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***Article***

# Human Renin Inhibitory Activity in Legumes

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**The effects of minor legume (legumes except soybeans and peanuts) extracts on human renin activity were investigated. All minor legume extracts showed renin inhibitory activity in this study. Minor legumes can be divided into two groups, high renin inhibitory (Group 1) and low renin inhibitory (Group 2) groups. Group 1 minor legumes include *Vigna angularis*, *V. anguiculata* subsp. *sesquipendalis*, and *Phascolus vulgaris*. Group 2 minor legumes include *P. coccineus* var. *albus*, *P. coccineus*, *Lablab purpureus*, *Canavalia gladiata*, *Vicia faba*, and *Pisum sativum*. This is the first demonstration of renin inhibitory activity in minor legumes.**

**Key words**: angiotensin; bean; inhibitor; legume; renin

## Introduction

The renin-angiotensin-aldosterone system (RAS) plays an essential role in blood pressure control in animals. Renin catalyzes the liberation of angiotensin I from plasma substrate angiotensinogen. The produced decapeptide angiotensin I is an inactive peptide activated by angiotensin converting enzyme (ACE).

ACE cleaves C-terminal dipeptide from angiotensin I. The produced angiotensin II raises blood pressure by vasoconstriction as well as stimulation of the synthesis and release of aldosterone.

Thus, renin is a key enzyme of RAS. The secretion of renin into the circulation is controlled by several stimuli [1]. Renin activity is also regulated by renin-binding protein (RnBP), an endogenous renin inhibitor first isolated from porcine kidney [2-7]. The expression and characterization of recombinant RnBPs has been reported [8-12]. RnBP inhibits renin activity by forming a complex of renin, called high molecular weight renin [2, 13-15].

ACE has been used as a target enzyme in RAS for screening inhibitors because of its simple assay method; however, renin is a rate-limiting enzyme in RAS, so it was not used

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The abbreviation used are: RAS, renin-angiotensin-aldosterone system; ACE, angiotensin converting enzyme; rh, recombinant human; RnBP, renin binding protein; Nma, *N*-methylanthranyl; Dnp, 2, 4 dinitrophenol.

because the measurement is very complicated. Recently, we expressed recombinant (rh) human renin in *Escherichia coli* cells, refolded and activated by trypsin [16]. Using rh-renin as a target enzyme, we screened the renin inhibitory activity in fermented soybean paste (miso) and found that soybean, the major ingredient of miso, had renin inhibitory activity [16]. In the present study, we screened renin inhibitory activity in minor legumes (legumes except soybean and peanut) and found that they had renin inhibitory activity. Minor legumes are classified into two groups, high renin inhibitory (Group 1) and low renin inhibitory (Group 2) groups.

**Materials and Methods**

Minor legumes were obtained from Fukutane Co. Ltd. (Fukui, Japan). The fluorogenic substrate for human renin *N*-methylanthranyl (Nma)-Ile-His- Pro-Phe-His-Leu-Val-Ile-Thr-His-Lys-2, 4 dinitrophenyl (Dnp)$NH_2$ was custom-synthesized at the Peptide Institute (Osaka, Japan). Recombinant human renin was prepared by the method of Takahashi *et al.* [16] or obtained from Cayman (LA, USA).

Twenty-five grams of legumes were soaked in 250 ml of distilled water at room temperature overnight, autoclaved at 115 °C for 30 min and then cooled to room temperature. The sample was homogenized in a food processor (MX-T10G, TOSHIBA, Tokyo, Japan) and centrifuged at 10,000 x g for 30min. The supernatant was applied to a Sep-Pac Vac C18 35 cc (Waters, Massachusetts, USA) that had been equilibrated with distilled water. The column had been washed with distilled water, and then the adsorbed materials were eluted with methanol. The eluate was evaporated to dryness, and the dry matter was dissolved in distilled water to evaluate the renin inhibitory activity.

The hydrolysis of the fluorogenic substrate at the Leu-Val bond was spectrophotometrically determined. The reaction mixture contained 38 µl of 50 mM sodium phosphate buffer, pH 6.5, 0.1 M NaCl, 0.02% $NaN_3$, 0.02% Tween 20, 2 µl of 1mM Nma-Ile-His-Pro-Phe- His-Leu-Val-Ile-Thr-His-Lys-Dnp$NH_2$ in DMSO, 5 µl of human renin solution (5 µg/ml of 20 mM sodium phosphate buffer, pH 6.5, 1 mM EDTA, 10 µM leupeptin, 0.1% heat-inactivated bovine serum albumin), and 5 µl of bean extracts in a total volume of 50 µl. The reaction mixture was incubated at 37 °C for 120 min and the reaction was terminated by adding 0.2 ml of distilled water. The increase of fluorescence intensity was measured at an emission wavelength of 440 nm upon excitation at 340 nm. The sample concentration required to inhibit 50% of renin activity under the assay condition was taken as the $IC_{50}$ value.

SDS-PAGE was performed by the method of Laemmli [17] using 5-20% polyacrylamide gel (E-T520L, ATTO, Tokyo, Japan). After electrophoresis, the proteins were stained with Coomassie brilliant blue R-250.

**Results and Discussion**

Fig. 1 shows the classification of minor legumes. Minor legumes used in this study were

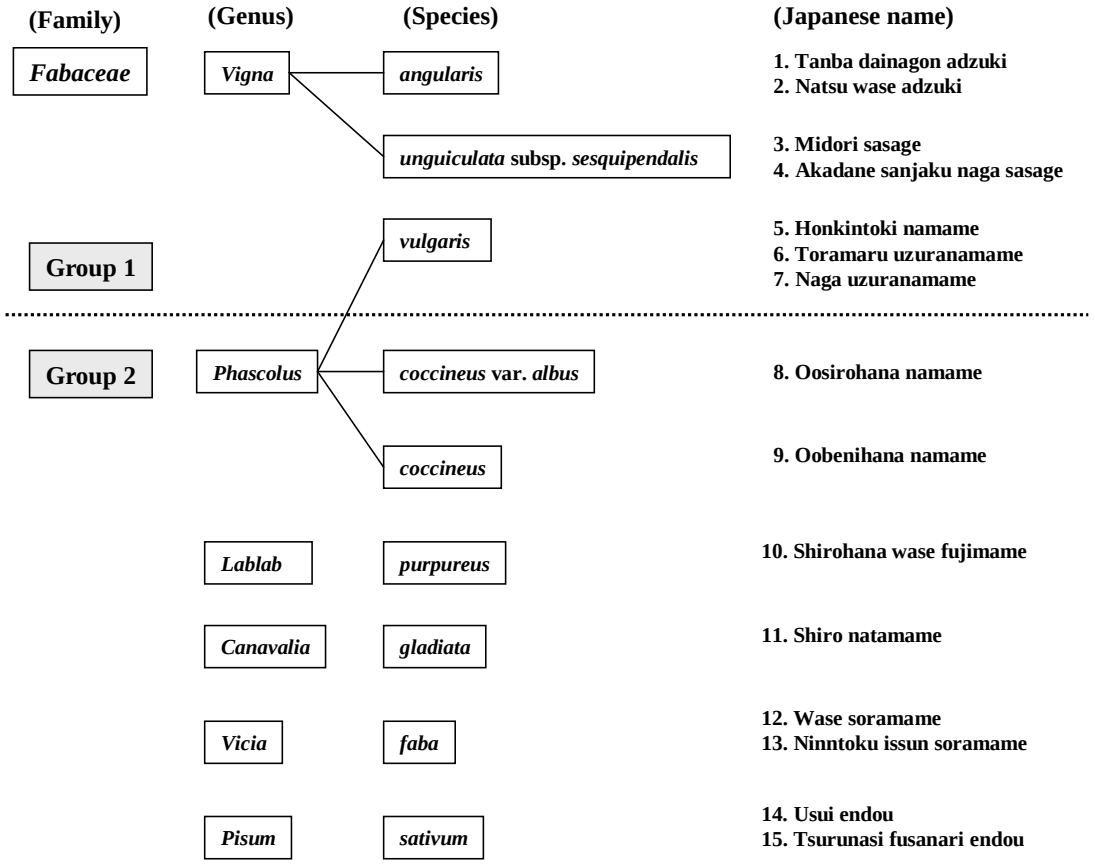


**Fig. 1.** Classification of Minor Legumes. Minor legumes classified into six groups.

classified as 6 genera, *Vigna*, *Phascolus*, *Lablab*, *Canavalia*, *Vicia*, and *Pisum*. In the present study, we used 15 minor legumes including 6 genera for renin inhibitory activity.

The renin inhibitory activities of 15 legume extracts were tested using rh-renin as the target enzyme and newly developed human renin substrate. In our previous study, we used hog angiotensinogen as a substrate and angiotensin I radioimmunoassay to evaluate human renin inhibitory activity. The assay method was very sensitive but we need a relatively long time of 2 days to measure renin activity. With the present assay method using a synthetic substrate, we can measure human renin activity within a few hours. Using a synthetic substrate and recombinant human renin, we evaluate the renin inhibitory activity of the legume extracts. Very interestingly, all minor legume extracts showed human renin inhibitory activity, as shown in Fig. 2. Partitional clustering analysis [18] of the inhibitory data clearly showed that minor legumes could be classified into two groups according to their renin inhibitory activity, a high renin inhibitory group (Group 1) and low renin inhibitory activity group (Group 2). Group 1 legumes included *Vigna angularis* (Tanba dainagon adzuki and Natsu wase adzuki), *V. anguiculata* subsp. *sesquipendalis* (Midori sasage and Akadane sanjaku naga sasage), and *Phascolus vulgaris* (Honkinntoki namame, Toramaru uzuranamame, and Naga

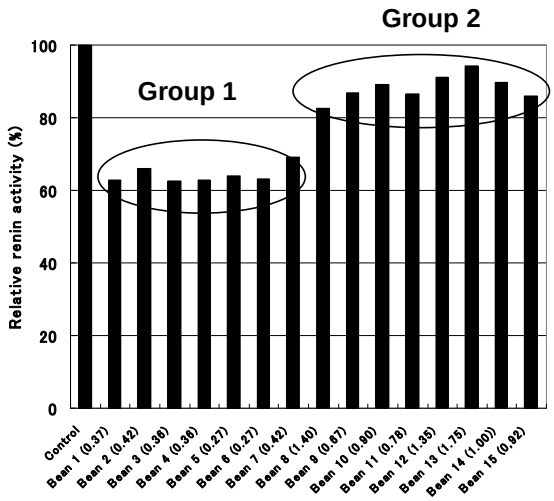


**Fig. 2.** Inhibition of Human Renin Activity by Minor Legume Extracts.

Legume extracts eluted from the Sep-Pak Vac C18 35 cc column were used for renin inhibition assay. The final concentrations of the extracts were 0.2 mg/ml. Renin activity without legume extracts was used as a control. Beans 1 to 15 are the same as bean names in Fig. 1. Beans 1 to 15 extracts (n = 5) showed significant difference compared to control at $p<0.05$ by student's t-test. $IC_{50}$ values of bean extracts (mg/ml) were indicated in the parentheses.

uzuranamame). Group 2 legumes included *P. coccineus* (Ooshirohana namame), *P. coccineus* var. *albus* (Oobenihan namame), *Lablab purpureus* (Shirohana wase fujimame), *Canavalia gladiata* (Shiro natamame), *Vicia faba* (Wase soramame and Ninntoku issun soramame), and *Pisum sativum* (Usui endou and Tsurunasi fusanari endou) (Fig. 2). The minor legume extracts showed several protein bands on SDS-PAGE (Fig. 3). Samples 1 to 7 showed major 30 kDa protein bands and minor proteins nearly the same mobility, but samples 8 to 15 showed various proteins with different motilities. These results suggest that beans could be divided into two groups according to their protein pattern.

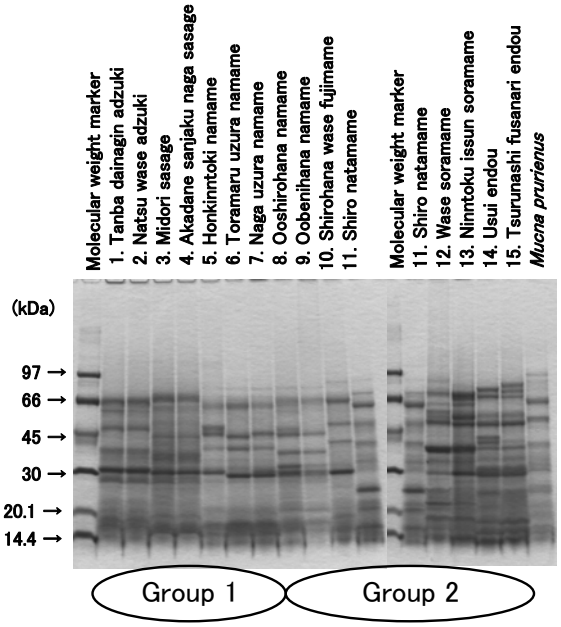


**Fig. 3.** SDS-PAGE of Minor Legume Extracts. Water extracts (10µl) of legumes were loaded on the gel. After electrophoresis, gels were stained with Coomassie Brilliant Blue R-250.

The grouping of minor legumes correlated very well with their protein pattern, shown in Fig. 3. $IC_{50}$ values of bean extracts were determined to be 0.27-0.42 mg/ml for Group 1 and 0.67-1.75 mg/ml for Group 2, respectively. Our previous results showed that fermented soybean, miso, had renin inhibitory activity derived from soybean. The $IC_{50}$ value of the soybean renin inhibitor was estimated to be 1.6-1.9 mg/ml [16]. These values are comparable to those of minor legumes. To our knowledge, this is the first demonstration of renin inhibitory activity in minor legumes. Our preliminary results showed that the molecular weights of renin inhibitory compound(s) in minor legumes were 500-1,500. The

identification of legume renin inhibitor may have scope to develop functional foods for regulating blood pressure.

**Conclusion**

Minor legumes, especially adzuki, have been used for traditional sweets for several centuries in Japan. In the present study, we discovered renin inhibitory activity in minor legume extracts. Among them, adzuki had relatively high renin inhibitory activity. The identification of active compound(s) will provide a possibility to develop antihypertensive functional foods using legumes.

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