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Effect of Solvent Extraction on the Anti-complementary Activities of Green and Ripe *Cucurbita moschata* Duch

– Research Note –

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Abstract

The edible part of *Cucurbita moschata* Duch, which is commonly used as a Korean traditional medicine as well as a popular food source, was studied to isolate anti-complementary substances. Extracts of *Cucurbita moschata* Duch showed significant anti-complementary activities on the classical pathway of the complement system. Especially, the ripe *Cucurbita moschata* Duch had more activity than that of the green one in terms of the overall anti-complementary activity. Among the extracts of various organic solvents of the ripe *Cucurbita moschata* Duch, chloroform and ethylacetate extracts, which are non-polar solvent extracts, showed the strongest activities. These results suggest that the major difference in the solvent extraction for the anti-complementary substances depends on the change in the chemical composition such as the fatty acid with the degree of ripening.

Key words: *Cucurbita moschata* Duch, anti-complementary activity, solvent extraction

INTRODUCTION

The complement system is known to be a major effector in inflammation activation. Complement is a series of proteins found in blood serum that attack bacterial cells or other foreign cells, causing lysis or leakage of cellular constituents as a result of damage to the cell membrane (1). These proteins play a critical role in aiding phagocytosis of immune complexes, which activate the complement system and these molecules are very significant in the regulation of cellular immune responsiveness. Thus, the human complement system plays an important role in the host defense system and activation of the system contributes to pathologic reactions in a variety of inflammatory and degenerative diseases (1).

Edible plants with anti-inflammatory properties have been considered as good sources for regulators of the complement system (2,3). Therefore, there have been many studies on the screening of anti-complementary substances from natural products (4-9).

Yamada et al. (4) characterized an anti-complementary pectic polysaccharide from the root of *Buleurum falcatum* L. Also, flavonoids from *Morinda morindoides* (5), cyclic octapeptide from the latex of *Jatropha curcas* L. (6), and triterpenes from *Crataeva nurvala* stem bark (8) were isolated as anti-complementary compounds.

Cucurbita spp. is an edible annual plant and divided into three groups; *Cucurbita moschata* Duch, *Cucurbita maximum* Duch, and *Cucurbita pepo* L. Among them, *Cucurbita moschata* Duch is dominant in Korea. A decoction of the edible part of *Cucurbita moschata* Duch is widely used as a Korean traditional medicine against various diseases, as well as a popular food source (10,11). Although it has pharmacological effects as well as nutritional value, little knowledge is available on the active compounds having medicinal effect. Therefore, to elucidate the pharmacological effects of *Cucurbita moschata* Duch, anti-complementary activities were examined after extracting the green and ripe *Cucurbita moschata* Duch using various solvents.

MATERIALS AND METHODS

Materials

The green and ripe *Cucurbita moschata* Duch were harvested in Taejon, Korea and the edible parts were used for extraction. Red blood cells of sheep were collected from the whole blood offered by the farm at Seoul National University. Normal human serum (NHS) was prepared from the blood that was donated from the healthy volunteers in the laboratory. Hemolysin was purchased from Sigma Chemical Company (St. Louis, MO, USA).

Extraction of *Cucurbita moschata* Duch

Cucurbita moschata Duch was extracted from non-polar solvents to polar solvents, in order of n-hexane, chloroform, ethylacetate, butanol, and water. Each extract was evaporated and the anti-complementary activity was measured.

Measurement of anti-complementary activity

Complement activity was assessed by the procedure described previously (7). Each fatty acid was dissolved in DMSO and the solution was diluted to 2.5% in GVB^{++} solution (gelatin veronal buffer, 1.8 mM sodium barbital, 3.1 mM barbituric acid, 0.14 M NaCl, 0.5 mM $MgCl_2$, 0.1% gelatin,

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and 0.3% sodium azide, pH 7.3). NHS was used as the complement source. Serum concentration was evaluated just before sample treatment. The optimal dilution range was 1/95 to 1/110. Sheep erythrocyte suspension (4.0×10^8 cells/mL) was sensitized by incubation with an equal volume of 1/100 diluted hemolysin at 37°C for 30 min (Fig. 1). Afterwards, sensitized erythrocytes were kept at 4°C and restored to formal concentration prior to use. Eighty micro-liter of optimal dilution of NHS were pre-incubated with 8 μL of sample solution at 37°C for 30 min, and then 4 μL of sensitized erythrocytes were added to them and incubated in the same conditions. The reaction mixture was centrifuged immediately and 100 μL of the supernatant were transferred to a flat-bottomed micro-plate and optical density was measured at 405 nm using a micro-plate reader. Anti-complementary activity was determined as a mean of triplicate tests per concentration and expressed as percent inhibition from complement-dependent hemolysis of the control.

RESULTS AND DISCUSSION

For measuring the anti-complementary activity, a hemolytic complement assay was performed. First of all, to optimize the complement fixation of the classical pathway, sheep erythrocyte suspension was sensitized by incubation with the diluted hemolysin solution. Hemolysine was diluted from 1/50 to 1/150. Fig. 1 shows that the optimal dilution was 1/100. Since the anti-complementary activity depends on the reaction conditions such as serum and hemolysin concentration, the optimization is necessary for the measurement of the activity.

The edible portions of green and ripe *Cucurbita moschata* Duch were extracted successively by n-hexane, chloroform, ethylacetate, butanol, and water. For the green *Cucurbita moschata* Duch, the hexane extract had the most inhibitory activity compared to those of other extracts (Fig. 2). In contrast with the green one, the ripe *Cucurbita moschata* Duch had more inhibitory activities in the extracts of chloroform and ethylacetate. Also, the ripe one was better in terms of anti-complementary activity overall. This is well accepted since the ripe *Cucurbita moschata* Duch only is used as an oriental medicine in Korea. To further examine the extracts of the ripe *Cucurbita moschata* Duch, their IC_{50} values were evaluated. Table 1 shows that chloroform and ethylacetate extracts had the lowest IC_{50} values, suggesting that rather non-polar solvent extracts of *Cucurbita moschata* Duch had anti-complementary activities.

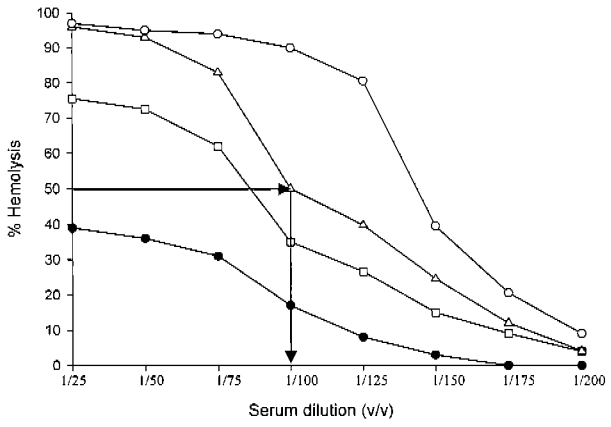

Fig. 1. Optimization of the complement fixation of the classical pathway. Sheep erythrocyte suspension was sensitized by incubation with the diluted hemolysin solution. Hemolysine was diluted to 1/50 (○), 1/75 (△), 1/100 (□), and 1/150 (●). Arrow indicates the serum concentration for 50% hemolysis.

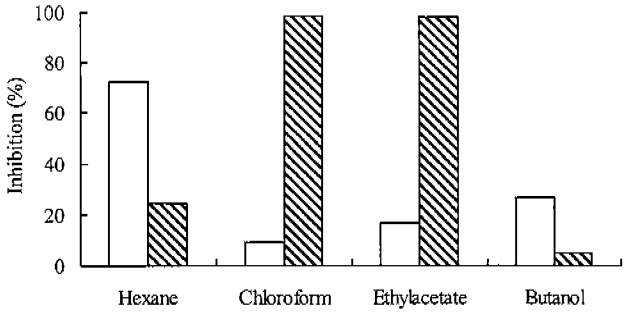

Fig. 2. Comparison between green and ripe *Cucurbita moschata* Duch in terms of anti-complementary activity. Symbols : □, green *Cucurbita moschata* Duch ; ▨, ripe *Cucurbita moschata* Duch.

It has been known that the anti-complementary substances could be one of the main constituents for the immunomodulating activity. Therefore, these results clearly explain the effective use of the ripe *Cucurbita moschata* Duch as a traditional medicine. Also, it should be taken into consideration that chloroform and ethyl acetate extracts of the ripe *Cucurbita moschata* Duch had the most potent inhibition of hemolysis on the classical pathway of the complement system. A preliminary study on the anti-complementary substances isolated from the ripe *Cucurbita moschata* Duch in our laboratory shows a type of fatty acid (data not shown), indicating that the major difference in the solvent extraction for the anti-complementary substances depends on the change in the chemical composition such as the fatty acid with the degree of ripening. Some unsaturated fatty acids are known to have anti-complementary activities (12,13). Eberhard et al. (13) reported that arachidonic acid metabolites exerted various biological effects on the initiation and resolution of inflammatory diseases. Therefore, to elucidate the role of these extracts, further study regarding the identification of anti-complementary substances from the chloroform and ethylacetate extracts of the ripe *Cucurbita moschata* Duch is currently under investigation.

Table 1. Anti-complementary activities of various solvent extracts from ripe *Cucurbita moschata* Duch

Extract	IC_{50} (μg/mL)
Hexane	47.2
Chloroform	13.6
Ethylacetate	26
Butanol	96.7
Water	-

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