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In vitro anti-coagulation activity of *Panax bipinnatifidus* Seem. and *Panax stipuleanatus* H.T. Tsai et K. M. Feng saponin enriched extracts

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Abstract: *Panax bipinnatifidus* Seem. and *Panax stipuleanatus* H.T. Tsai et K.M. Feng are rich saponin containing medicinal plants primarily grown in the North-West Moutain region in Vietnam. The present study aims to explore the anticoagulant activity of saponin enriched extracts isolated from these herbal plants using prothrombin time (PT) and activated partial thrombin time (aPTT). Heparin (0.1 IU/mL) was used as positive control, while DMSO (0.04 %) and NaCl (0.9 %) were used as negative and solvent control. Heparin showed clearly effect on aPTT prolongation the time up to 58.14 seconds while those in DMSO and NaCl group were 34.86 and 34.63 seconds, respectively. In contrast to our previous study using crude extracts, the saponin-enriched extracts of *Panax bipinnatifidus* Seem. and *Panax stipuleanatus* H.T. Tsai et K.M. Feng did not express any anticoagulant activity ($p>0.05$). In conclusion, our study demonstated that the anticoagulant activity of crude extracts of *Panax bipinnatifidus* Seem. and *Panax stipuleanatus* H.T. Tsai et K.M. Feng was not caused by saponin compound.

Keywords: Anti-coagulant activity, *Panax bipinnatifidus* Seem., *Panax stipuleanatus* H.T. Tsai et K. M. Feng, saponin-enriched extract

INTRODUCTION

Cardiovascular diseases related to coagulation pathophysiology such as cardiac injury, stroke or thrombosis could cause high risk of death in affected patients. Furthermore, blood cloting limited the blood flow and acute ischemic in tissue or organ. Combination of anticoagulant and anti-platelet aggregation drugs like clopidogrel, acenocoumarol and warfarin co-treated with aspirin were first medicinal therapies for patients, especially after coronary artery stenting or heart valve replacement (Cordier, 2012). Despite the anticoagulant activity, these drugs could also have side effect of bleeding. Thus, search for finding alternative medicinal plants possesing anticoagulant activity broaden the horizon for drug replacement as per the patient's choice.

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Panax bipinnatifidus Seem. and *Panax stipuleanatus* H.T. Tsai et K.M. Feng have been used for long-term treatment of patients with cardiovascular diseases. It is well known that these *panax* extracts contain high saponin content with dammarane and oleanan structures in their leaves and roots (Yang, 1985). Previous study showed that some crude extracts of *Panax bipinnatifidus* Seem. and *Panax stipuleanatus* H.T. Tsai et K.M. Feng have anticoagulant activity (Trinh, 2018). The question is whether the same result will be observed in saponin-enriched extracts of these two medicinal plants. To answer this question, anticoagulant activity of saponin-riched extracts of *Panax bipinnatifidus* Seem. and *Panax stipuleanatus* H.T. Tsai et K.M. Feng is investigated using pro-thrombin time (PT) and activated partial thrombin time (aPTT).

MATERIAL AND METHODS

Plant extracts: Panax bipinnatifidus Seem. and *Panax stipuleanatus* H.T. Tsai et K.M. Feng root and stem were collected at Hoang Su Phi, Ha Giang province, Vietnam. Saponin extracts were provided by Dr. Nguyen Huu Tung from Department of Pharmaceutical Chemistry, School of Medicine and Pharmacy, VNU-Hanoi and Assoc. Prof. Dr. Do Thi Ha from Department of Plant Chemistry, National Institute of Medicinal Materials, Vietnam.

Blood collecting: Venous blood (4 ml) drawn from healthy volunteers of age from 21 to 25 years were stored in tubes containing 3.8% sodium citrate. Blood tubes were then centrifuged at 3000 rpm for 10 minutes. Selection criteria of patients included no history of hematological disease, non-smoking, and not using anti-coagulant drug.

Anti-coagulant activity assay: Plasma samples were divided into 5 tubes with 450 µl in each tube. 50 µl of saponin extracts of *Panax bipinnatifidus* Seem. and *Panax stipuleanatus* H.T. Tsai et K.M. Feng were added with the dose range of 0; 100; 200; 400; 800 µg/mL into serum, respectively. DMSO 0.04% and heparin 0.1 IU/mL were used as negative and positive controls. Anti-coagulant activity assay was performed by using PT and aPTT kits (Sysmex CA 530, Japan) following the manufacture assay guideline. Each assay was repeated 7 times.

Statistical analysis: Data was entered, edited and analysed using SPSS software version 16.0 (IBM, USA)- with one-way ANOVA test along with post-hoc comparison. The processed data were shown in mean ± SE; $p \leq 0.05$ expressed for significant difference.

Ethic approval: This study was approved by the ethic committee of School of Medicine and Pharmacy, code IRB-VN01016.

RESULTS

Effect of saponin extract of Panax bipinnatifidus Seem. and *Panax stipuleanatus* H.T. Tsai et K.M. Feng *on prothrombin time (PT):* The effect of saponin extracts from *Panax bipinnatifidus* Seem. and *Panax stipuleanatus* H.T. Tsai et K.M. Feng on PT for *in vitro* study is shown in Table 1. The saponin riched extracts at all concentrations were not modulated in PT compared with both DMSO 0.04% and NaCl 0.9%. Surprisingly, heparin as positive control also revealed no effect as expected ($p>0.05$).

Effect of saponin extract of Panax bipinnatifidus Seem. and Panax stipuleanatus H.T. Tsai et K.M. Feng on activated partial thrombin time (aPTT): Similar results were observed with aPTT (Table 2) that no saponin extracts of *Panax bipinnatifidus* Seem. changed the aPTT *in vitro*. Heparin which served as positive control was not showed clearly effect on PT but dominant effect on aPTT ($p<0.000$).

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Table 1. Effect of saponin extract from *Panax bipinnatifidus* Seem. and *Panax stipuleanatus* H.T. Tsai et K.M. Feng on PT

Groups	n	PT (s)	PT (%)	INR
DMSO 0.04%	7	12.00 ± 0.24	88.43 ± 2.67	1.09 ± 0.021
NaCl 0.9%	7	11.95 ± 0.25	88.86 ± 2.74	1.08 ± 0.022
HP 0.1 IU/ml	7	11.99 ± 0.26	88.57 ± 2.83	1.09 ± 0.023
PbS 100 µg/ml	7	11.75 ± 0.25	91.00 ± 2.78	1.07 ± 0.021
PbS 200 µg/ml	7	11.91 ± 0.25	89.29 ± 2.78	1.08 ± 0.022
PbS 400 µg/ml	7	11.99 ± 0.23	88.43 ± 2.51	1.09 ± 0.021
PbS 800 µg/ml	7	11.69 ± 0.24	92.00 ± 2.81	1.06 ± 0.021
PS 100 µg/ml	7	11.70 ± 0.21	91.50 ± 2.39	1.06 ± 0.019
PS 200 µg/ml	7	11.80 ± 0.21	90.43 ± 2.30	1.07 ± 0.018
PS 400 µg/ml	7	11.84 ± 0.23	90.00 ± 2.55	1.07 ± 0.020
PS 800 µg/ml	7	11.70 ± 0.23	91.71 ± 2.64	1.06 ± 0.020
p (ANOVA)		> 0.05	> 0.05	> 0.05

(HP: Heparin; PbS: Panax bipinnatifidus Seem. saponin extracts; PS: Panax stipuleanatus H.T. Tsai et K.M. Feng *saponin extracts; PT: Prothrombin time; INR: International Normal Ratio)*

Table 2. Effect of saponin extract from *Panax bipinnatifidus* Seem. *and Panax stipuleanatus* H.T. Tsai et K.M. Feng on aPTT

Groups	n	APTT (s)	Ratio
DMSO 0.04%	7	34.86 ± 1.00	1.12 ± 0.032
NaCl 0.9%	7	34.63 ± 0.95	1.12 ± 0.031
HP 0.1 IU/ml	7	58.14 ± 5.11 *	1.87 ± 0.165 *
PbS 100 µg/ml	7	34.47 ± 0.99	1.12 ± 0.032
PbS 200 µg/ml	7	35.03 ± 0.96	1.13 ± 0.030
PbS 400 µg/ml	7	35.20 ± 0.93	1.14 ± 0.030
PbS 800 µg/ml	7	35.81 ± 1.01	1.15 ± 0.033
PS 100 µg/ml	7	34.92 ± 1.01	1.13 ± 0.034
PS 200 µg/ml	7	35.30 ± 1.00	1.14 ± 0.033
PS 400 µg/ml	7	35.44 ± 1.02	1.14 ± 0.033
PS 800 µg/ml	7	35.69 ± 1.07	1.15 ± 0.036
p ANOVA		p = 0.000	p = 0.000

(**p=0.000 vs DMSO 0.04%; PbS: Panax bipinnatifidus Seem. saponin extracts; HP: Heparin; aPTT: activated partial thrombin time)*

DISCUSSION

In our study, we used heparin with concentration of 0.1 IU/ml as positive control. Previous studies have reported that heparin affected aPTT activity by activation of antithrobin III, inactivation of thrombin and IXh, Xh, XIh, XIIh factors. In such assay, it was recommended that anti-vitamin K acting as anti-coagulant drugs should not be used since they needed to be converted by liver and, therefore, they would be ineffective for *in vitro* models (Chen, 2018). Our results were in agreement with previous studies (Lau, 2009; Liang, 2010). Lau et al. (2009) studied anti-platelet and anti-coagulant activites of *Panax notoginseng, Panax ginseng and Panax quinquefolium* in Korea. Amond these three types of *Panax*, only *Panax notoginseng* stem extracts showed dominant anti-platelet and anti-coagulant effects while *Panax ginseng and Panax quinquefolium* had no effect on

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these activites. The same results were observed in our previous study on Vietnam *Panax notoginseng* with the prolongation of coagulation as well as anti-thrombosis activites (Thao, 2016).

Concerning anti-coagulant activity of *Panax bipinnatifidus* Seem. *and Panax stipuleanatus* H.T. Tsai et K.M. Feng *saponin extracts,* as shown in Table 1 and 2, at all concentration there was no effect of saponin riched extracts on both PT and aPTT. In Vietnam, these two medicinal plants belonging to *Panax* L. are commonly used as pain killers, and for protection of cardiovascular diseases (Luận, 2009). Li (2013) showed that the genus *Panax* had potential anticoagulant activity. Our previous studies on anticoagulant activity of n-butanol, ethyl acetat extracts from *Panax bipinnatifidus* Seem. as well as n-hexan and water extracts from *Panax stipuleanatus* H.T. Tsai et K.M. Feng expressed clearly effects on aPTT value (Trinh, 2018; Thom, 2018[a]). In addition, our previous study demonstrated that *Panax stipuleanatus* H.T. Tsai et K.M. Feng induced the smooth muscle relaxation via NO release (Thom, 2018[b]). From our previous results, we expected that ethyl acetate, n-hexan or n-butanol extracts should contain higher saponin contents with subsequent enhanced anticoagulant activity. Unfortunately, in our current study, saponin-enriched extract did not show any effect at all concentration on either PT or aPTT. Looking at the chemical properties of *Panax bipinnatifidus* Seem. and *Panax stipuleanatus* H.T. Tsai et K.M. Feng, previous studies showed that these *Panax* extracts contained many other identified compounds such as triterpenoid, amino acid, reduced sugar, organic acid and minerals (Luan, 2002, 2009). Therefore, the compound that acts as anticoagulant in our previous studies might not result from saponin but from other unidentified compounds that deserve further investigation in the near future.

CONCLUSION

Saponin-enriched extracts of of *Panax bipinnatifidus* Seem. and *Panax stipuleanatus* H.T. Tsai et K.M. Feng did not show any effect on anticoagulant activity *in vitro* model.

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DECLARATION OF CONFLICT OF INTEREST

None of conflict of interest

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