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Antithrombotic Therapeutic Effects Of Aloe Vera Gel And Skin Extracts Through In-Vitro Biological Activity Evaluation

Dong-Myong Kim[1, 5†*], Yeo-Jin Lee[2†], Seo-Hyeon Hwang[3], Chae-Yun Yang[4], Hyung-Kon Lee[1], Yong-Seong Kwon[1], Yeon-Mea Choi[5], Chang Ok Kim[6] Gwang Jin Cho[6]

[1]*R&D Center Of Kjmbio Co. Ltd, Seoul, Korea*
[2] *Department Of Biomedical Engineering, UNIST, Ulsan (44919), Korea*
[3]*Department Of Biological Sciences, KAIST, Daejeon (34051), Korea*
[4]*Department Of Chemical And Biomolecular Engineering, KAIST, Daejeon (34051), Korea*
[5]*kimjeongmoon Aloe Ltd., 15 Saimdang-Ro, Seocho-Gu, Seoul (06649), Korea*
[6]*Department Of Dermatology, Seoul National University Bundang Hospital, Seongnam-Si, Gyeonggi-Do, Korea*

Abstract
Background: *Aloe is a succulent plant known for its diverse medicinal properties, traditionally used in wound healing, anti-inflammatory, and skin care treatments. The bioactive compounds present in both the gel and skin of Aloe vera, including polyphenols, are believed to contribute to its therapeutic effects. Despite its widespread use, there is a need for scientific validation of its potential antithrombotic and antioxidant activities, which could support the development of new functional biomaterials for medical applications.*
Materials and Methods: *In vitro biological activities of Aloe vera gel and skin extracts were evaluated. The total polyphenol content of Aloe vera skin was measured. Antioxidant activities were assessed using DPPH radical scavenging and superoxide dismutase (SOD) assays. The antithrombotic activities were determined using activated partial thromboplastin time (APTT) and prothrombin time (PT) assays.*
Results: *The total polyphenol content of Aloe vera skin was 41.12 mg/g. The DPPH radical scavenging activities were 55% for Aloe vera skin-70% EtOH extract, 38% for Aloe vera skin-D.W. extract, 11% for Aloe vera gel-70% EtOH extract, and 10% for Aloe vera gel-D.W. extract. The SOD antioxidant activity was higher in the Aloe vera-70% EtOH extract compared to the D.W. extract. Aloe vera skin D.W. extract exhibited significant APTT and PT anticoagulant activities, indicating antithrombotic activity in both intrinsic and extrinsic pathways.*
Conclusion: *Aloe vera gel and skin extracts possess significant antioxidant and antithrombotic activities, particularly in the 70% EtOH extracts of the skin. These findings suggest that Aloe vera extracts, especially from the skin, have potential as functional biomaterials for developing therapeutic agents due to their high antioxidant properties and antithrombotic effects.*
Key Word*: Intrathecal, Bupivacaine, Buprenorphine, Nalbuphine, Postoperative analgesia.*

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I. Introduction

Aloe (*Aloe vera Barbadensis* Miller) is a tropical plant belonging to the genus *Aloe* of the *Liliaceae* family that lives in tropical regions[1,2,3]. There are about 300 varieties of aloe that have been discovered to date, and there are 6-7 varieties of aloe that are used in the food industry and for medicinal purposes. Edible aloe includes *Aloe vera*, which is grown in large quantities mainly in Florida, Texas, New Mexico in the United States, and Indonesia, and *Aloe arborescence*, which grows naturally in Kyushu, Japan, *Aloe saponaria*, which grows naturally in the United States and Hawaii. Medicinal aloe includes *Scotra Aloe*, which grows mainly on Scotra Island in South Africa, and *Aloe ferox*, which grows naturally in Cape Town, East Africa. In Korea, *Aloe vera* and *Aloe arborescence* have been cultivated in the Jeju Island area since 1976[4]. In order to apply aloe to food and medicine, bioactive substances in each part of aloe must be identified and research on its biochemical actions must be conducted first. Aloe is known to contain over 200 compounds, including fatty acids, organic acids, and flavonoids, as well as glycoproteins, polysaccharides, and various types of low-molecular-weight substances such as anthraquinones, anthrones, chromones, and it is reported that it contains over 80 types of ingredients, including pyrones, amino acids, vitamins and minerals[5,6,7].

In the Korean Pharmacopoeia, most of the main ingredients are anthraquinones (aloin, emodin, isobarbaloin), which are contained in a liquid pressed from aloe leaves and have a strong constipation-relieving effect. In particular, aloin is known as an indicator that distinguishes between medicine and food. Aloe that can be used as food must contain less than 5% of this aloin ingredient, and most of this aloin ingredient exists in the yellow sap layer and also has an antibacterial effect[8]. Most of aloe's anthraquinones and polyphenol substances have hydroxyl groups, and in particular, substances containing phenolic acid esters are reported to have high antioxidant activity[9]. Aloe glycoprotein (NY 945), a glycoprotein isolated and purified from aloe vera, has been reported to have anti-allergic effects. In particular, it has been reported to inhibit histamine secretion and the infiltration of eosinophils into tissues[10]. It has been reported that alprogen, a single substance extracted from aloe, prevents commonly known allergic hypersensitivity reactions by binding to IgE antibodies present in the cell membranes of mast cells and normal cells[11]. In addition, aloe gel extract promotes the growth of fibroblasts, increases the elasticity of regenerated tissue, and promotes collagen decomposition and regeneration[12]. In addition, aloe extract is reported to have a protective effect against ultraviolet rays by increasing macrophage activity and enhancing the function of langrthans cells, thus promoting collagen biosynthesis in the body[13].

Most research on the efficacy of aloe is focused on the efficacy and ingredient analysis of aloe gel ingredients, and research on the physiological activity and related ingredients of aloe *skin* is still insufficient and is being discarded entirely. Therefore, in this study, we extracted aloe vera gel and skin, which have already proven to have physiological activity, using water and ethanol, and proposed the possibility of developing new treatments or medicines based on their antithrombotic activity.

II. Material And Methods

Experimental materials and reagents

Aloe (*Aloe vera Barbadensis* Miller) fresh leaves grown from KimJeongMoon Aloe (Jeju, Korea) and AloveBali (Bali, Indonesia) agricultural factory were used as experimental materials. The solvent used to extract aloe vera gel and skin was distilled water and 70% ethanol (Korea Alcohol Industrial Co., Korea). Reagents used for bioactivity analysis were Folin-ciocalteu's phenol reagent, ursolic acid, ascorbic acid, 1,1-diphenyl-2-picrylhyd-razyl (DPPH), N-succinyl-Ala-Ala-Ala-p-nitroanilide (SANA), elastase, collagenase, and 4-pheny-lazobenzyl-oxycarbonyl-pro-leu-gly-pro-d-ar were products from Sigma (MO, USA). All other reagents were of grade 1 or higher.

Preparation of Aloe Vera Extract

Aloe vera leaves were washed thoroughly using running water to remove foreign substances, the aloe vera gel and skin were separated, crushed using a grinder, and the particle size of the aloe was made uniform with a 20-mesh sieve. Add 20 times distilled water (D.W.) and 70% (v/v) ethanol to each aloe vera gel and skin with a certain particle size, extract in a shaking water bath (70°C, 110 rpm) for 4 hours, and then extract with filter paper. It was filtered using (Whatman No. 1). The filtered extract was freeze-dried, powdered, and used as a raw material for functional evaluation.

Total polyphenol content analysis

Total polyphenol content was analyzed using the Folin-Denis assay[14]. 50 ㎕ of folin-ciocalteu reagent was added to 0.1 ㎖ of each extracted and dried aloe vera gel and skin extract, mixed by dilution, reacted at room temperature for 4 minutes, and then mixed with 1.5 ml of 20% sodium carbonate anhydrous saturated solution. ㎖ was added and reacted for 2 minutes, and the absorbance was measured at 760 nm using a microplate reader (VERSAmax, Molecular Device, CA, USA). Total polyphenol content was calculated using a standard curve analyzing chlorogenic acid, a control material. The experimental results were measured three times and the content was expressed.

DPPH radical scavenging activity analysis

DPPH radical scavenging ability was determined by adding 160 ㎕ of DPPH solution dissolved in 0.4 mM methanol and 40 ㎕ of sample according to the method of Heo *et al*.[15], leaving it in the dark for 30 minutes to react, and then measuring the absorbance at 515 nm using a microplate reader. Measured. DPPH radical scavenging ability (%) was expressed as a reduction rate in absorbance of the sample solution with and without addition as follows.

DPPH radical scavenging ability (%) = 1-(Absorbance of sample addition group/Absorbance of additive-free group) x 100

SOD activity analysis

SOD activity was determined by adding 40 μl of sample and 40 μl of 7.2 mM pyrogallol to 120 μl of Tris-HCl buffer solution (pH 8.5) according to the method of Lee *et al*.[16], reacting at 25℃ for 10 minutes, and measuring the absorbance of the sample at 420 nm. SOD activity (%) was expressed as the rate of decrease in absorbance of the sample solution added and not added as follows.

SOD activity (%) = 1-(Absorbance of sample addition group/Absorbance of additive-free group) x 100

Antithrombotic activity assay

1) Thrombin time (TT) analysis

TT assay was used to confirm activity in the common pathway among antithrombotic activities. 50 ㎕ of plasma solution mixed with sample and plasma (TEControl N, plasma, TECO, Neufahrn, Germany) at a ratio of 1:9 was preheated at 37°C for 1 minute, and then preheated at 37°C with thrombin.

After adding 50 ㎕ of diagnostic reagent, the time until coagulation was recorded using a coagulometer (Coatron M1, TECO, Neufahrn, Germany). For the control group, 25 ㎕ of pure plasma was used to measure the coagulation time.

2) Activated partial thromboplastin time (APTT) analysis

APTT assay was used to confirm activity in the intrinsic pathway among antithrombotic activities. Mix sample and plasma (TEControl N, plasma, TECO, Neufahrn, Germany) at a ratio of 1:9 with 25 ㎕ of plasma solution and 25 ㎕ of APTT diagnostic reagent, preheat at 37°C for 5 minutes, and then at 37°C. After adding 25 ㎕ of preheated 20mM $CaCl_2$, the time until coagulation was measured using a coagulometer.

3) Prothrombin time (PT) analysis

PT assay was used to confirm activity in the extrinsic pathway among antithrombotic activities. 25 ㎕ of plasma solution, which is a mixture of sample and plasma (TEControl N, plasma, TECO, Neufahrn, Germany) at a ratio of 1:9, was preheated at 37°C for 2 minutes, and then preheated at 37°C for 50 minutes with the prothrombin time diagnostic reagent. After adding 50 ㎕, the time until coagulation was measured using a coagulometer (Coatron M4, Teco, Germany).

Statistical processing

For comparative analysis between each experimental group, ANOVA analysis was performed using the SPSS 14.01 program (Version 14.01, SPSS Inc., USA), and significance was verified using Duncan's multiple range test at α=0.05.

III. Result And Discussion

Total polyphenol content

To investigate the effect of different extraction solvents on the antioxidant power of aloe vera extract, the total polyphenol content of aloe vera extracted with D.W. and 70% ethanol was analyzed and shown in Table.

Table 1. Total polyphenol contents of aloe vera gel and skin extracts.[1),2)]

	Aloe vera gel extracts		Aloe vera skin extracts	
Polyphenol(mg/g)	D.W.	70% ethanol	D.W.	70% ethanol
	10.49±0.06	15.38±0.45	38.81±0.12	41.12±0.47

[1)]Values are means ± SD, n=3.

[2)]Values in row not sharing a common superscript differ significantly ($p < 0.05$)

Among the many phytochemicals present in plants, polyphenol compounds and flavonoids are widely distributed in various foods and medicines, and studies have been conducted to show that they can act as natural antioxidants[17,18,19]. As a result of the experiment, the total polyphenol content was determined by D.W. In the case of extracts, aloe vera skin and gel existed in that order, and the contents were 38.81 mg/g and 10.49 mg/g, respectively. In the case of 70% ethanol extract, aloe vera skin and gel were measured to be highest in that order, and the respective contents were 41.12 mg/g and 15.38 mg/g. According to a study by Lee *et al.*[20], the total polyphenol content of aloe was reported to be 1.88 mg/g and 2.00 mg/g for 50% MeOH extract and 80% MeOH extract, respectively, which is about 7~7 mg/g compared to the aloe extract in this study. It showed 20 times lower polyphenol content. From these results, it was confirmed that the polyphenol content varies greatly depending on the extraction method, and based on these results, it is judged that the total polyphenol content of aloe vera did

not show a significant difference compared to previous studies. It has been reported that aloe's phenolic compounds have the effect of scavenging free radicals or hydroxyl radicals[21], and the total polyphenol content of each extract can be determined from the aloe vera gel and skin used in this study. It was confirmed that antioxidant activity due to polyphenols can be expected.

Antioxidant activity

DPPH radical is a stable free radical that has the property of accepting electrons or protons from other atoms and molecules and changing into a stable molecule. Using these DPPH radicals, the scavenging activity of aloe extract against DPPH was analyzed at various concentrations, and the activity is shown in Fig. 1. The control group, ascorbic acid, showed more than 90% activity at a concentration of 120 ㎍/㎖.

When the concentration of 70% ethanol extract of aloe vera skin was 1,000, 2,000, and 4,000 ㎍/㎖, the DPPH radical scavenging ability was 35%, 45%, and 55%, respectively. These results confirmed that the activity was similar to that reported by Shin *et al*.[22], where the palm cactus extract, a cactus plant, showed 44.57% DPPH radical scavenging ability at a concentration of 3,200 ㎍/㎖. On the other hand, the 70% ethanol extract of aloe vera gel showed a low DPPH radical scavenging activity of 11% only at a concentration of 4,000 ㎍/㎖, and the DPPH radical scavenging activity could not be confirmed at lower concentrations.

The D.W. extract of aloe vera skin showed a DPPH radical scavenging activity of 28% and 38% at concentrations of 2,000 and 4,000 ㎍/㎖, respectively, and the D.W. extract of aloe vera gel showed a DPPH radical scavenging activity of 10% only at a concentration of 4,000 ㎍/㎖.

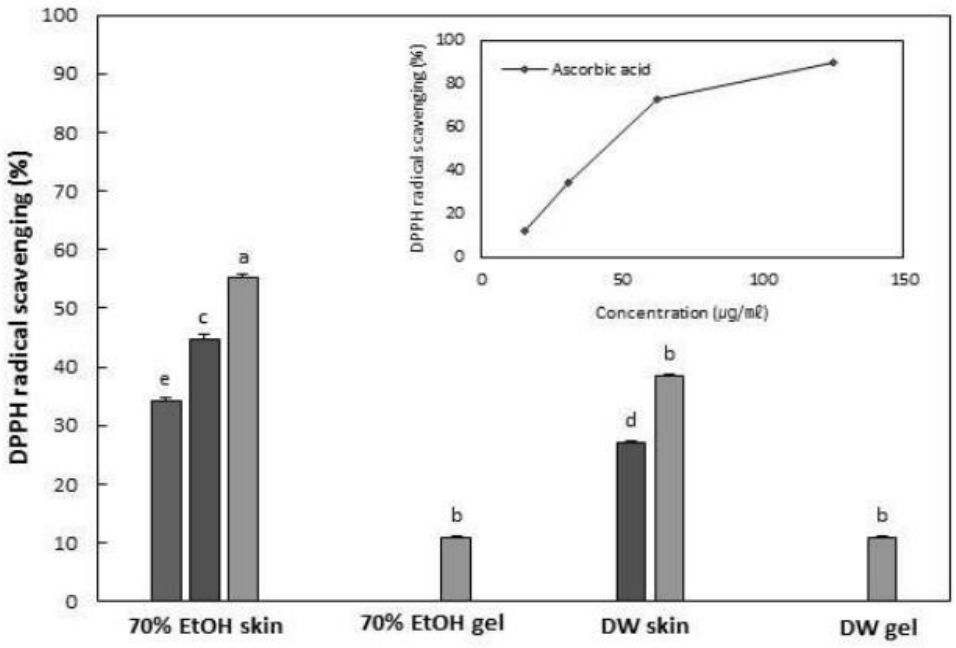

Figure 1. DPPH free radical scavenging activities of aloe vera gel and peel (■, 1,000 ㎍/㎖; ■, 2,000 ㎍/㎖; ■, 4,000 ㎍/㎖).

In conclusion, by region, aloe vera skin had a higher DPPH radical scavenging ability than aloe vera gel, and by solvent, the 70% ethanol extract showed a higher DPPH radical scavenging ability than the D.W. extract. As seen in Table 1, considering that the 70% ethanol extract has a higher polyphenol content than the D.W. extract, it is believed that the activity appeared dependent on the polyphenol concentration.

As another method of analyzing antioxidant activity, SOD activity was measured by measuring the auto-oxidation reaction of pyrogallol, which reacts with superoxide to produce browning substances[23,24]. The 70% ethanol extract of aloe vera skin showed 50% activity at a concentration of 4,000 ㎍/㎖, which is similar to the activity shown at the control ascorbic acid at a concentration of 120 ㎍/㎖ (Fig. 2).

In addition, the D.W. extract of aloe vera skin, 70% ethanol extract of aloe vera gel, and D.W. extract of aloe vera gel showed SOD activities of 44%, 27%, and 16%, respectively. The research results of Lim[25] showed

that SOD activity significantly increased in the aloe and fermented aloe group compared to the control group. In this study, SOD activity was shown in the following order: 70% ethanol extract of aloe vera skin, D.W. extract of aloe vera skin, 70% ethanol extract of aloe vera gel, and D.W. extract of aloe vera gel.

The experimental results related to antioxidant activity obtained in this study also show that among the compounds showing physiological activity of aloe, polyphenol compounds mostly have a hydroxyl group, and this is believed to have an effect on the antioxidant effect[26]. In addition, the relatively low inhibition rate of antioxidant activity of aloe vera gel, excluding aloe vera skin, appears to be correlated with the fact that many antioxidant substances are found in the skin[27]. Considering that the antioxidant activity of plant extracts is due to phenolics or flavonoid substances[28], the high antioxidant activity of aloe vera skin extract is also believed to be due to the total phenol and flavonoid content contained therein.

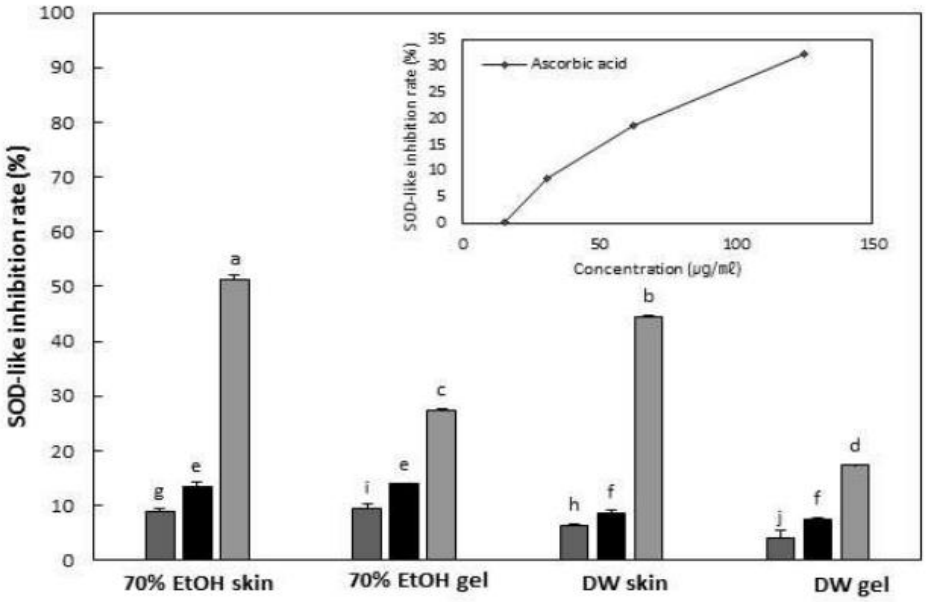

Figure 2. SOD activity of Aloe vera gel and skin extracts
(, 1,000 μg/mℓ; , 2,000 μg/mℓ; , 4,000 μg/mℓ).

Antithrombotic activity

Blood clotting is an activity within the human body to prevent excessive bleeding, and antithrombotic activity measurement methods are divided into the intrinsic pathway, extrinsic pathway, and common pathway depending on the coagulation mechanism. In this study, the anticoagulant activity of aloe extract was measured to determine its effect (Fig. 3, 4, and 5). In the intrinsic pathway, heparin, a positive control, showed excellent blood coagulation inhibitory activity of over 300 seconds at a concentration of 1.6 μg/mℓ, and in the case of D.W. extract of aloe vera skin, 333 μg/mℓ compared to the blank test group (39 seconds). It showed a delay effect of about 17% in concentration. Aloe vera skin 70% ethanol extract, aloe vera gel D.W. extract, and aloe vera gel 70% ethanol extract showed a thrombus formation delaying effect of 13%, 9%, and 7%, respectively. As for the extrinsic pathway, in the case of heparin, which is a positive control, the blood coagulation inhibitory activity was excellent at 140 seconds at a concentration of 12.5 μg/mℓ, and in the case of D.W. extract of aloe vera skin, at a concentration of 333 μg/mℓ compared to the blank test group (19 seconds). It showed a delaying effect of about 15%, and the thrombus formation delaying effect of 16%, 13%, and 13% was shown in aloe vera skin 70% ethanol extract, aloe vera gel D.W. extract, and aloe vera gel 70% ethanol extract, respectively. In the case of heparin, a positive control of the common pathway, the blood coagulation inhibitory activity was excellent at a concentration of 2.5 μg/mℓ for more than 300 seconds, and the aloe extract did not show a delay in thrombus formation compared to the blank test group (10 seconds). In common, antithrombotic activity was shown in the D.W. extract of aloe vera skin in both intrinsic and extrinsic pathways. According to a report by Shin *et al*.[29], the antithrombotic activity of *Fritillaria ussuriensis* Maxim, a member of the *Liliaceae* family, was excellent in the methanol extract, and the hexane, ethyl acetate, and butanol fractions showed activity of more than 300 seconds at a concentration of 2.4 mg/ml.

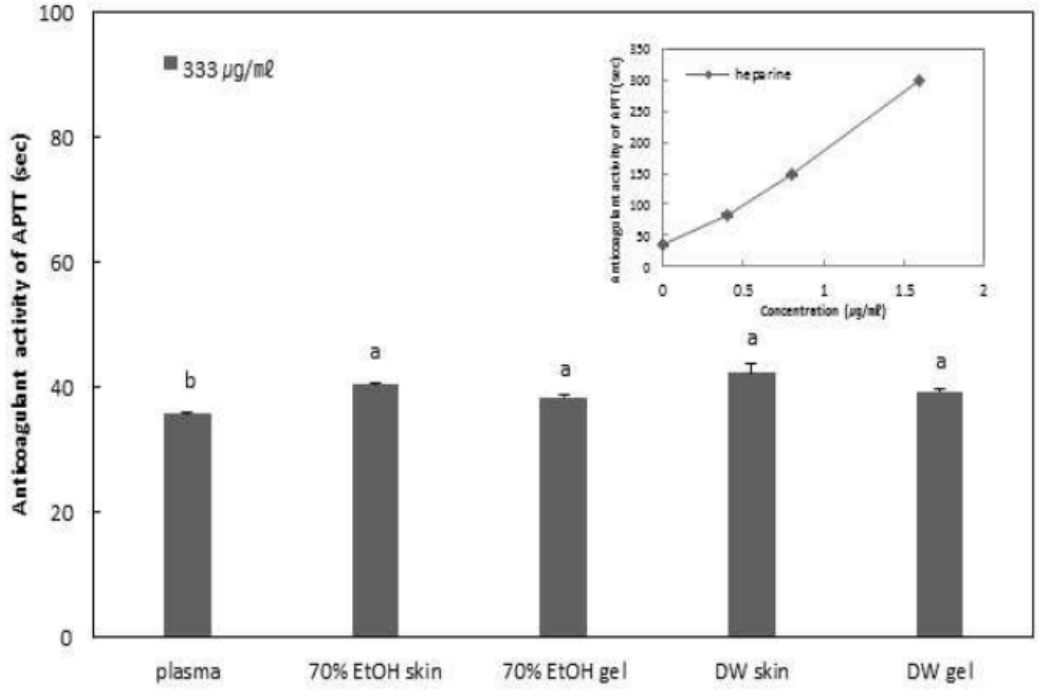

Figure 3. Anticoagulant activity of Aloe vera gel and skin extracts measured by activated partial thromboplastin time (APTT) activity assay.

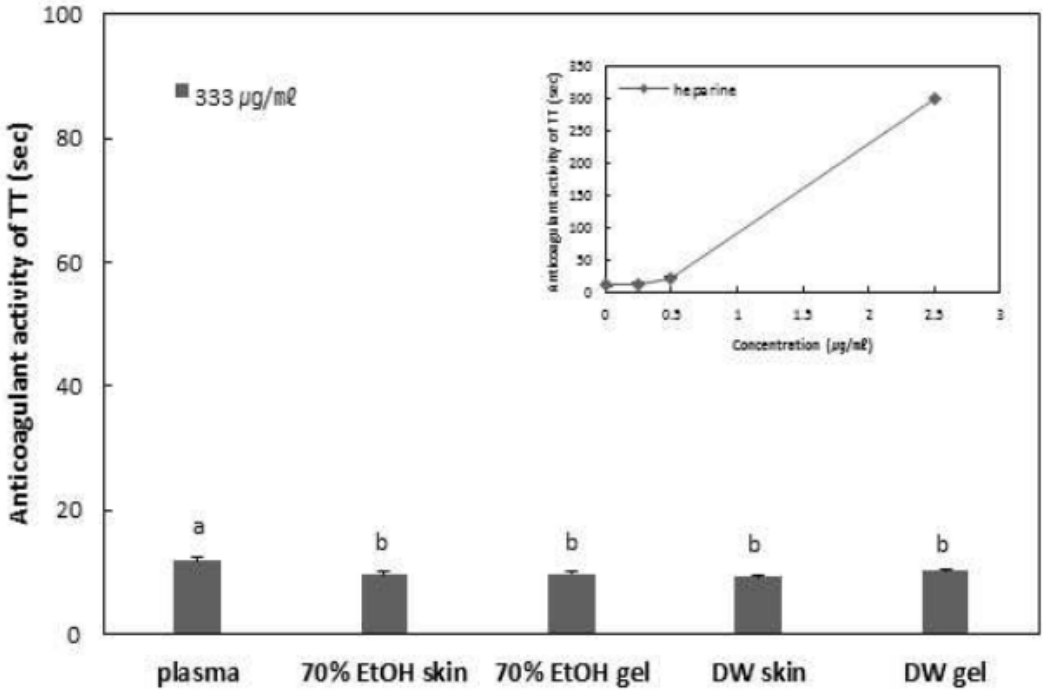

Figure 4. Anticoagulant activity of Aloe vera gel and skin extracts measured by thrombin time (TT) activity assay.

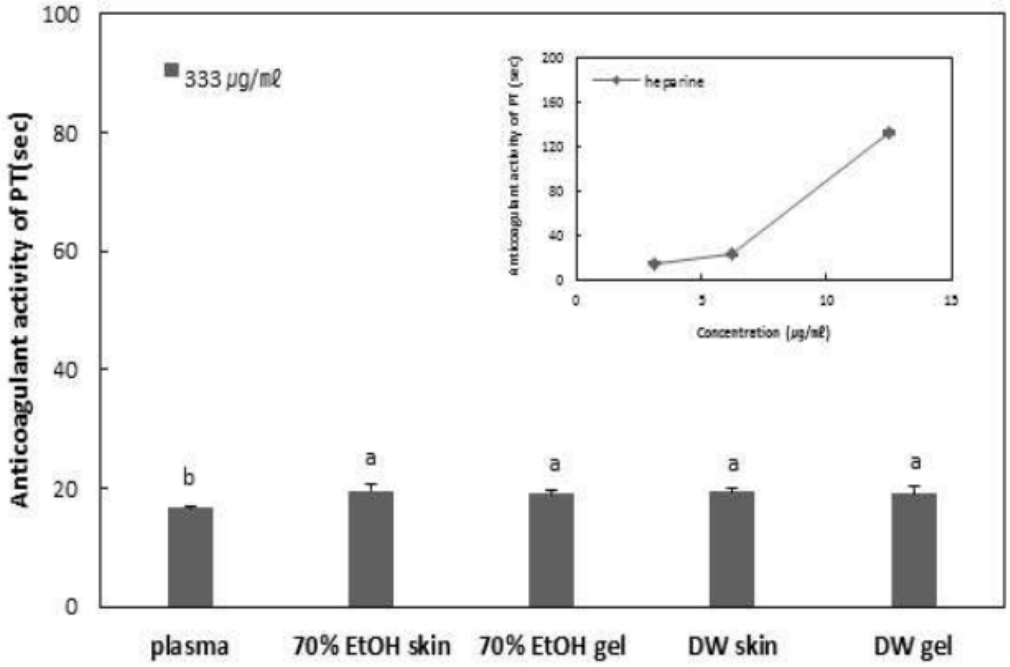

Figure 5. Anticoagulant activity of Aloe vera gel and skin extracts measured by prothrombin time (PT) activity assay.

IV. Conclusion

Aloe (Aloe vera Barbadensis Miller) is a tropical plant belonging to the genus Aloe of the Liliaceae family that lives in tropical regions, and most studies are conducted on ingredients extracted from aloe gel. Therefore, in this study, we compared and analyzed the physiological activities of aloe vera gel and skin using aloe vera extract, which is not a substance whose efficacy has already been proven. The total polyphenol content of D.W. extract was highest in that order, followed by aloe vera skin and gel, and the contents were 38.81 mg/g and 10.49 mg/g, respectively. As a result of measuring antioxidant activity, the 70% ethanol extract of aloe vera skin showed DPPH radical scavenging activity of 55%, and the SOD activity was also high compared to other extracts, with 50% activity in the 70% ethanol extract of aloe vera skin. Antithrombotic activity measurement results showed that the D.W. extract of aloe vera skin had antithrombotic activity in both the intrinsic and extrinsic pathways. Based on these results, it was confirmed that aloe vera gel and skin extract have anti-thrombotic activity due to their antioxidant properties, and showed potential in the development of treatments or medicines.

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