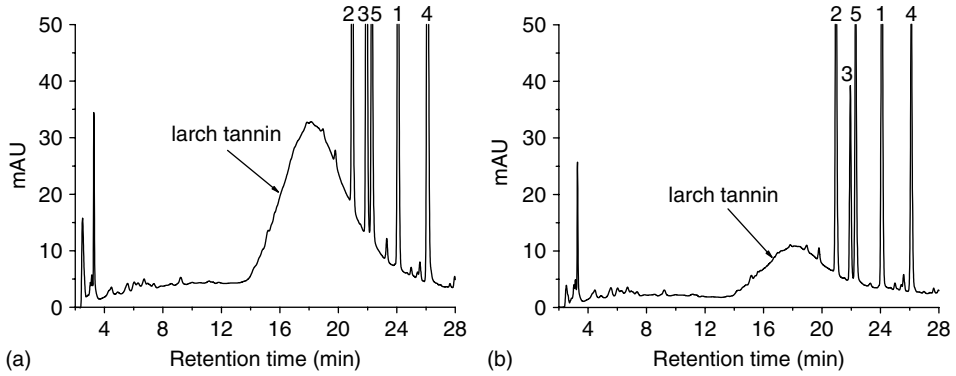

Figure 5. HPLC pattern of larch tannin–active constituents solution adsorbed by polyamide (chromatographic conditions were as the same as Fig 2). (a) Before adsorption; (b) after adsorption.

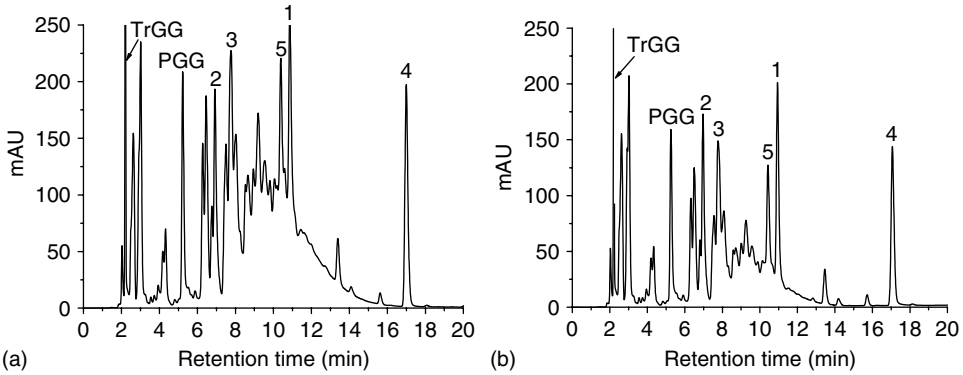

Figure 6. HPLC pattern of tannic acid–active constituents solution adsorbed by polyamide (chromatographic conditions were as the same as Fig 4). (a) Before adsorption (b) after adsorption.

Table 3. Extent of adsorption of tannins and active constituents on polyamide

	Extent of adsorption (%)					
System	Tannins	Naringin (**2**)	Genistin (**3**)	Resveratrol (**5**)	Baicalin (**1**)	Genistein (**4**)
Active constituents	—	10.6	77.7	31.4	0.0	71.0
Larch tannin–active constituents	69.0	7.2	68.5	25.9	6.4	63.6
Black wattle tannin–active constituents	82.7	7.5	67.9	24.7	0.0	60.5
Bayberry tannin–active constituents	74.6	7.5	71.5	26.6	0.0	61.9

When the tannic acid–active constituents solution was adsorbed by polyamide, the components of tannic acid with larger molecular size were adsorbed to a certain degree, but not completely, as shown in Fig 6. At the same time, the active constituents were also significantly adsorbed by polyamide. This fact indicated that the adsorption capacity and adsorption selectivity of polyamide to hydrolyzable tannins are also limited.

CONCLUSION

Collagen fiber, crosslinked by glutaraldehyde, could effectively remove condensed tannins and hydrolyzable tannins from medicinal plant extracts. In comparison with conventionally used polyamide, its advantage in adsorption capacity and selectivity are remarkable.

In this study, a certain amount of collagen fiber adsorbent was used for all the imitative systems in which the ratio of tannin content and active constituent content was fixed. In these conditions, all kinds of the tannins could be completely removed. However, the adsorption extent of some active constituents was considerable. In consideration of competition of the adsorptions, the conditions of using the collagen fiber adsorbent should be further optimized. For example, the amount of adsorbent used is an important factor influencing adsorption selectivity, and, in fact, the adsorbent used should be changed according to the actual content of tannins in the medicinal plant extracts. This research is being undertaken in the authors' laboratory.

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