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Biological Activities of the Water Soluble Fraction from the Overground Part of *Panax ginseng*

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Abstract

In the screening of those components with comparatively strong xanthine oxidase inhibition activities from *Panax ginseng* folium, trifolin, panasenoside, kaempferol and so far unconfirmed phenol whose chemical structure still without elucidation have been discovered as such components. Specific inhibitors of xanthine oxidase are thought to be therapeutically useful for the treatment of gout. Such opinion is considered to be useful to support the thought that ginseng folium is effective for the adjustment of gout. Although trifolin, panasenoside, and kaempferol are well known flavonoids, further studies on unidentified new phenols should be continued.

Introduction

It is assumed that efforts to reach the final goal of ginseng studies to declear its entity, there still remain approaches such as chemical and biological needed to be conducted on the overground part of *Panax ginseng* accumulated from 1955 to 1976[1-7] to make up the gap created inbetween. Studies conducted during this period only reported flavonoids, dammaran glycosides, organic acid, etc., however, biological and pharmacological studies on these compounts have been neglected for a long time with the exception of the study of *Panax ginseng* folium polysaccharide isolation and its anticomplementary activities.[8] In Korea and some parts of Japan, ginseng leaf decoctors are used as folk medicine for the alleviation of gout symptom. This study is intended to scientifically validate medicinal efficacy of this common folk medicine for gout. For the purpose of explaining this contention this researcher has screend from *Panax ginseng* folium those components that can effectively inhibit the enzymic activities of xanthine oxidase. As a result, the investigator has found that trifolin, panasenoside, kaempferol and new kinds of phenols whose chemical structure is still pending elucidation (hereinafter referred to as phenol A) are the very components in this category. In the course of this study it is also discovered that ginsenoside Rg_1, Re, Rd contained in *Panax ginseng* folium are excluded from the effective extent of xanthine oxidase inhibition activities.

Along with this experiment, from the watery extract of overground part of *Panax ginseng*, two polysaccarides, neutral and acidic, were isolated by the sephacryl S-400, DEAE-Sepharose CL-6B gel filtration techniques.

Experiment

Xanthine Oxidase Inhibition Test

Material : trifolin 10μg, panasenoside 10μg, kaempferol 10μ, phenol A 10μg, comparative test component (each 10μg), ginsenoside Re, Rg, Rd, chiisanoside, syringin, AKF (A flavonoid isolated from the folium of *Acanthopanax Koreanum)*

Xanthine oxidase and chemicals : xanthine oxidase from cow's milk was obtained from Sigma Chemical Co., sodium phosophast dibasic 12 hydrate and potassium phosphate monobasic were obtained from Sigma Chemical Company. The buffer used was Hasting-Sendroy's 1/15M potassium phosphate--sodium phosphate buffer, pH 7.5. The substrate solution, 0.15m M xanthine in water, was prepared immediately before use. Xanthine oxidase solution containing about 0.04 unit per ml in 1/5 M phosphate buffer, pH 7.5, was prepared immediately before use.

Sample Solution for Xanthine Oxidase Inhibition Test The test samples were prepared with 10μg/ml concentration respectively.

Assay of Enzyme Activity : The xanthine oxidase activity with xanthine was measured spectrophotometrically by the method of Kalckar[9] and Noro's Modification.[10] The assay mixture consisted of 1.0 ml of xanthine oxidase solution.

This mixture for assay was preincubated for 15 minutes at 30°C. Then, the substrate (0.15mM xanthine in water) was added and subjected to the incubation for 30minutes at 30°C.

The reaction was stopped by adding 1.0ml of IN HCl, and the absorbance of the assay mixture at 290 nm was measured spectrophotometrically. A blank was prepared in the same manner without enzyme solution added to the assay mixture after adding IN HCl. One unit of xanthine oxidase defined as the amount of xanthine oxidase producing 1μmol uric acid per min. at 25°C theoretically.

Estimation of Xanthine Oxidase Inhibition Activity : Xanthine oxidase inhibition activity in this test has been indicated by percentage of xanthine oxidase inhibition and calculated by the (1-B/A) x 100 formula. In this formula A represents the activity of the enzyme before the test samples were added ; while B, the activity of the enzyme after the addition of the test samples.

Results and Discussions

The chart reflects the relative values of the xanthine oxidase inhibition percentage, based on allopurinol, of trifolin, panasenoside and kaempferol already reported by Gomatsu, et al., and new phenol A, whose structure elucidation still undetermined, separated from *Panax ginseng* folium, the following significances can be noticed. Phenol A, trifolin and panasenoside possess the strongest inhibition activity. Kaempferol which is a genin of trifolin possesses the moderate value in the percentage, while ginsenoside Rg, Re, etc. or dammaran glycosides of the ginseng do not come in the effective range in terms

Table 1. Xanthine oxidase inhibition percent of the phenols from ginseng leaves and related compounds

Compound	^290nm[a)]	% inhibition
Normal	0.015	—
Trifolin	0.002	93
Panasenoside	0.001	93
Phenol A	0.001	94
Kaempferol	0.005	70
Morin	0.005	70
Ginsenoside Re	0.011	27
Syringin	0.015-12	0-20
Chiisanoside	0.007	33
Allopurinol[b)]	0.001-0.000	100

a) the absorbances of the assay samples measured at 290 nm spectrophotometrically
b) commercially available for gout treatment in clinical

of statistical significance.

Consequently, the effective components of ginseng leaves that can be for the reduction of an pathological symptom of gout are flavonoides such as trifolin, panasenoside, kampferol and new phenol A, not belong flavonoid family which chemical structure elucidation is still undetermined . The findings flavonoids except trifolin, panasenoside, kaempferol and phenol A possess the xanthine oxidase activities were already reported by Noro[11-12], however, it can said that the relationship of its chemical *structure can fluctuate the extent of its efficacy*. Additional studies should be pursued on trifolin, panasenoside, and kaempferol and also on phenol A which belong to the category of the same contention.

It is, however, necessary to keep in mind that the chemical structures of these components do not have any structural relationship with that of Allopurinol[13], which has been developed as a drug, for the purpose of xanthine oxidase inhibition in clinically.

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H. Oura : I want to hear about the polysaccharide study.

D. R. Hahn : Polysaccharide studies are already presented by Japanse pharmacologists such as professor Tanaka and professor Yamada but in Korea it was not proceeded intensively exception of author's work. Studies on the purification and biological activity to the immune system will be done in a near future.

인삼잎의 수용성 분획의 생리활성

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인삼잎의 수용성 분획에서 플라보노이드와 다당류를 확인하였다. 플라보노이드 분획은 xanthine oxidase 활성을 억제하였고, 다당류 분획은 세망내피계의 macrophage 기능을 촉진하였다.