Radical Scavenging and Inhibition of Platelet Function by a Polyphenol-Rich Fraction from *Salvia miltiorrhiza* Bunge

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Abstract: A diet rich in polyphenols has previously been shown to be associated with a lower risk for cardiovascular disease. Specifically, inhibition of platelet hyperactivation by polyphenols decreases platelet-dependent thrombosis, reducing the risk for cardiovascular disease. However, whether polyphenols in *Salvia miltiorrhiza* Bunge (SMB) affect platelet functions has not been clearly determined. Here, we investigated the effects of methanol and ethyl acetate (EtOAc) extracts from SMB on free radical scavenging activities, and platelet aggregation and adhesion. We found that the EtOAc fraction was the most effective at scavenging 1,1-diphenyl-2-picrylhydrazyl (DPPH) and 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) free radicals (concentration for 50% reduction, $RC_{50} = 2.4 ± 1.5$ and $27.9 ± 1.4 \mu g/mL$, respectively), and that, especially for DPPH radicals, the EtOAc fraction showed better scavenging activity than butylated hydroxyanisole (BHA, $RC_{50} = 5.5 ± 0.1 \mu g/mL$). Measurements with a turbidimetric aggregometer revealed that the EtOAc fraction was the most potent inhibitor of ADP- and collagen-induced rat platelet aggregation. In addition, the methanol and EtOAc fraction dose-dependently inhibited thrombin-stimulated platelet adhesion to collagen or fibrinogen. Collectively, these results suggest that the polyphenol-rich EtOAc fraction from SMB can reduce platelet hyperactivation by scavenging free radicals. Thus, the EtOAc fraction of SMB is a potential source of inhibitors of platelet-dependent thrombosis.

Keywords: *Salvia miltiorrhiza* Bunge, adhesion, platelet aggregation, antioxidant, polyphenol.

INTRODUCTION

Platelets, which are key players in thrombosis, are the smallest enucleated blood cells derived from megakaryocytes. These cells respond to a variety of activators, including thrombin, epinephrine, serotonin, ADP, thromboxane A₂ and collagen, which can affect platelet adhesion, shape, and aggregation as well as their secretion of storage granules [1, 2]. Platelet hyperactivity at the site of injury or in atherosclerotic vessel walls plays a major role in thrombosis and the pathogenesis of cardiovascular diseases, and inhibition of platelet function decreases thrombosis and therefore heart disease [3].

Given the importance of thrombosis in cardiovascular disorders, natural products, for example, extracts from Ginkgo biloba [4], garlic [5], and onion [6], as well as omega-3 fatty acids from fish oil [7], have been screened for anti-platelet activity. Likewise, flavonoid-rich foods such as grapes juice [8] and cocoa [9], as well as catechin, epicatechin, and procyanidine have been shown to modify platelet function [10]. In general, the in vitro inhibitory effects of dietary antioxidants and the flavonoids on platelet function have been extensively investigated [11].

*Salvia miltiorrhiza* Bunge (SMB), a popular traditional Chinese medicinal plant, has been used extensively not only in China, but also in Korea for the treatment of coronary heart disease, cerebrovascular disease, and inflammation, and it is cytotoxic to human tumor cell lines [12, 13]. SMB is mainly used as a decoction in traditional Chinese medicine. Thus, much attention has recently been focused on polyphenols, which are the biologically active water-soluble components. The putative active components are salvianolic acid B, danshensu, lithospermic acids, protocatechuic acid, and rosmarinic acid [14]. In recent years, an increasing number of phenolic components from the water-soluble fraction of SMB have been reported to prevent cardiovascular disease.

Despite these findings, the relationship between the antioxidant activity and the effects of platelet function of various SMB extracts has not been clarified. Thus, in the current study, we investigated the antioxidant and anti-platelet activities of different solvent fractions of SMB. The results should help in the production of functional foods with antioxidant and anti-platelet function.

MATERIALS AND METHODS

Materials

SMB roots were purchased from a Chinese drug store in Daegu, Korea. DPPH, ABTS, dimethyl sulfoxide (DMSO), fibrinogen, thrombin, bicinchoninic acid, HEPES and NaCl were purchased from Sigma-Aldrich (Sigma, St. Louis, MO, USA). Collagen and ADP were obtained from Chrono-Log

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(Chrono-Log Co., Ltd., Havertown, PA, USA). EtOAc, hexane, chloroform, methanol, and butanol were purchased from Duksan (Duksan Pure Chemical Co., Ltd., Ansan, Kyungido, Korea).

**Extraction and Fractionation of SMB**

SMB (1 kg) was extracted three times with 10 L of 80% methanol at room temperature for 24 h. The extract was filtered through No. 3 filter paper (Whatman, Maidstone, UK), and the combined filtrate was concentrated in a rotary evaporator (EYELA NVC-2000; Tokyo Rikakikai Co. Ltd., Tokyo, Japan) at 55°C and then freeze-dried, providing 396.2 g of methanol extract (39.6% yield). The methanol extract (100 g) was resuspended in distilled water and sequentially partitioned into hexane (0.65 g), chloroform (13.84 g), EtOAc (1.78 g), butanol (2.79 g), and water (57.04 g) fractions. The organic solvent fractions were concentrated to dryness by rotary evaporation at 55°C, and the water fraction was freeze-dried.

**DPPH Radical Scavenging Activity**

Radical activities were determined using DPPH as a free radical as described by Moreno et al. [15] with some modifications. Briefly, in each well of a 96-well plate, 160 μL of various concentrations of sample in methanol were added to 40 μL of 0.15 mM DPPH in methanol. After a 30-min incubation in the dark at room temperature, the decrease in absorbance was measured at 517 nm. Methanol was used as the blank solution, and DPPH solution without any added sample extract served as a control. The radical scavenging activity was calculated from a standard curve of known concentrations of BHA. The concentration required for a 50% reduction of DPPH levels after 30 min (RC50) was calculated from the reduction in the absorbance at 517 nm. The experiment was performed in triplicate.

**ABTS Radical Scavenging Activity**

ABTS radical scavenging activity was measured by the ABTS cation decolorization assay as described by Re et al. [16]. Briefly, 14 mM ABTS was mixed with an equal volume of 4.95 mM potassium persulfate. After 24 h incubation at room temperature in the dark, the ABTS radical cation decolorization assay was described by Re et al. [16]. Briefly, 14 mM ABTS was mixed with an equal volume of 4.95 mM potassium persulfate. After 24 h incubation at room temperature in the dark, the resulting ABTS radical cation was measured. The percentage of ABTS radical cation decolorization was calculated as the difference between the absorbance at 414 nm and the absorbance at 734 nm. The experiment was performed in triplicate.

**Total Phenolics**

The total polyphenol content of the extract and the fractions was determined using the Folin-Denis method [17] adapted to 96-well plates. In each well of a 96-well plate, 60 μL of sample dissolved in distilled water was mixed with 60 μL of 2-fold diluted Folin reagent. After 3 min, 60 μL of aqueous 10% Na2CO3 was added, and the mixture was mixed and allowed to stand at room temperature in the dark for 1 h. The absorbance was read at 700 nm, and the total polyphenol concentration was calculated from a calibration curve generated using tannic acid as a standard. The results were expressed as mg/L of tannic acid equivalents.

**Total Flavonoid Content**

The total flavonoid content of the samples was determined using a previously described colorimetric method [17] with some modification. A 0.1-mL sample of 10-fold diluted solutions in 80% ethanol was mixed with 20 μL of 10% Al(NO3)3, 20 μL of 1 M CH3COOK, and 860 μL of 80% ethanol. After 40 min reaction at room temperature, the increase in absorbance was measured at 415 nm. The total flavonoid content was calculated by comparison with a calibration curve generated using quercetin as a standard. The results were expressed as mg/L of quercetin equivalents.

**Preparation of Washed Platelets**

Male Sprague-Dawley rats (200 to 250 g) were purchased from OrientBio (Orient Bio INC, Korea) and housed in an air-conditioned room that was kept at 21±2°C. Blood was collected from the abdominal aorta using plastic syringes containing 0.15 M sodium citrate (1:9 v/v) as an anticoagulant. Platelet-rich plasma was obtained after centrifugation of the blood at 120 × g for 10 min, and washed platelets were prepared as described previously [18]. Briefly, platelet-rich plasma was centrifuged at 200 × g for 15 min at room temperature, and the sediments platelet pellets were washed twice with modified Tyrode-HEPES buffer (137 mM NaCl, 2.9 mM KCl, 1 mM MgCl2, 5 mM glucose, 12 mM NaHCO3, 0.34 mM Na2HPO4, 20 mM HEPES, and 0.25% bovine serum albumin, pH 7.4) containing 1 mM EDTA. Finally, the platelets were gently resuspended in Tyrode-HEPES buffer, counted using a cell counter (Hema-vet HV950FS; Drew scientific, Oxford, CT, USA), and adjusted to a concentration of 3 × 108 platelets/mL.

**Platelet Aggregation**

Platelet aggregation experiments were performed with 0.5 mL of washed platelet suspension in polystyrene cuvettes using a turbidimetric aggregometer (Chrono-log Co., Ltd., Havertown, PA, USA). The washed platelet suspensions were incubated at 37°C for 3 min in the aggregometer with stirring at 1,100 rpm. The platelets were then stimulated with ADP and collagen. Platelet aggregation was monitored as the change in light transmission for 7 min and the rates were calculated at 5 min point. The anti-aggregation effects of the samples were tested by preincubation of the washed platelets with samples for 2 min before the addition of the stimulating agents.

**Platelet Adhesion**

Adhesion of activated platelets to fibrinogen and collagen was determined as described by Tuszyński and Murphy [19]. The platelets were preincubated for 10 min at 37°C with test samples at a final concentration of 10, 25, 50, 100, and 200 μg/mL or without test sample (control) and then activated with 0.2 U/mL thrombin. Wells of a 96-well microtiter plate were incubated for 2 to 3 h with 50 μL of 2 mg/mL fibrinogen in PBS (pH 7.5) or 40 μg/mL collagen dissolved in 0.05% CH3COOH. The solution was removed by aspiration, incubated for 1 h with 200 μL of PBS containing 1% bovine serum albumin, and then washed three times with 200 μL of PBS. Immediately after washing, 50 μL of 0.2 U/mL thrombin in PBS was added to each well, followed by 100 μL of platelet suspension. After 1 h incubation at 37°C, nonadherent cells were removed by aspiration, and the wells were
washed three times with 200 μL of PBS. The total cell-associate protein was determined by dissolving the attached platelets in 200 μL of bicinchoninic acid working solution (Sigma) for 1 h at 37°C. After cooling to room temperature, the absorbance of each well was determined at 562 nm with a Spectramax 340 microplate reader (Molecular Devices, Sunnyvale, CA, USA).

**Statistical Analysis**

All the results are expressed as means ± SEM or SD, and the significance of differences between the means of two groups was determined using an unpaired Student’s *t*-test. The differences were considered significant when *P* was less than 0.05. All the experiments were performed at least three times.

**RESULTS AND DISCUSSION**

Increasing epidemiological evidence indicates that a diet rich in polyphenols is associated with reduced mortality due to cardiovascular disease [9] and stroke [20]. There is an obvious hypothesis about the antioxidant properties of polyphenols protecting blood vessels against the effects of oxidative stress associated with cardiovascular risk factors. The polyphenolic compounds from red wine and green tea, for example, have been shown to prevent atherosclerosis in animal models [21, 22]. Thus, we first tested the anti-oxidative activities of various fractions from SMB on platelet function.

**DPPH and ABTS Radical-Scavenging Activities**

We examined the antioxidant activities of the methanol extract and the solvent fractions of SMB by measuring their abilities to transfer hydrogen to the stable free radicals DPPH and ABTS. In these experiments, BHA and trolox were used as control compounds, respectively. The tested samples reacted with DPPH, causing a decrease in the absorbance at 517 nm. We also examined the reduction of ABTS free radicals by measuring the absorbance at 732 nm. These methods are commonly used for the screening of antioxidant activity and can be applied to both lipophilic and hydrophilic antioxidants. The radical-scavenging activities, as indicated by their RC₅₀ values, differed between the methanol extract and the various solvent fractions (Table 1), and the scavenging activity increased in a concentration-dependent manner for both radical species (data not shown). The results indicate that among the SMB fractions, the EtOAc fraction was the most potent scavenger of DPPH free radicals (RC₅₀ = 2.4 ± 1.5 μg/mL). This was approximately two-fold more potent than the synthetic antioxidant BHA (RC₅₀ = 5.5 ± 0.1 μg/mL). The methanol extract and the butanol fractions also exhibited lower RC₅₀ values, indicating better radical-scavenging activity than BHA, whereas the hexane, chloroform, and water fractions had higher RC₅₀ values and, thus, relatively low activity. Furthermore, the EtOAc fraction was the strongest antioxidant amongst the various SMB fractions in the ABTS assay system, with an RC₅₀ of 27.9 ± 1.4 μg/mL. The RC₅₀ of trolox was found to be 18.6 ± 0.3 μg/mL under the same condition (Table 1). These results along with those from the DPPH assay confirm that some constituents in the EtOAc fraction from SMB have radical scavenging activity.

Lin *et al.* [23] reported that salvianolic acid B, a water-soluble polyphenolic antioxidant from SMB, exhibits DPPH radical-scavenging activity. The EtOAc fraction of this plant was also reported to potently induce cell death and dopamine release in PC12 cells [24] and to inhibit matrix metalloproteinase-9 activity and the migration of human aortic smooth muscle cells [25]. Along with these reports, our results suggest that the EtOAc fraction contains highly concentrated antioxidants that have a variety of beneficial effects on human health.

**Total Polyphenolic and Flavonoids Content**

Because the antioxidative activities of the methanol extract and the solvent fractions of SMB differed, we determined the total polyphenol and flavonoid contents to identify correlations between the various antioxidant compounds and the antioxidant activity. Table 2 shows the polyphenol and flavonoid contents in the methanol extract and the solvent

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**Table 1. RC₅₀ Values of SMB Extract and Its Solvent Fractions**

<table>
<thead>
<tr>
<th>Sample</th>
<th>RC₅₀ DPPH (mg/mL)</th>
<th>RC₅₀ ABTS (mg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methanol extract</td>
<td>8.6 ± 0.7</td>
<td>54.7 ± 3.5</td>
</tr>
<tr>
<td>Hexane fraction</td>
<td>68.2 ± 7.1</td>
<td>113.2 ± 19.1</td>
</tr>
<tr>
<td>Chloroform fraction</td>
<td>54.7 ± 3.2</td>
<td>80.3 ± 3.2</td>
</tr>
<tr>
<td>EtOAc fraction</td>
<td>2.4 ± 1.5</td>
<td>27.9 ± 1.4</td>
</tr>
<tr>
<td>Butanol fraction</td>
<td>5.1 ± 0.1</td>
<td>35.8 ± 3.8</td>
</tr>
<tr>
<td>Water fraction</td>
<td>46.9 ± 3.0</td>
<td>110.9 ± 21.7</td>
</tr>
<tr>
<td>Trolox</td>
<td>-</td>
<td>18.61 ± 0.3</td>
</tr>
<tr>
<td>BHA</td>
<td>5.5 ± 0.1</td>
<td>-</td>
</tr>
</tbody>
</table>

*Concentration required for 50% reduction of DPPH (0.15 mM) or ABTS (7 mM) radicals.

*Sample concentration is 100 μg/mL.

*Values are means ± SEM (n=5).

*Standard compound for ABTS assay.

*Standard compound for DPPH assay.
fractions. The polyphenol content was 578.3 ± 42.7 mg/mL in the EtOAc fraction. This was the highest concentration and was approximately 7-fold higher than that in the methanol extract (84.5 ± 4.5 μg/mL). The polyphenol content decreased in the order of butanol fraction > methanol extract > water fraction > chloroform fraction > hexane fraction. On the other hand, the flavonoid content in the methanol extract was much lower than in the EtOAc or chloroform fraction.

The results in Tables 1 and 2 reveal a trend for an increase in radical scavenging capacity with the increase in polyphenol content. This agrees with the fact that polyphenolic compounds are responsible for much of the antioxidant capacity of plants.

### Platelet Aggregation

We next examined the inhibitory effects of various fractions obtained from the methanol extract of SMB on the aggregation of washed rat platelets induced by ADP (6 μM) or collagen (2 μg/mL) (Table 3).

In these experiments, the fractions were tested at concentrations of 100 and 50 μg/mL, respectively. The EtOAc fraction was a much more potent inhibitor of both ADP- and collagen-induced platelet aggregation compared to the methanol extract, butanol fraction, and water fraction. Pretreatment of platelets with the EtOAc fraction for 2 min dose-dependently inhibited ADP- and collagen-induced platelet aggregation (Fig. 1), with IC_{50} values of 34.2 ± 5.6 and 25.3 ± 1.5 μg/mL, respectively (Table 4).

These results suggest that the ability of the EtOAc fraction to block ADP- or collagen-induced platelet aggregation was significantly increased by fractionation of the methanol extract. This may be due to the concentration of polyphenolic compounds in the EtOAc fraction. The results also show that higher concentrations of the methanol extract and the EtOAc fraction were needed to inhibit ADP-induced aggregation than collagen-induced aggregation. In contrast, the hexane and chloroform fractions induced platelet aggregation in the absence of added agonist; therefore, they were not examined in the aggregation test. On the basis of the results in Fig. 1 and Table 1, we conclude that free radicals

<table>
<thead>
<tr>
<th>Sample</th>
<th>Aggregation (%)</th>
<th>Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ADP (μm)</td>
<td>Collagen (%)</td>
</tr>
<tr>
<td>Control</td>
<td>75.3 ± 1.5</td>
<td>76.0 ± 1.7</td>
</tr>
<tr>
<td>Methanol extract</td>
<td>62.3 ± 3.1</td>
<td>32.3 ± 4.0</td>
</tr>
<tr>
<td>Hexane fraction</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Chloroform fraction</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>EtOAc fraction</td>
<td>16.3 ± 2.3</td>
<td>6.7 ± 1.2</td>
</tr>
<tr>
<td>Butanol fraction</td>
<td>40.3 ± 4.6</td>
<td>16.0 ± 1.7</td>
</tr>
<tr>
<td>H₂O fraction</td>
<td>64.7 ± 1.5</td>
<td>61.3 ± 1.5</td>
</tr>
</tbody>
</table>

### Table 2. Total Content of Polyphenols and Flavonoids in Solvent Fractions from SMB

<table>
<thead>
<tr>
<th>Solvent Fraction</th>
<th>Polyphenols</th>
<th>Flavonoids</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methanol extract</td>
<td>84.5 ± 4.5</td>
<td>4.0 ± 0.3</td>
</tr>
<tr>
<td>Hexane fraction</td>
<td>54.3 ± 3.6</td>
<td>37.4 ± 1.3</td>
</tr>
<tr>
<td>chloroform fraction</td>
<td>54.7 ± 7.6</td>
<td>14.0 ± 0.8</td>
</tr>
<tr>
<td>EtOAc fraction</td>
<td>578.3 ± 42.7</td>
<td>29.4 ± 1.3</td>
</tr>
<tr>
<td>Butanol fraction</td>
<td>251.3 ± 11.1</td>
<td>5.2 ± 1.1</td>
</tr>
<tr>
<td>Water fraction</td>
<td>60.9 ± 7.7</td>
<td>1.3 ± 0.1</td>
</tr>
</tbody>
</table>

*Values are means ± SD (n > 3).
*mg of total polyphenol content/g of plant based on tannic acid as standard.
*mg of total flavonoid content/g of plant based on quercetin as standard.

### Table 3. Effects of Methanol Extract and Various Fractions Obtained from SMB on Platelet Aggregation Induced by ADP and Collagen

<table>
<thead>
<tr>
<th>Sample</th>
<th>Aggregation (%)</th>
<th>Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ADP (μm)</td>
<td>Collagen (%)</td>
</tr>
<tr>
<td>Control</td>
<td>75.3 ± 1.5</td>
<td>76.0 ± 1.7</td>
</tr>
<tr>
<td>Methanol</td>
<td>62.3 ± 3.1</td>
<td>32.3 ± 4.0</td>
</tr>
<tr>
<td>Hexane</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Chloroform</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>EtOAc</td>
<td>16.3 ± 2.3</td>
<td>6.7 ± 1.2</td>
</tr>
<tr>
<td>Butanol</td>
<td>40.3 ± 4.6</td>
<td>16.0 ± 1.7</td>
</tr>
<tr>
<td>H₂O</td>
<td>64.7 ± 1.5</td>
<td>61.3 ± 1.5</td>
</tr>
</tbody>
</table>

*Sample concentration was 100 μg/mL for ADP and 50 μg/mL for collagen.
*Aggregation is presented as means ± SEM (n=3).
*Inhibition (%) = [(A-B)/A]×100, where A is the percent aggregation in the control and B is the percent aggregation in the sample (%).
*[ADP] = 6 μM.
*[Collagen] = 2 μg/mL.
*ND, not determined.
Fig. (1). Effect of EtOAc fraction on rat platelet aggregation induced by ADP or collagen. Washed rat platelets were incubated with sample or DMSO (0.2%) at 37°C for 2 min and then stimulated with ADP (6 μM) or collagen (2 μg/mL). The extent of aggregation was monitored for 7 min after the addition of an inducer.

Pignatelli et al. [26] reported that the formation of platelet aggregates is associated with a transient H2O2 burst, which triggers oxidative stress in platelets. Resveratrol has been shown to inhibit ADP- or thrombin-induced platelet aggregation [27] and secretion [28] and to reduce the production of reactive oxygen species in thrombin-stimulated platelets [29]. In addition, salvianolic acid B, a component of SMB, has been shown to inhibit high shear stress-induced platelet aggregation [30], but the mechanism of action is unclear and whether it is the most potent compound in the polyphenol-rich fraction of this herb is not known.

Table 4. IC50 Values of Methanol Extract and EtOAc Fraction on the Aggregation of Rat Washed Platelets Induced by ADP and Collagen

<table>
<thead>
<tr>
<th>Agonist</th>
<th>Methanol extract IC50 (μg/mL)</th>
<th>EtOAc fraction IC50 (μg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADPb</td>
<td>472.3 ± 46.5</td>
<td>34.2 ± 5.6</td>
</tr>
<tr>
<td>Collagenc</td>
<td>68.4 ± 13.2</td>
<td>25.3 ± 1.5</td>
</tr>
</tbody>
</table>

aValues are means ± SD (n = 3).
b[ADP] = 6 μM.
c[Collagen] = 2 μg/mL.

distributed during platelet activation induce platelet aggregation and that bioactive antioxidants in SMB can block this process.

Platelet Adhesion

Platelet adhesion mediated by collagen or other adhesive proteins, such as fibrinogen, von Willebrand factor, fibronectin, or vitronectin, is the first step in platelet activation. Interactions between platelet receptors and adhesive proteins mediate platelet adhesion, intracellular signaling, and activation. This is accompanied by the synthesis and release of platelet activators such as thromboxane A2 and ADP [1]. Our results show that the methanol extract and EtOAc fraction of SMB modulate platelet adhesion to collagengen and fibrinogen. We found that preincubation of rat washed platelets for 10 min at 37°C with 25 to 200 μg/mL of either the methanol extract or the EtOAc fraction dose-dependently inhibited the adhesion of platelets to fibrinogen and collagen following stimulation with 0.2 U/mL thrombin (Fig. 2). The highest concentrations of methanol extract and EtOAc fraction used in the experiments (200 μg/mL) reduced adhesion by 59% and 49%, respectively. Furthermore, the EtOAc fraction was substantially more potent than the methanol extract at inhibiting platelet adhesion. The butanol and water fractions had little inhibitory effect on platelet adhesion to both collagen and fibrinogen (data not shown). Platelet adhesion has also been shown to be inhibited by polyphenolic compounds from Yucca schidigera Roezl. bark [31] and resveratrol [32], but this is the first report of an effect of SMB on platelet adhesion.

CONCLUSIONS

In the current study, we found that the polyphenol-rich EtOAc fraction of SMB potently inhibits platelet aggregation and adhesion in vitro, possibly due to free radical scavenging. On the basis of our results, we conclude that the EtOAc
fraction of SMB may be an important source of polyphenols with antioxidant activity that can inhibit platelet activation and therefore reduce thrombosis and cardiovascular disease.

ACKNOWLEDGEMENTS

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REFERENCES


Fig. (2). Effects of the methanol extract and EtOAc fraction on platelet adhesion to fibrinogen or collagen. Washed rat platelets were incubated with sample or DMSO (control; 1%) for 10 min at 37°C. After incubation, platelets were activated by thrombin (0.2 U/mL), and the level of adhesion was measured (n = 3). #P < 0.05; *P < 0.01.
Antiplatelet Function of S. miltiorrhiza Bunge


