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INHIBITORY EFFECT ON HUMAN BLOOD COAGULATION OF AERIAL *CANNA EDULIS* KER GAWL

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Summary

Inhibitory Effect on Human Blood Coagulation of Aerial *Canna edulis* Ker Gawl

Canna edulis Ker Gawl has been traditionally used to treat heart diseases with a lack of scientific evidence. This study reports for the first time the anticoagulant effect of the aerial part of *C. edulis*. The aerial part of *C. edulis* was extracted with methanol and then fractionated with *n*-hexane, ethyl acetate, and water. Three assays: prothrombin time (PT), activated partial thromboplastin time (APTT) and thrombin time (TT) were performed to evaluate the anticoagulant activity of various extracts on human blood samples. The results showed that the methanol extract and the ethyl acetate fraction significantly prolonged APTT and TT, suggesting their impact on coagulation factors involved in the intrinsic and common pathway of the coagulation cascade. The water fraction also significantly prolonged APTT compared to the negative control ($p < 0.05$). *C. edulis* could be a promising natural resource for finding novel antithrombotic agents and to develop supplementary products for treating and preventing thrombosis-related diseases. Further studies should be done to investigate new bioactive molecules from this interesting plant and to understand their mechanisms of antithrombotic action.

Keywords: *Anticoagulant, Canna edulis, Aerial, Clotting time.*

1. Introduction

Cardiovascular diseases (CVDs) are the leading cause of morbidity and mortality worldwide [1]. Antithrombotic drugs remain the cornerstone in the treatment of CVDs. However, many adverse effects are associated with conventional antithrombotic drug regimens [2]. Alternative therapies with high efficacy and fewer side effects are thus needed. Medicinal plants are important natural resources for drug development due to their nutritional, safety, and therapeutic values [3],[4].

Canna edulis Ker Gawl belongs to *Canna*, the only genus of *Cannaceae* plant family. Its starchy rhizome is an important source of noodles in Vietnam [5]. Both aerial and rhizome parts of this plant were used for the treatment of various conditions such as ulcers, pain, bruises, hepatitis, diarrhea, and heart-related diseases in folk medicine. However, scientific evidence about the phytochemicals and pharmacological effects of this plant is still limited. *C. edulis* rhizome was shown to have good antioxidant activity. Some phenolic compounds with antioxidant effect [6], lignan with antidiabetic effect [7], and arabinoxylan with inhibitory effects on pepsin and lipase activity [8] were isolated from the rhizome. Recently, we reported anticoagulant and antiplatelet activity and isolated bioactive compounds possessing these effects from *C. edulis* rhizome [3]. It could be seen that current studies mainly focus on the rhizome but not the aerial part of this interesting plant. This study aimed to investigate for the first time the anticoagulant effect of various extracts of the aerial part of *C. edulis*.

2. Materials and methods

2.1. Materials

The edible *canna* plant was collected in Son La province, Vietnam in July 2022, and identified with the scientific name *Canna edulis* Ker Gawl. A voucher specimen (No. CE.A.TN02) was kept at the Department of Life Sciences, University of Science and Technology of Hanoi, Hanoi, Vietnam.

Chemicals used in this study were dimethyl sulfoxide (DMSO) from Sigma-Aldrich, prothrombin time (PT), activated partial thromboplastin time (APTT), and thrombin time (TT) reagents from Dade Behring Marburg GmbH (Marburg, Germany). Solvents for plant extraction included methanol (MeOH), *n*-hexane, and ethyl acetate (EtOAc) from China.

2.2. Preparation of aerial Canna edulis extracts

The raw aerial part of *C. edulis* was cleaned, cut into pieces, and dried at room temperature. Then, it was ground into fine powder. The powder (0.8 kg) was macerated in MeOH three times at room temperature, and the solvent was then evaporated. The total methanol extract (CE.A.Me) was then fractionated with *n*-hexane, ethyl acetate, and water. Each fraction was extracted three times. The extracts were combined, filtered, and evaporated under reduced pressure to completely remove the solvent. The fractions of *n*-hexane (CE.A.H), ethyl acetate (CE.A.EA), and water (CE.A.W) were obtained and kept at 4-6 C for further use. The

extracts were dissolved in DMSO and then diluted into concentrations of 20, 40, and 80 mg/mL. The final DMSO concentration in the tested samples did not exceed 0.1%.

2.3. Preparation of platelet-poor plasma

The ethics approval was obtained from the Research Ethics Committee, School of Medicine and Pharmacy, Vietnam National University, Hanoi, Vietnam (document number 022020/CN-HĐĐĐ). Healthy volunteers who had not taken any medicines for at least 3 weeks, were nonsmokers, aged between 18 and 35, and fasting overnight were recruited for the study. Their complete blood counts were checked before anticoagulant experiments. Only blood samples that had normal blood cell counts were used for experiments. The venous blood was taken and put in a tube containing 3.2% sodium citrate as an anticoagulant. Then, the blood sample was centrifuged at 3000 rpm for 10 min, and the supernatant, named platelet-poor plasma (PPP), was collected. The experiments were performed at the National Institute of Hematology and Blood Transfusion.

2.4. Activated partial thromboplastin time assay

The mixtures of 50 µL of plant extracts at different concentrations and 450 µL of PPP were prepared and incubated at 37°C for 5 minutes. Activated partial thromboplastin time (APTT) reagents were then added, and APTT values were measured, using the Sysmex CS-2100i machine (Japan). APTT refers to the time from the addition of calcium chloride until the plasma clots. DMSO at 0.1% and heparin at 0.2 IU/mL was used as the negative and positive control, respectively.

2.5. Prothrombin time assay

The mixtures of 50 µL of plant extracts and 450 µL of PPP were prepared and incubated at 37°C for 5 minutes. Prothrombin time (PT) reagents were then added into the mixtures, and the PT values of samples were measured by the Sysmex CS-2100i machine (Japan). PT refers to the time from the addition of calcium chloride until the plasma clots. DMSO at 0.1% and heparin at 0.2 IU/mL was used as the negative and positive control, respectively.

2.6. Thrombin time assay

First, 50 µL of plant extracts were added into 450 µL of PPP and the mixtures were then incubated at 37°C for 5 minutes. In the Sysmex CS-2100i machine (Japan), thrombin time (TT) reagent was added and TT values were measured for different samples. TT refers to the time from the addition of TT reagent until the plasma clots. DMSO at 0.1% and heparin at 2.0 IU/mL was used as the negative and positive control, respectively.

2.7. Data analysis

Data were presented as mean ± standard deviation (SD). Statistical analysis was performed using R Programming. Pearson correlation coefficient (r) test was used to test the associations between variables, and independent sample t-test and ANOVA test were used to compare the differences between samples. $P < 0.05$ was regarded as statistically significant.

3. Results and discussion

Effect of extracts on activated partial thromboplastin time

The results showed positive correlations between APTT values and tested extract concentrations for the total methanol extract, the ethyl acetate, and water fractions (Pearson correlation coefficient, $r > 0.9$, $p < 0.05$). The total methanol extract at both concentrations of 8 and 4 mg/mL significantly prolonged APTT compared to the negative control (mean APTT, at 8 and 4 mg/mL, 40.37 ± 1.08 and 32.5 ± 0.60 vs. 27.63 ± 0.84 s, respectively, $p < 0.05$). Moreover, the ethyl acetate showed the highest ability to prolong APTT compared to other extracts ($p < 0.05$). Notably, the APTT value of the ethyl acetate fraction at 8 mg/mL was similar to the one of the positive control heparin 0.2 IU/mL (mean APTT, 42.70 ± 1.11 vs. 45.23 ± 3.09 s, $p > 0.05$). The water fraction also showed significantly higher APTT than the negative control DMSO 0.1% (mean APTT, at 8 and 4 mg/mL, 36.10 ± 1.23 and 31.80 ± 0.61 vs. 27.63 ± 0.84 s, respectively, $p < 0.05$). However, the *n*-hexane did not affect APTT (Table 1).

Table 1. Effect of extracts from the aerial part of *C. edulis* on the clotting time in the APTT, PT and TT assay

Sample	Conc. (mg/mL)	APTT (s)	PT (s)	TT (s)
CE.A.Mc	8	40.37 ± 1.03*#	12.70 ± 0.59#	24.80 ± 2.04*#
	4	32.50 ± 0.60*#	12.70 ± 0.28#	22.93 ± 0.15*#
	2	26.83 ± 0.31#	12.43 ± 0.29#	14.50 ± 0.69#
CE.A.EA	8	42.70 ± 1.11*	12.83 ± 0.25#	29.40 ± 2.04*
	4	32.50 ± 0.87*#	12.23 ± 0.19#	21.50 ± 0.87*#
	2	27.23 ± 0.23#	12.07 ± 0.31#	14.77 ± 0.55#

Sample	Conc. (mg/mL)	APTT (s)	PT (s)	TT (s)
CE.A.H	8	27.60 ± 0.46#	12.50 ± 0.33#	14.50 ± 0.60#
	4	27.27 ± 0.47#	12.67 ± 0.31#	14.20 ± 0.95#
	2	27.13 ± 0.25#	12.00 ± 0.22#	14.40 ± 0.75#
CE.A.W	8	36.10 ± 1.23*#	12.67 ± 0.83#	15.63 ± 0.63#
	4	31.80 ± 0.61*#	12.67 ± 0.42#	14.77 ± 0.42#
	2	27.97 ± 0.50#	12.07 ± .60#	14.73 ± 0.35#
DMSO	0.1%	27.63 ± 0.84#	11.93 ± 0.58#	14.23 ± 0.34#
Heparin	0.2 IU	44.03 ± 1.41		31.93 ± 1.51
	2 IU		29.17 ± 0.57	

APTT: activated partial thromboplastin time, PT: prothrombin time, TT: thrombin time, CE.A.Me: the total methanol extract, CE.A.EA: the ethyl acetate fraction, CE.A.H: the *n*-hexane fraction, CE.A.W: the water fraction, conc.: concentration, DMSO: the negative control, heparin: the positive control, *: $p < 0.05$ when compared with the negative control, #: $p < 0.05$ when compared with the positive control

Effect of extracts on prothrombin time

It was observed that all extracts did not prolong the PT values at all tested concentrations compared to the negative control (Table 1). This means that these extracts of the aerial part of *C. edulis* did not affect the coagulation factors involved in the extrinsic pathway.

Effect of extracts on thrombin time

It is noted that the total methanol extract at both concentrations of 8 and 4 mg/mL had significantly higher TT values than the negative control DMSO 0.1% (mean TT, at 8 and 4 mg/mL, 24.80 ± 2.04 and 22.93 ± 0.15 vs. 14.23 ± 0.34 s, respectively, $p < 0.05$). The ethyl acetate fraction at 8 mg/mL showed similar TT compared to the positive control heparin 0.2 IU/mL (mean TT, 29.40 ± 0.82 vs. 31.93 ± 2.51 s, $p > 0.05$). Moreover, the ethyl acetate extract at 4 mg/mL also significantly prolonged TT compared to the negative control DMSO 0.1% (mean TT, 21.50 ± 0.87 vs. 14.23 ± 0.34 s, $p > 0.05$). Both extracts prolonged TT in a dose-dependent manner (Pearson correlation coefficient, $r > 0.9$ for both extracts, $p < 0.05$), in which an increase in the amount of extracts led to an increase in the coagulation time. The n-hexane and the water fraction did not prolong the TT value (Table 1).

Discussion

APTT, PT, and TT are common laboratory tests to evaluate coagulation disorders of patients in clinical practice. These assays are also commonly used to investigate the anticoagulant capacity of medicinal plants [3],[4],[9]. The APPT assay is the main screening test for the intrinsic pathway of hemostasis. The PT test measures the time taken by the PPP to form a blood clot in the presence of sufficient concentration of calcium. The TT is a coagulation assay that measures the fibrin formation from fibrinogen caused by the action of thrombin reagent. This is the last step in the coagulation cascade. This study demonstrated for the first time the anticoagulant effect of extracts from the aerial part of *C. edulis*. The prolonged APTT observed in this study reflects the anticoagulant effects of the methanol, ethyl acetate, and water extracts on the intrinsic pathway of blood coagulation. In addition, the methanol extract and the ethyl acetate fraction might affect the transferring process from fibrinogen to fibrin in the final phase of the coagulation cascade.

The anticoagulant results of the extracts from the aerial part of *C. edulis* were similar to the previously reported findings on the extracts from *C. edulis* rhizome and other *Canna* species [3],[4],[9],[10]. The ethyl acetate fraction of *C. edulis* rhizome at 4 mg/mL was also reported to prolong blood clotting time better than the *n*-hexane and water ones. The study by Nguyen et al. documented that the ethyl acetate fraction from both rhizome and aerial parts of *C. warszewiczii* had good anticoagulant activity at 16 mg/mL [10]. Similarly, the aerial part of *C. generalis* exhibited an anticoagulant effect, and the acetyl acetate fraction at 4 mg/mL showed the best activity with prolonged APTT among fractions [9]. Bioactive compounds with antithrombotic effects were successfully isolated from the aerial part of *C. generalis* and *C. edulis* rhizome [3],[9]. Our new finding on the anticoagulant effect of the aerial part of *C. edulis* in the present study suggests that the aerial part of *C. edulis* could be also a potential source to search for novel antithrombotic molecules.

Current antithrombotic agents play an important role in the treatment and prevention of CVDs. However, people are concerned about

their side effects and drug resistance [2]. Therefore, the need to find safe and effective alternative sources of antithrombotic agents has been increasing. In Vietnam, researchers have been also interested in this topic and reported the anticoagulant effects of the extracts from other plants such as *Panax bipinnatifidus, Kaempferia parviflora, Pseuderanthemum palatiferum*, etc. [11],[12],[13].

Heparin is an anticoagulant medication that binds to antithrombin and inactivates thrombin. It inhibits a number of coagulation cascade factors, prevents fibrin formation, and reduces clotting time [14]. Therefore, this drug was used as the positive control in the study. This study confirmed the strong effects of heparin on the intrinsic and common pathway with prolonged APTT and TT values at 0.2 IU/mL. The ethyl acetate fraction of the aerial part of *C. edulis* also exhibited the anticoagulant effect by prolonging APTT and TT, thus, this fraction might have similar mechanisms of action to heparin. However, more thorough experiments are required to understand the mechanism of action of this plant.

In this study, the tested concentrations for *in vitro* anticoagulant assays were chosen following previous studies [3],[4],[9],[10]. Because this is the first study reporting the anticoagulant effect of the aerial part of *C. edulis*, and no *in vivo* studies on this plant have been carried out, the effective doses for *in vivo* studies need to be explored in future studies.

Several limitations exist in this study. First, although the anticoagulant assays were performed with human blood, they were still *in vitro* tests. Second, the bioactive phytoconsituents of the aerial part of *C. edulis* were not investigated. Therefore, further *in vitro* and *in vivo* studies should be done to confirm the anticoagulant activity and investigate the bioactive molecules of the aerial part of *C. edulis*.

4. Conclusion

This is the first experimental study to demonstrate the anticoagulant effect of the aerial part of *C. edulis*. The total methanol extract and the ethyl acetate fraction exhibited better anticoagulant activity compared to the others. They significantly prolonged APTT and TT values. Active fractions and bioactive molecules from the aerial part of *C. edulis* might affect the intrinsic and the common, but not the extrinsic pathway of the coagulation cascade. *C. edulis* could be a promising natural resource for the discovery of novel antithrombotic agents and the development of supplementary products for treating and preventing thrombosis-related diseases. Further studies should be carried out to investigate new bioactive molecules from this interesting plant and understand their mechanisms of antithrombotic action.

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