Asian Journal of Pharmacognosy

Research Article

Novel finding on anticoagulant activity of *Canna warszewiczii* extracts

Thi Van Anh Nguyen[a], Huong Duong Thi Ly[b], Thi Thom Vu[b], Thi Thu Nguyen[a], Hong Luyen Le[a*]

[a] *Department of Pharmacological, Medical and Agronomical Biotechnology, University of Science and Technology of Hanoi (USTH), Vietnam Academy of Science and Technology (VAST), Hanoi, Vietnam.*
[b] *School of Medicine and Pharmacy, Vietnam National University, Hanoi, Vietnam.*

Received December 10, 2018; accepted March 10, 2018; published online April 15, 2018.

Hong Luyen Le:*le-hong.luyen@usth.edu.vn

Abstract

Canna warszewiczii is a traditional medicine used to treat cardiovascular diseases like coronary artery disease, myocardial ischemia. However, the pharmacological effects of this plant have not been studied yet. This study aimed to investigate for the first time the anticoagulant activity of *Canna warszewiczii* extracts. Rhizomes and the upper parts (leaves and shoots) of the plant were extracted by 90% ethanol, then fractioned by n-hexane, dichloromethane, ethyl acetate and distilled water. Human plasma collected from healthy volunteers was treated with the ethyl acetate and aqueous extracts at different concentrations (2, 4, 8, 16 mg/mL), then the prothrombin time (PT) and activated partial thromboplastin time (APTT) were measured. The results showed a novel finding that the crude ethyl acetate extracts of both rhizomes and upper parts prolonged significantly both PT and APTT at the concentration of 8 mg/mL and 16 mg/mL *($P < 0.05$)*. These preliminary results suggest that *Canna warszewiczii* extracts can be a potential source of anticoagulant compounds and may be useful in the treatment and prevention of thrombotic disorders.

Key words: medicinal plants, anticoagulant, *Canna warszewiczii*

Introduction

Thrombosis, the formation of blood clots in the blood vessels, represents the most common underlying pathology of cardiovascular diseases and is a major contributor to the global disease burden (Ashwini and Asha, 2017). Anticoagulant drugs, which are chemically or biologically synthesized such as heparin, aspirin, warfarin, vitamin K-antagonists and their derivatives, play an important role in the treatment and prevention of thrombosis (Hirsh, O'Donnell *et al.*, 2005). Unfortunately, these drugs have a variety of life-threatening adverse effects including hemorrhage, neutropenia, thrombocytopenia, birth defects, miscarriage, gastrointestinal upset and drug-drug interactions (Hurlen , Abdelnoor *et al.*, 2002; Koenig-Oberhuber and Filipovic, 2016). Thus, there is still an urge for new effective and safe anticoagulant drugs for the treatment and prevention of thrombotic disorders and cardiovascular diseases. Researchers are interested in searching medicinal plants as a potential source of natural products and herbal medicines, which have safety and eco-friendly image (Ashwini and Asha, 2017; Chua and Koh, 2006).

 eISSN-0128-1119

Canna warszewiczii, a species of the *Canna* genus, belonging to the family Cannaceae, is cultivated in tropical regions as a source of starch. In Vietnam, this plant has been used as a traditional medicine to treat cardiovascular diseases like coronary artery disease, myocardial ischemia. However, anticoagulant activity of this plant has not been studied. However, plant parts of edible *canna* like rhizomes, leaves, and flowers are believed to have considerable medicinal properties such as diuretic, demulcent and diaphoretic and they are also used to treat women health related problems (Duke and Ayensu, 1985). Recently, the rhizome extract of *Canna edulis* has been reported to have antioxidant properties (Mishra, Goyal *et al.*, 2011). It is evidenced that consumption of dietary anticoagulants reduces the risk of thrombotic disorders (Matsubara, Matsuura *et al.*, 2001; Guglielmone, Agnese *et al.*, 2002). The present study aimed to evaluate anticoagulant activity of *Canna warszewiczii* extracts. This would give preliminary data to guide further studies for searching potential natural products for prevention and treatment of thrombosis and cardiovascular diseases.

Material and methods

Plant extractions

The *Canna warszewiczii* plant, harvested in Thai Nguyen province, Vietnam, was air-dried, grounded into fine powder and separated into two parts, rhizomes and the upper parts including leaves and shoots. Both parts were extracted with 90% ethanol (x 3 times) at room temperature, filtered, and then concentrated to dryness using a rotary evaporator. The obtained residue was suspended in distilled water and then extracted with n-hexane, dichlomethane and ethyl acetate and aqueous. After evaporating solvents with the rotary evaporator, the obtained residues were diluted in DMSO 10% to give concentrations of 20, 40, 80 and 160 mg/mL.

Blood collection and plasma preparation

Blood samples were collected from healthy volunteers, aged 18-25 years, with no history of thrombosis or abnormal bleeding, active neoplasia or active inflammatory disease. Drugs that affected the blood coagulation process had not been taken within two weeks and no food intake had been done at least 3 hours before taking blood. 3 mL venous blood withdrawn from each volunteer was added to a plastic centrifuge tube containing 3.2% trisodium citrate as an anticoagulant (9:1 ratio). Plasma was obtained by centrifugation (3000 x g, 10 minutes).

In vitro anticoagulant assays

The anticoagulation activity of ethyl acetate and aqueous extracts was determined by measuring PT (prothrombin time) and activated partial prothrombin time (APTT) using Sysmex CS-2100i machine (Japan). For both tests, 450 µL plasma was mixed with 50 µL of plant extracts to give final concentrations of 2, 4, 8, 16 mg/mL, then incubated at 37°C for 5 minutes before measuring PT and APTT. Heparin 0.2IU and DMSO 1% in plasma was used as positive and a negative control, respectively (Li, Wang *et al.*, 2013) (Li *et al*. 2013). The experiments were repeated 5 times.

Data analysis

Data were analyzed using Microsoft Excel 2007 and GraphPad Prism 7.0. The independent Student's t-test and the one-way ANOVA test were used to assess the differences among different concentrations, and between the test and control groups. Correlations between the extraction concentration and PT or APTT values were analyzed using the Pearson's rank correlation test. P value ≤ 0.05 was considered statistically significant.

 eISSN-0128-1119

Results

Effect of the ethyl acetate extract of the plant rhizomes on PT and APTT

The data indicate that the effect on PT and APTT of the ethyl acetate extract of rhizomes was dependent on concentrations of the plant extracts *($p < 0.05$).* Higher concentrations were associated with higher values of PT and APTT (Pearson's rank correlation test, r = 0.9761, *P* = 0.0239 for PT, and r = 0.9766, P = 0.0234 for APTT). The ethyl acetate rhizome extracts at the concentrations of 2 and 4 mg/ml did not prolong PT or APTT (P > 0.05). However, as can be seen in Table 1, both PT and APTT were prolonged significantly at concentrations of 8 mg/mL and 16 mg/mL *($P < 0.05$).*

Table 1. Effect of the ethyl acetate extract of the rhizome on PT and APTT

Sample	N	PT (s)	P-value (Compared to DMSO 1%)	APTT(s)	P-value (compared to DMSO 1%)
DMSO 1%	5	11.56 ± 0.65		29.64 ± 1.79	
Heparin 0.2IU	5	12.24 ± 0.76	0.1689	45.7 ± 5.62	**0.0009**
EAC 2 mg/mL	5	11.96 ± 0.64	0.9677	30.42 ± 2.06	0.5412
EAC 4 mg/mL	5	12.26 ± 0.61	0.9306	31.54 ± 2.2	0.1799
EAC 8mg/mL	5	**13.62 ± 1.05**	**0.0058***	**36.42 ± 3.28**	**0.0037***
EAC 16 mg/mL	5	**19.72 ± 0.87**	**< 0.001***	**58.14 ± 8,16**	**< 0.001***
P value (ANOVA test)		**0.0023***		**< 0.0001***	

EAC: ethyl acetate extract of rhizome, data were expressed as mean ±SD

Effect of the ethyl acetate extract of the plant aerial parts on PT and APTT

Similarly, both PT and APTT values were positively associated with the concentrations (r = 0.97, $p = 0.03$, and r = 0.967 $p = 0.032$, respectively). As shown in Table 2, at the concentration of 2 and 4 mg/mL, there was no difference in both PT and APTT between the negative control and the ethyl acetate extract of the upper plant parts *($P > 0.05$).* However, the ethyl acetate extracts at the concentration of 8 and 16 mg/mL prolonged PT and APTT significantly *($p < 0.05$).*

Table 2. Effect of the ethyl acetate extract of the aerial part on PT and APTT values

Samples	N	PT (s)	P-value (Compare to DMSO 1%	APTT (s)	P-value (compare to DMSO 1%)
DMSO 1%	5	11.85±0.78		29.8 ± 1.67	
Heparin 0.2IU	5	12.24 ± 0.76	0.1689	45.7 ± 5.62	**0.0009***
EAL 2 mg/ml	5	11.34 ± 0.44	0.5514	28.86 ± 1.48	0.4747
EAL 4 mg/ml	5	12.12 ± 0.37	0.1343	31.68 ± 2.3	0.1572
EAL 8 mg/ml	**5**	**14.68 ± 0.53**	**<0.0001***	**42.64 ± 6.12**	**0.0019***
EAL 16 mg/ml	**5**	**29.24 ± 1.94**	**<0.0001***	**107.84 ± 11.59**	**<0.001***
P value (ANOVA test)		**< 0.0001***		**< 0.0001***	

EAL: Ethyl acetate extracts of Canna warszewiczii upper ground parts
Data were expressed as mean ± SD

 eISSN-0128-1119

Effect of the aqueous extract of the plant on PT and APTT values

The effect of the aqueous extracts of rhizomes on both PT and APTT was not different among different concentrations and in comparison with the negative control ($P > 0.05$). Similarly, the aqueous extracts of the upper parts (leaves and shoots) did not affect both PT and APTT parameters ($P > 0.05$).

Comparison of PT and APTT values between extracts

As shown in figure 1 and figure 2, among 4 extracts tested, the ethyl acetate extract of the upper parts showed the highest values of PT and APTT parameters, followed by ethyl acetate extract of rhizomes ($P < 0.05$). Moreover, at 16 mg/mL, the ethyl acetate extract of the upper parts showed very high APTT value (107.84 seconds), which means that this extract concentration may cause a bleeding risk. The water extracts of both rhizome and leaves did not have any effect on both PT and APTT.

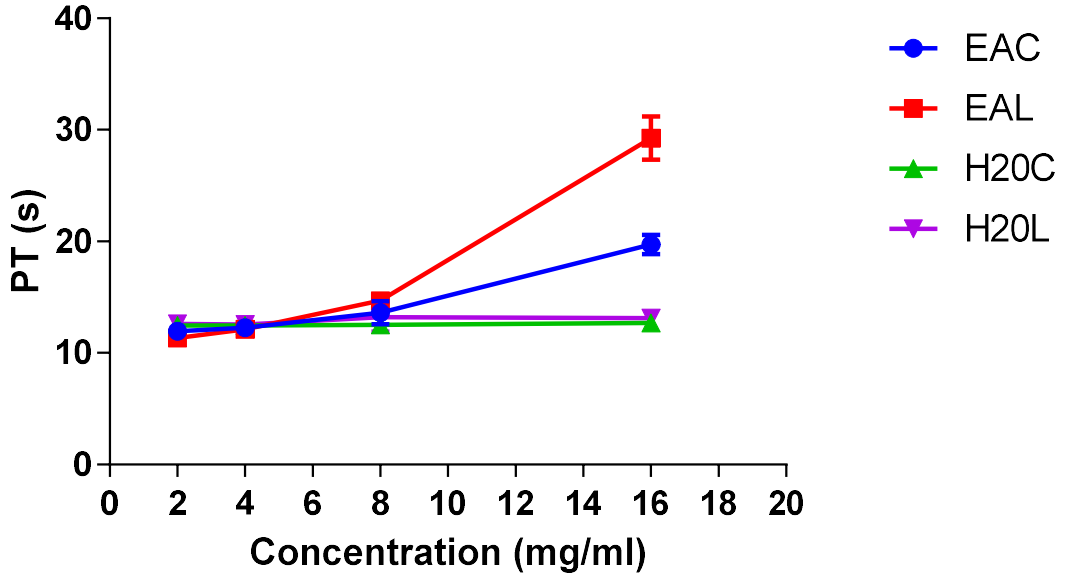

Figure 1.The activity of 4 extracts from *Canna warszewiczii* on PT. EAC: ethyl acetate extracts of rhizomes, EAL: ethyl acetate extracts of upper parts, H2OC: water extracts of rhizome, H20L: water extracts of upper parts

eISSN-0128-1119

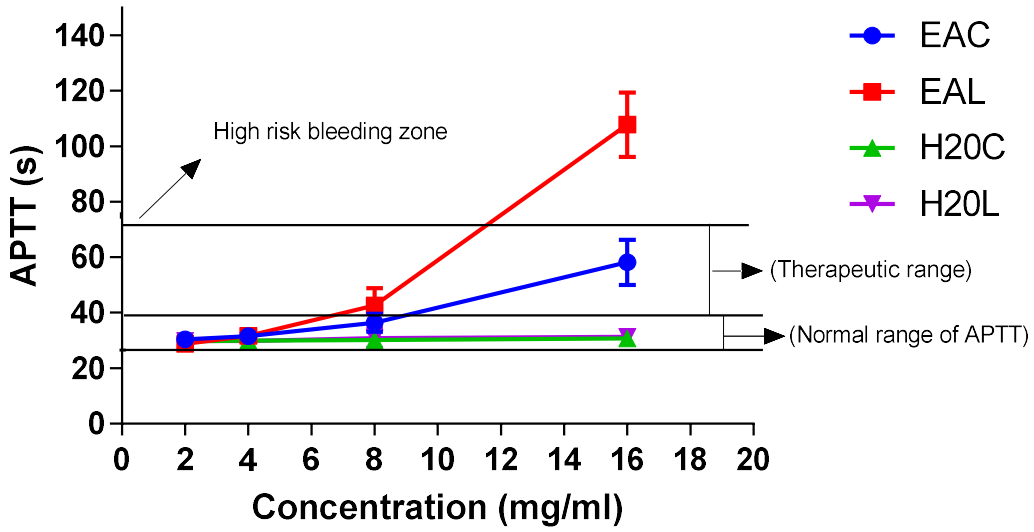

Figure 2. The activity of 4 extracts from *Canna warszewiczii* on APTT. EAC: ethyl acetate extracts of rhizomes, EAL: ethyl acetate extracts of upper parts, H2OC: water extracts of rhizome, H20L: water extracts of upper parts

Discussion

A rich potential source of novel antithrombotic agents is hidden in herbal medicines and many excellent pharmacological effects have been studied (Tsai, Lin *et al.*, 2000; Ballabeni, Tognolini *et al.*, 2007). Recently, there has been an increasing interest to isolate thrombolytic agents and anti-thrombotic compounds from food and natural sources, which are presumed to be safe and effective. For example, chrysophanol-8-O-glucoside, an anthraquinone derivative of *Rhubarb* found in 2011, had anticoagulation and anti-platelet aggregation activity. This substance unchanged PT and only affected APTT, indicating that it could influence on the intrinsic pathway of coagulation (Seo, Ngoc *et al.*, 2012). The present study showed for the first time that the ethyl acetate extracts of both rhizome part and the upper parts (leaves and shoots) of *Canna warszewiczii* prolonged coagulation parameters PT and APTT parameters. The PT test measures the blood clotting time of the extrinsic pathway, while the APTT test measures the intrinsic pathway of coagulation. The normal range of the PT value is from 11 to 13 seconds, and this of APTT is from 28.5 to 38.5 seconds (Pagana and Pagana, 2017).The therapeutic value for both PT and APTT is about 1.5 to 2.5 times above the normal values. In this study, both the ethyl acetate extract of rhizomes and the upper parts *of Canna warszewiczii* exerted the anticoagulant effect at 8 mg/mL of concentration. At 16 mg/ml of the upper parts, the *in vitro* test showed that both PT and APTT were very high (mean, 29.24 and 107.84 seconds, respectively), which may suggest a high risk of bleeding. These preliminary results indicate that *Canna warszewiczii* exhibited anticoagulant activity and influenced on both extrinsic and intrinsic pathway or even the common pathway of coagulation. However, to clarify which factors involved in the mechanism of anticoagulant activity, further studies are needed. Furthermore, this study was conducted with crude extracts; thus, intensive extractions, enrichment of active compounds, and testing of lower concentrations should be carried out in further experiments. Particularly, determining the main compounds with potent anticoagulation activity is required. Natural agents that inhibit coagulation process are of potential interest for the prevention of atherosclerosis and coronary artery disease (Ashwini and Asha, 2017).

Conclusion

In conclusion, the present study reported a novel finding on anticoagulant properties of the

 eISSN-0128-1119

ethyl acetate extract of leaves, shoots and rhizomes of *Canna warszewiczii,* and the upper parts showed better anticoagulant activity than rhizomes. These extracts prolonged both PT and APTT parameters and as such, could be a potential source of anticoagulant and anti-thrombotic agents and may act as a promising alternative in the treatment and prevention of thrombotic disorders.

Acknowledgment

This project was funded by University of Science and Technology of Hanoi, Vietnam Academy of Science and Technology.

Declaration of Conflict of Interest

No conflict of interest associated with this work.

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 eISSN-0128-1119