

## Anticoagulant and Fibrinolytic Activities of Hwanggeumchal Sorghum *In Vitro*

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Received November 13, 2013 / Revised December 13, 2013 / Accepted December 17, 2013

To examine whether miscellaneous cereal grains have an antithrombotic effect, we investigated the anticoagulant activity of 80% ethanol extracts from eleven selected miscellaneous cereal grains. The 80% ethanol extract of hwanggeumchal sorghum (*Sorghum bicolor*) showed the highest anticoagulant activity, followed by that of green foxtail millet grains, in terms of thrombin time (TT). When the ethanol extract of hwanggeumchal sorghum was sequentially fractionated with n-hexane, methylene chloride, ethyl acetate, and n-butanol, the majority of the TT-inhibitory activity was detected in the hexane and methylene chloride fractions. Whereas aspirin (final conc. 480 µg/ml) prolonged TT by 2-fold, the ethanol extract, hexane fraction, and methylene chloride fraction in the same dose prolonged TT by 2.2-fold, 2.9-fold, and 2.5-fold, respectively. The ethanol extract of hwanggeumchal sorghum could delay activated partial thromboplastin time (APTT) as well as prothrombin time (PT). Although the APTT-inhibitory activity of the ethanol extract was mainly partitioned into the hexane and methylene chloride fractions, the PT-inhibitory activity of the ethanol extract was solely partitioned into the hexane fraction. The APTT- and PT-inhibitory activities of these organic solvent fractions were more potent than those of the control warfarin (final conc. 3.13 mg/ml). The TT-inhibitory activity of the ethanol extract was heat-stable and acid-stable. The ethanol extract, hexane fraction, and methylene chloride fraction of hwanggeumchal sorghum appeared to possess a direct fibrinolytic activity toward fibrin clotting. These results show that hwanggeumchal sorghum can exert anticoagulant and fibrinolytic effects and, thus, have the potential to be applicable as antithrombotic dietary sources.

**Key words** : Anticoagulant activity, antithrombotic dietary source, fibrinolytic activity, hwanggeumchal sorghum (*Sorghum bicolor*) grains, miscellaneous cereal grains

### Introduction

Thrombus (blood clot) as the product of abnormal coagulation process in the artery and vein system causes vascular blockage and leads to serious arterial and venous thrombotic disorders. In particular, thrombus-induced arterial thrombotic disease including myocardial infarction, coronary artery disease, and ischemic stroke is a serious threat to human health and has become one of the major causes of morbidity and mortality in the developed country [1, 22]. Recently, sig-

nificant advances have been made in understanding of the mechanisms regulating the hemostatic and thrombolytic pathways. Thrombus is known to be generated in the circulatory system, when the hemostatic pathway is strongly activated so that it may exceed the normal regulatory counterbalance performed by anticoagulant mechanisms [23, 43]. Under normal healthy conditions, hemostasis is tightly controlled by several anticoagulant mechanisms to prevent inappropriate vascular blood clotting. If the blood loss occurs from damaged vessel, the balance between pro- and anti-coagulant factors becomes rapidly shifted to mediate coagulation. In blood coagulation, a cascade reaction composed of many clotting factors is known to be involved and culminates in generation of thrombin which is the key enzyme responsible for conversion of soluble fibrinogen to insoluble fibrin clot [34]. Because of the critical role of thrombin in thrombus formation, propagation and stabilization,

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an effective inhibition of thrombin can be a prerequisite for prevention and treatment of thrombotic disorders [6, 13, 26].

Many studies have reported the relationship between the genetics of hemostatic factors and pathogenic thrombosis [14, 22]. It has also been reported that transient or long-lasting environmental influences are involved in perturbing hemostasis and modulating risk for thrombosis. The inappropriate high-fat diet is known to be one of the critical environmental factors that play causative roles in the development of thrombotic diseases. An involvement of diet in ameliorating thrombotic disease is well evidenced by the French Paradox that mortality from cardiovascular diseases is significantly lower in French people who consume a high-fat diet than in people from other countries who consume similar high-fat diet [41]. This status has promoted many experimental studies to seek antithrombotic fruits and vegetables. In this context, it has been shown that fruits and vegetables possessing antithrombotic effect could reduce risk factor for coronary heart disease and stroke in human [4, 10, 16, 17, 25, 45]. However, little information has been known regarding anticoagulant and antithrombotic effect of cereal grains [38].

In recent years, due to increased demand for nutritional well-being and healthy diets, the interest in miscellaneous cereal grains, as crude fibers and bioactive phytochemical sources that benefit human health, and thus the consumption of miscellaneous cereal grains, are also increasing in Korea. Although several studies have been performed to extend our understanding on nutritional importance, antioxidant, antimicrobial, antimutagenic and anticarcinogenic, and antidiabetic properties of miscellaneous cereal grains [3, 8, 12, 18-21, 30], the systematic study on their bioactive components associated with the antithrombotic efficacy is still rare. If miscellaneous cereal grains are proven as a proper source of antithrombotic substances, it is likely that these grains become highly effective in preventing thrombotic cardiovascular disease by consuming as either diet or dietary supplements [27, 35-37]. Furthermore, it is generally accepted that natural antithrombotic phytochemicals derived from edible plants may be more safely applicable for human health, as compared with proteinous anticoagulants.

Recently, we have investigated anticoagulant effects of 80% ethanol extracts using the thrombin time (TT) assay in order to compare antithrombotic effects of Korean miscellaneous cereal grains, such as proso millet, yellow glutinous proso millet, glutinous sorghum, hwanggeumchal sorghum,

white glutinous sorghum, yellow glutinous foxtail millet, non-glutinous foxtail millet, green glutinous foxtail millet, golden foxtail millet, barnyard millet, and adlay. Since the 80% ethanol extract of hwanggeumchal sorghum (*Sorghum bicolor*) grains exhibited a marked prolongation of TT, it was sequentially fractionated by n-hexane, methylene chloride, ethyl acetate, and n-butanol, and the individual organic solvent fractions were compared for their anticoagulant activities in terms of thrombin time (TT), activated partial thrombin time (APTT) and prothrombin time (PT). In addition, we have investigated fibrinolytic effect of the 80% ethanol extract and its organic solvent fractions (n-hexane, methylene chloride, ethyl acetate, and n-butanol) of hwanggeumchal sorghum grains.

The results show that the 80% ethanol extract of hwanggeumchal sorghum grains possesses not only anticoagulant activity in terms of TT, APTT and PT, but also fibrinolytic activity.

## Materials and Methods

### Sample extraction

Eleven miscellaneous cereal grains, including proso millet (polished grains), yellow glutinous proso millet (unpolished grains), glutinous sorghum (polished grains), hwanggeumchal sorghum (unpolished grains), white glutinous sorghum (unpolished grains), yellow glutinous foxtail millet (polished grains), non-glutinous foxtail millet (polished grains), green glutinous foxtail millet (unpolished grains), golden foxtail millet (unpolished grains), barnyard millet (unpolished grains), and adlay (polished grains) harvested in Korea were provided by National Institute of Crop Science, Rural Development Administration, Miryang, Gyeongnam 627-803, Korea. As shown in Fig. 1, individual dried grains were milled on a Blender 7012 (Dynamics Corporation, USA), and then extracted with 80% ethanol for 3 h at 80°C. The ethanol extract was evaporated, dissolved in water, and then sequentially extracted with n-hexane, methylene chloride, ethyl acetate, and n-butanol. Each organic solvent fractionation was repeated three times. Each organic solvent fraction as well as the remnant aqueous fraction was centrifuged at 7,500 rpm for 15 min to remove insoluble substances. The recovered supernatant of each fraction was then concentrated by rotary vacuum evaporator (Heidolph LR 4000, Germany). Individual dry weights of the 80% ethanol extracts and organic solvent fractions were described in Table 1.

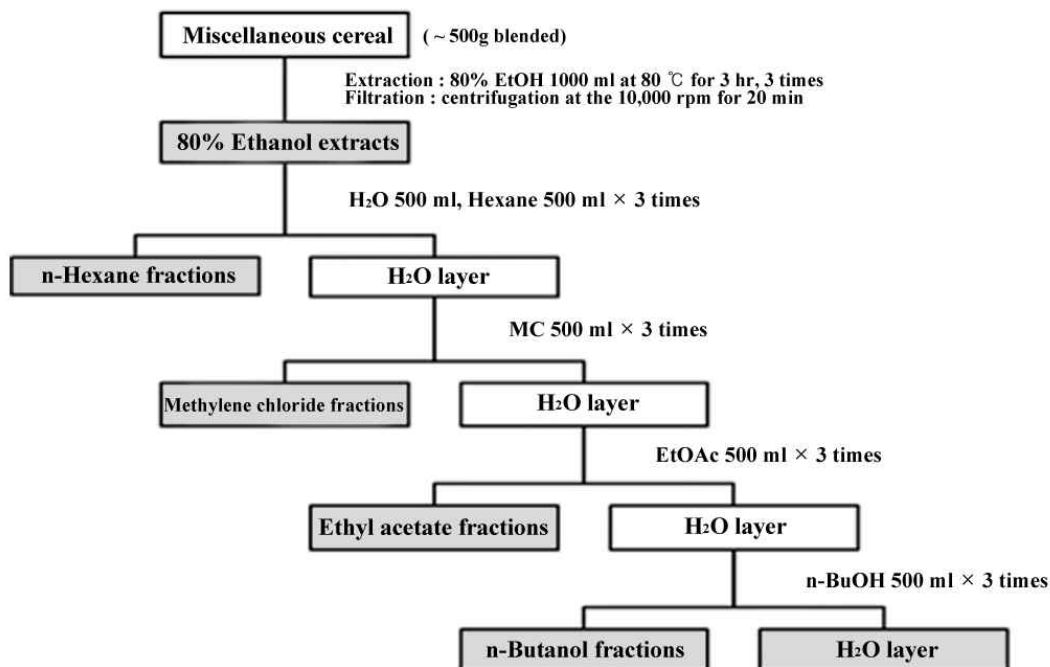


Fig. 1. Procedure for preparation of the 80% ethanol extracts and their organic solvent fractions from eleven miscellaneous cereal grains.

Thrombin time (TT) assay

To assess anticoagulant activity of sample, the effect of sample on thrombin time (TT) was determined using an Auto Blood Coagulation Analyzer (Sysmex CA-540, Japan),

according to the manufacturer’s instructions. Briefly, 50 µl of human thrombin (Sigma, St. Louis, MO, USA) was pre-incubated for 10 min at 37°C with 10 µl of individual samples dissolved in DMSO before mixing with 50 µl of 20 mM

Table 1. Dry weights of the 80% ethanol extracts and their organic solvent fractions prepared from eleven miscellaneous cereal grains

No.	Miscellaneous cereal grains	Dry weight (g)	Dry weight (g)					
			EtOH	Hex	MC	EtOAc	BuOH	H <sub>2</sub> O
1	Proso millet (polished)	400	38.20	2.38	2.57	0.16	0.70	5.10
2	Yellow glutinous proso millet (unpolished)	500	26.48	3.08	0.75	0.97	0.80	3.30
3	Glutinous sorghum (polished),	400	23.21	1.82	1.25	0.18	0.83	1.98
4	Hwanggeumchal sorghum (unpolished)	400	23.24	2.28	0.34	0.77	1.52	13.47
5	White glutinous sorghum (unpolished)	500	22.17	2.45	1.75	0.27	1.40	8.13
6	Yellow glutinous foxtail millet (polished)	250	15.01	1.23	0.79	0.67	2.61	3.84
7	Non-glutinous foxtail millet (polished)	500	34.30	2.86	7.18	1.69	1.99	3.27
8	Green glutinous foxtail millet (unpolished)	500	17.63	2.29	0.85	0.33	3.19	9.48
9	Hwanggeum millet (unpolished)	500	29.01	1.47	0.51	0.31	1.37	5.37
10	Barnyard millet (unpolished)	63	2.24	0.58	0.27	0.13	0.54	0.77
11	Adlay (polished)	500	37.96	0.89	1.65	0.72	0.68	6.96

CaCl<sub>2</sub> and 100 µl of standard human plasma (Siemens, Marburg, Germany). DMSO and aspirin dissolved in DMSO were used as negative and positive controls, respectively. The time period required for clot formation was measured by the Auto Analyzer. The thrombin time (TT)-inhibitory activity (%)=(coagulation time of sample/coagulation time of negative control)×100. The experiment was performed in triplicates.

#### Activated partial thromboplastin time (APTT) assay

To assess inhibitory effect of the sample on the intrinsic pathway of coagulation cascade, activated partial thromboplastin time (APTT) was determined using an Auto Blood Coagulation Analyzer (Sysmex CA-540), according to the manufacturer's instructions. Briefly, 50 µl of the Dade<sup>®</sup> Actin<sup>®</sup> Activated Cephaloplastin reagent (Siemens) was pre-incubated for 10 min at 37°C with 10 µl of individual samples. Sequentially, 50 µl of standard human plasma (Siemens) and 50 µl of 25 mM CaCl<sub>2</sub> were added, and then the time period required for clot formation was measured by the Auto Analyzer. DMSO and warfarin dissolved in DMSO were used as negative and positive controls, respectively. The activated partial thromboplastin time (APTT)-inhibitory activity (%)=(coagulation time of sample/coagulation time of negative control)×100. The experiment was performed in triplicates.

#### Prothrombin time (PT) assay

To assess inhibitory effect of the sample on the extrinsic pathway of coagulation cascade, prothrombin time (PT) was determined using an Auto Blood Coagulation Analyzer (Sysmex CA-540), according to the manufacturer's instructions. Briefly, 50 µl of the Thromborel<sup>®</sup> S reagent (Siemens) was preincubated for 10 min at 37°C with 10 µl of individual samples prior to mixing with 100 µl of standard human plasma (Siemens). The time period required for clot formation was measured by the Auto Analyzer. DMSO and warfarin dissolved in DMSO were used as negative and positive controls, respectively. The prothrombin time (PT)-inhibitory activity (%)=(coagulation time of sample/coagulation time of negative control)×100. The experiment was performed in triplicates.

#### Fibrinolytic activity assay

Fibrinolytic activity was detected by a fibrin plate method as described previously [2, 31]. To prepare the fibrin plate,

6.6 ml of 0.75% human fibrinogen (Sigma) in 50 mM sodium phosphate buffer (pH 7.4) was mixed with 130 µl of 50 U/ml thrombin (Sigma), and the mixture was quickly poured into the petri dish (60 mm diam.). The plate was left until the mixture solidified. Three microliter of plasmin (Sigma) dissolved in 50 mM Tris-HCl buffer as the positive control and individual samples dissolved in DMSO were loaded on the fibrin plate using sterile filter-paper discs (3 mm diam.). The plate was then incubated at 37°C for 3 h. The fibrinolytic activity of samples was detected as lytic area surrounding the disc on the fibrin plate compared to standard plasmin (15 U/ml and 30 U/ml).

#### Statistical analysis

Unless otherwise indicated, each result in this paper is representative of at least three separate experiments. Values represent the mean ± standard deviation (SD) of these experiments. The statistical significance was calculated with Student's *t*-test. *P* values less than 0.05 were considered significant.

## Results and Discussion

Effect of the 80% ethanol extracts of individual miscellaneous cereal grains on thrombin time (TT) *in vitro*

The action of thrombin, which is known to be critical for blood coagulation, can be inhibited indirectly or directly. Traditional anticoagulants, such as aspirin, heparin (unfractionated and fractionated) and vitamin K antagonists are indirect inhibitors of thrombin. Although these traditional anticoagulant agents are effective if appropriately used, they also have many limitations, such as unpredictable anticoagulant response, need for routine dose adjustments and anticoagulant monitoring, heparin-induced thrombocytopenia (HIT), genetic variations in response, binding to various proteins and cells, and lack of inhibition of clot bound thrombin [11, 15, 24, 40]. In this regard, many efforts have been performed in order to explore alternative anticoagulants for prevention and treatment of thrombotic disorders.

In order to screen eleven miscellaneous cereal grains for anticoagulant effect on thrombin-mediated coagulation, the 80% ethanol extracts from individual miscellaneous cereal grains were dissolved in DMSO, and then their effects on thrombin time (TT) were investigated. As shown in Fig. 2,

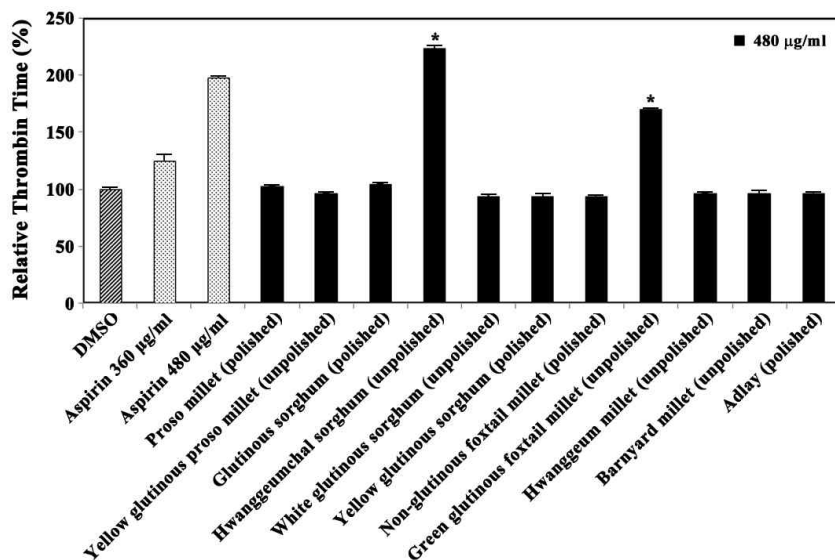


Fig. 2. Thrombin time (TT)-inhibitory activity of 80% ethanol extracts of individual miscellaneous cereal grains. TT of individual samples was measured at a final concentration of 480 µg/ml as described in Materials and Methods. The experiment was performed in triplicates. Symbol: \* $p < 0.05$  as compared with the control.

the ethanol extract obtained from hwanggeumchal sorghum grains appeared to prolong TT by 2.2-fold at a concentration of 480 µg/ml. Under the same conditions, the extract obtained from green foxtail millet grains was able to extend TT by 1.7 fold at a concentration of 480 µg/ml. However, the ethanol extracts, which were obtained from the other miscellaneous cereals grains including proso millet (polished grains), yellow glutinous proso millet (unpolished grains), glutinous sorghum (polished grains), white glutinous sorghum (unpolished grains), yellow glutinous foxtail millet (polished grains), non-glutinous foxtail millet (polished grains), golden foxtail millet (unpolished grains), barnyard millet (unpolished grains), and adlay (polished grains) failed to prolong TT remarkably. In addition, the positive control aspirin at a concentration of 480 µg/ml appeared to extend TT by 2.0-fold.

These results showed that the 80% ethanol extracts obtained from hwanggeumchal sorghum grains and green foxtail millet grains exerted a remarkable anticoagulant effect among the eleven miscellaneous cereal grains tested. Current results also suggested that the anticoagulant activity of the 80% ethanol extract of hwanggeumchal sorghum grains, which was assessed by TT assay, was more potent than that of the positive control aspirin in the same dose (final conc. 480 µg/ml).

Anticoagulant activity of the 80% ethanol extract of hwanggeumchal sorghum grains

To examine further anticoagulant ingredients in the 80% ethanol extracts of hwanggeumchal sorghum grains, the

ethanol extract was dissolved in water and then sequentially fractionated with various organic solvents such as n-hexane, methylene chloride, ethyl acetate, and n-butanol. The individual organic solvent fractions as well as the remnant aqueous fraction were tested for their anticoagulant activities by TT, APTT, and PT assays. When the effect of each organic solvent fraction on TT at a concentration of 480 µg/ml, the thrombin-induced coagulation reaction was not completed in the presence of either hexane or methylene chloride fractions (Fig. 3). However, the presence of ethyl acetate fraction, butanol fraction or aqueous fraction did not disturb significantly the thrombin-induced coagulation reaction. At the same time, the positive control aspirin at concentrations of 360 µg/ml and 480 µg/ml was able to prolong TT by 1.2-fold and 2.0-fold, respectively. These results show that the majority of the anticoagulant ingredients contained in the 80% ethanol extract of hwanggeumchal sorghum grains were partitioned into hexane and methylene chloride fractions.

To compare further the anticoagulant activities between n-hexane fraction, methylene chloride fraction, and the positive control aspirin, we decided to examine their inhibitory effects on the TT at various diluted concentrations of 120, 240 and 360 µg/ml. As shown in Fig. 4, n-hexane fraction at concentrations of 120, 240 and 360 µg/ml appeared to extend TT by 1.5-fold, 1.8-fold and 2.9-fold, whereas methylene chloride fraction at concentrations of 120, 240 and 360 µg/ml extended TT by 1.4-fold, 1.6-fold and 2.5-fold, respectively. Although 80% ethanol extract at a concentration of 120 µg/ml failed to prolong TT, the ethanol extract at concentrations

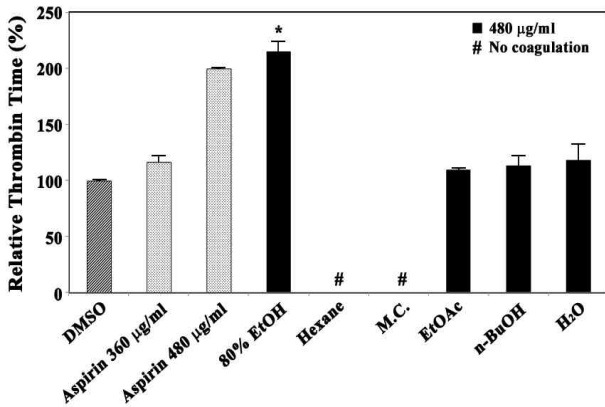


Fig. 3. TT-inhibitory activity of 80% ethanol extract of hwanggeumchal sorghum grains. TT of individual samples was measured at a final concentration of 480 µg/ml as described in Materials and Methods. The experiment was performed in triplicates. Symbol: # no coagulation; \* $p < 0.05$  as compared with the control.

of 240 and 360 µg/ml could extend TT by 1.6-fold, and 2.3-fold, respectively. With this data, we repeatedly confirmed that hexane and methylene chloride fractions of 80% ethanol extracts from hwanggeumchal sorghum grains were containing the major antithrombotic components. Again, the positive control aspirin at concentrations of 360 µg/ml and 480 µg/ml could prolong TT by 1.2-fold and 2.0-fold, respectively, whereas aspirin at a concentration of 240 µg/ml was unable to prolong TT. These results confirm that the anticoagulant activity of 80% ethanol extract of hwanggeumchal sorghum grains is more potent than that of aspirin, and suggest that the anticoagulant activities of hexane fraction and methylene chloride fraction might be at least 3-fold stronger than that of aspirin.

Since the anticoagulant activity assessed by TT assay is

to investigate the inhibitory effect of sample on thrombin activity crucial for conversion of fibrinogen to fibrin [9], we decided to examine whether the 80% ethanol extract and organic solvent fractions from hwanggeumchal sorghum grains can inhibit the intrinsic pathway [34] and the extrinsic pathway [34] of coagulation cascade by employing APTT assay and PT assay, respectively. As shown in Fig. 5, APTT assay showed that the majority of anticoagulant activity detectable in the 80% ethanol extract was partitioned into both hexane and methylene chloride fractions, which appeared to possess a slightly higher efficacy than that of the positive control warfarin at a concentration of 3.13 mg/ml. It is interesting to note that the anticoagulant activity of the 80% ethanol extract, which was evaluated by PT assay, was mostly contained in the hexane fraction but not in the other organic solvent fractions including the methylene chloride fraction (Fig. 6). Again, the inhibitory effect of the 80% ethanol extract and the hexane fraction on the extrinsic pathway of coagulation cascade appeared to be stronger than that of the positive control warfarin at a concentration of 3.13 mg/ml.

Consequently, current results suggest that the anticoagulant activity of 80% ethanol extract of hwanggeumchal sorghum grains can be exerted via direct inhibition of thrombin action as well as via inhibition of the intrinsic and extrinsic pathways of blood coagulation.

Heat and acid stability of TT-inhibitory activities of the 80% ethanol extract of hwanggeumchal sorghum grains

The 80% ethanol extract as well as the hexane fraction of hwanggeumchal sorghum grains dissolved in DMSO at a concentration of 10 mg/ml were treated at 100°C for 30

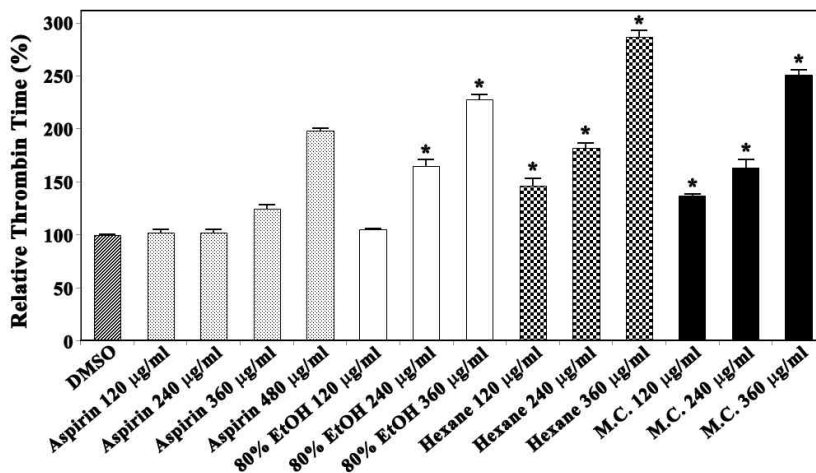


Fig. 4. Dose-dependent effect of 80% ethanol extract, hexane fraction, and methylene chloride fraction of hwanggeumchal sorghum grains on Thrombin time (TT). TT of individual samples was measured at various final concentrations ranging from 120-360 µg/ml as described in Materials and Methods. The experiment was performed in triplicates. Symbol: \* $p < 0.05$  as compared with the control.

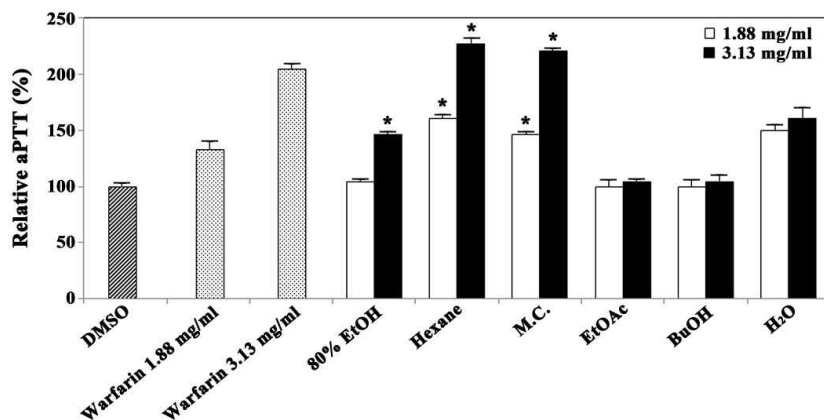


Fig. 5. Activated partial thromboplastin time (APTT)-inhibitory activity of 80% ethanol extract and its organic solvent fractions of hwanggeumchal sorghum grains. APTT of individual samples was measured at final concentrations of 1.88 mg/ml and 3.13 mg/ml as described in Materials and Methods. The experiment was performed in triplicates. Symbol: \* $p < 0.05$  as compared with the control.

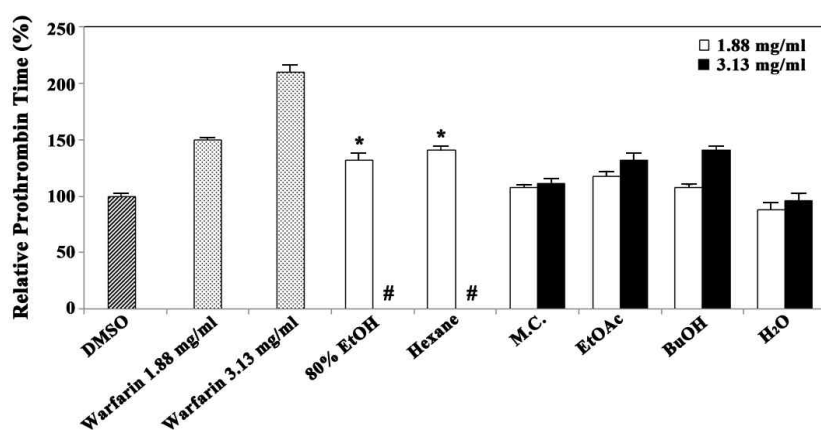


Fig. 6. Prothrombin time (PT)-inhibitory activity of 80% ethanol extract and its organic solvent fractions of hwanggeumchal sorghum grains. PT of individual samples was measured at final concentrations of 1.88 mg/ml and 3.13 mg/ml as described in Materials and Methods. The experiment was performed in triplicates. Symbol: # no coagulation; \* $p < 0.05$  as compared with the control.

min or 60 min, and then their TT-inhibitory activities were compared with those of the untreated samples and the positive control aspirin. As shown in Fig. 7A, the anticoagulant activity of the 80% ethanol extract in terms of TT was not reduced by the heat treatment at 100°C for up to 60 min. To examine the acid stability of the 80% ethanol extract and the hexane fraction of hwanggeumchal sorghum grains, the pH of the samples (10 mg/ml DMSO) were adjusted to 2.0 and incubated at 37°C for 60 min or 120 min. After the pH of the samples was readjusted to 7.0, their TT-inhibitory activities were compared with those of the untreated samples and the positive control aspirin. As shown in Fig. 7B, the anticoagulant activities of the 80% ethanol extract was not significantly influenced by the treatment at pH 2.0 for 120 min. Consequently, these results suggested that the anticoagulant components contained in the 80% ethanol extract and the hexane fraction were heat-stable and acid-stable substances.

In relation to active components of herbal plants possessing anticoagulant activities, several phytochemicals have been implicated. These include phenolic compounds, such as myricetin, rosmarinic acid and caffeic acid phenethyl ester

[28], and *p*-coumaric acid, ferulic acid, caffeic acid, vanillic acid, and protocatechuic acid found in Korean red ginseng [44]. Previously, it has been reported that sorghum is a rich source of various phytochemicals including phenolic compounds, which have been mainly studied for antioxidant effects and relevant health benefits [3]. Since the presence of *p*-coumaric acid, ferulic acid, caffeic acid and myricetin, has also been detected in sorghum as antioxidant phenolic components, these previous and current results raised the possibility that the heat-stable and acid-stable anticoagulant activities detected in the 80% ethanol extract of hwanggeumchal sorghum grains might be, at least in part, due to some anticoagulant phenolic components.

Fibrinolytic activity of the 80% ethanol extract and organic solvent fractions of hwanggeumchal sorghum grains

Since the pathological thrombus formation in the circulatory blood vessel is known to be provoked by imbalance between the hemostatic pathway and the thrombolytic pathway [7], the ideal antithrombotic agents need to have not only anticoagulant activity but also fibrinolytic activity. If

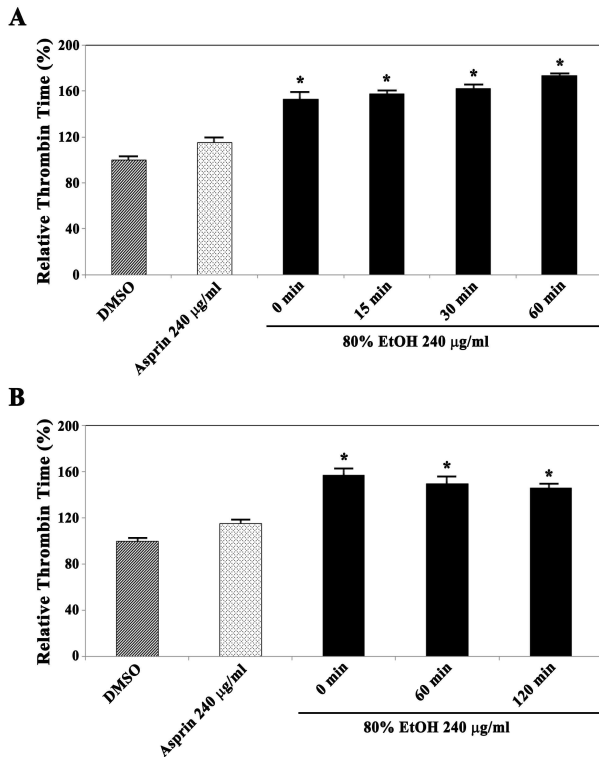


Fig. 7. Heat-stability (A) and acid-stability (B) of TT-inhibitory activity of 80% ethanol extract of hwanggeumchal sorghum grains. For heat treatment, the 80% ethanol extract of hwanggeumchal sorghum grains (5 mg/ml DMSO) was treated at 100°C for 15 min, 30 min or 60 min. For acid treatment, the 80% ethanol extract (5 mg/ml DMSO) were adjusted to pH 2.0 and then incubated at 37°C for 60 min or 120 min prior to adjustment to pH 7.0. The TT-inhibitory activity of individual samples was measured at a final concentration of 240 µg/ml as described in Material and Methods. The experiment was performed in triplicates. Symbol: \* $p < 0.05$  as compared with the control.

hwanggeumchal sorghum grains exert only the anticoagulant activity that can interfere with thrombus formation, their efficacy would have a limitation in treatment of thrombotic diseases due to the absence of thrombolytic activity. To examine whether the 80% ethanol extract and organic solvent fractions from hwanggeumchal sorghum grains have a fibrinolytic activity, 3 µl of each sample dissolved in DMSO at a concentration of 10 mg/ml was placed on the fibrin plate using filter-paper discs. When the diameter of the clear area around disc was measured as fibrinolytic activity after incubation for 3 h at 37°C, the 80% ethanol extract from hwanggeumchal sorghum grains showed 1.5 to 2 times greater activity as compared with that of the positive control plasmin (15 U/ml or 30 U/ml) (Fig. 8). In addition,

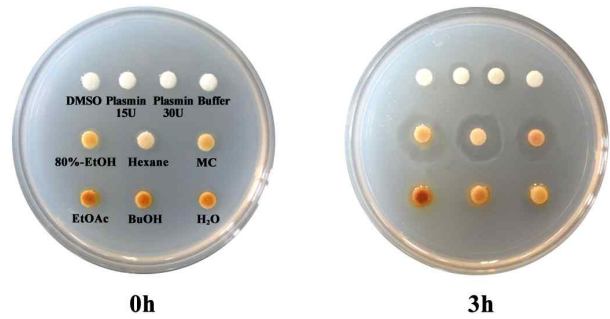


Fig. 8. Fibrinolytic activity of 80% ethanol extract and its organic solvent fractions of hwanggeumchal sorghum grains. Three microliter of plasmin dissolved in 50 mM Tris-HCl buffer as the positive control and individual samples (10 mg/ml DMSO) were loaded on the fibrin plate using filter-paper discs (3 mm diam.). Sequentially, the fibrin plate was incubated at 37°C for 3 h. The fibrinolytic activity of samples was detected as lytic area surrounding the disc compared to standard plasmin (15 U/ml and 30 U/ml). A representative study is shown and two additional experiments yielded similar results.

the fibrinolytic activity was observed in the hexane and methylene chloride fractions, but not in the ethyl acetate, butanol, and aqueous fractions.

These results indicated that hwanggeumchal sorghum grains possessed a direct fibrinolytic activity against blood clot. These results also suggested that the fibrinolytic ingredients in hwanggeumchal sorghum grains, which could be extracted by 80% ethanol, were mainly partitioned in the hexane and methylene chloride fractions. While there were no reports regarding anticoagulant effect of sorghum grains, it was reported that the methanol extract of aged sorghum vinegar could exert antithrombotic and fibrinolytic activities [7]. However, because vinegar generation from sorghum grains proceeded via two fermentation steps, yeast ethanol fermentation and bacterial acetic acid fermentation, it remained obscure whether the anticoagulant phenolics and flavonoids found in the vinegar extract were derived from either the initial materials, sorghum grains or the microbial fermentation products.

Previously, it has been shown that the enzymic substances that resemble serine proteases like plasmin can play roles as the active components of plant extracts/products-derived fibrinolytic activities [5, 29, 39, 42]. In addition, a plant phenolic compound, kaempferol has been shown to possess the fibrinolytic activity [32]. Although characteristics of the anticoagulant ingredients of hwanggeumchal sorghum grains remain to be elucidated, it seems likely that the 80% ethanol



extract of hwanggeumchal sorghum grains might have potential antithrombotic effects in that it could exert fibrinolytic as well as anticoagulant activities.

In conclusion, current results show that the 80% ethanol extract from hwanggeumchal sorghum grains could exert not only anticoagulant activity in terms of TT, APTT and PT, but also fibrinolytic activity. These results suggest that hwanggeumchal sorghum grains might have a potential to be applicable as antithrombotic dietary source.

#### Acknowledgment

This work was supported by Bio-industry Technology Development Program, Ministry of Agriculture, Food and Rural Affairs (Grant #110133-3).

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초록 : 황금찰수수의 혈액응고저해 및 혈전용해 효과

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잡곡류의 항혈전작용을 조사하기 위해 국내산 11종의 잡곡으로부터 80% 에탄올 추출물을 얻어 혈액응고저해 활성을 thrombin time (TT)법으로 측정한 결과, 황금찰수수의 에탄올 추출물이 가장 높은 혈액응고저해활성을 보였으며, 뒤이어 청차조 유래의 에탄올 추출물도 혈액응고저해활성을 보였다. 황금찰수수의 에탄올 추출물을 n-hexane, methylene chloride, ethyl acetate 및 n-butanol로 분획하였을 때, 대부분의 TT-저해활성은 hexane과 methylene chloride 분획에 분포하였으며, 이들 분획의 활성은 동일 농도의 aspirin (최종 농도 480 µg/ml)보다 더 높은 것으로 나타났다. 또한 황금찰수수의 에탄올 추출물의 혈액응고저해활성은 activated partial thromboplastin time (APTT)법 및 prothrombin time (PT)법으로도 확인되었다. 이때 APTT-저해활성은 hexane 및 methylene chloride 분획에 주로 분포하였으나 PT-저해활성은 hexane 분획에 주로 분포하였다. 이들 분획들의 APTT-저해활성 및 PT-저해활성은 warfarin (최종농도 3.13 mg/ml) 보다 더 높은 것으로 확인되었다. 황금찰수수 유래 에탄올 추출물의 TT-저해활성은 열 안정성 및 pH 안정성이 매우 우수하였다. 한편, 이와 같은 황금찰수수 유래 에탄올 추출물, hexane 분획 및 methylene chloride 분획의 경우는 피브린 응괴를 가용화할 수 있는 피브린용해활성을 지닌 것으로 나타났다. 이러한 결과들은 황금찰수수가 혈액응고저해활성과 혈전용해활성을 지니고 있음을 보여 주며, 아울러 항혈전 식이요법의 소재가 될 수 있음을 시사한다.