Pharmacogn. Res.
A multifaceted peer reviewed journal in the field of Pharmacognosy and Natural Products
www.phcogres.com | www.phcog.net

ORIGINAL ARTICLE

Inhibitory Effect on Human Platelet Aggregation, Antioxidant Activity, and Phytochemicals of *Canna warszewiczii* (A. Dietr) Nb. Tanaka

Le Hong Luyen, Vu Thi Thom[1], Le Thi Thanh Huong[2], Duong Thi Ly Huong[3], Nguyen Thi Van Anh

Department of Life Sciences, University of Science and Technology of Hanoi, Vietnam Academy of Science and Technology, Hanoi, Vietnam, [1]Department of Basic Sciences in Medicine and Pharmacy, VNU-H-School of Medicine and Pharmacy, Hanoi, Vietnam, [2]Department of Biotechnology, Thai Nguyen University of Sciences, Thai Nguyen, Vietnam, [3]Department of Pharmacology and Clinical Pharmacy, VNU-H-School of Medicine and Pharmacy, Hanoi, Vietnam

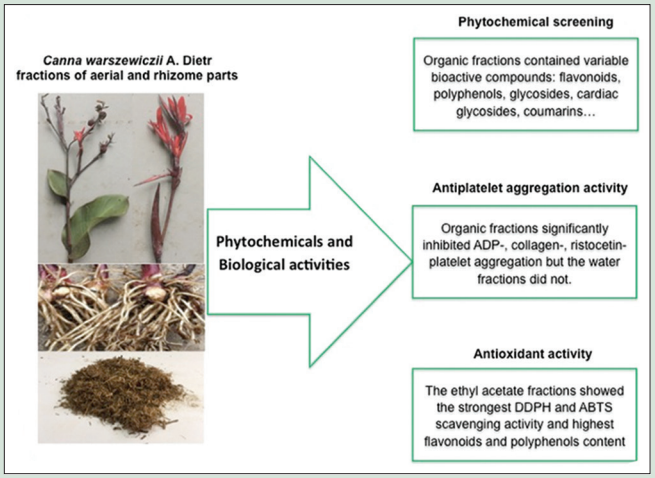
ABSTRACT

Background: *Canna warszewiczii* (A. Dietr) Nb. Tanaka has been traditionally used to treat heart diseases in Vietnam, but there is a lack of scientific evidence. **Objectives:** This study investigated the inhibitory effect on human platelet aggregation, antioxidant activity, and main phytochemicals of fractions from the aerial and rhizome parts of the plant. **Materials and Methods:** Human platelets were prepared and incubated with different fraction doses (0.33, 0.75, and 1.5 mg/mL). Platelet aggregation was triggered by different agonists (adenosine diphosphate [ADP], collagen, and ristocetin). 2,2-diphenyl-1-pycrylhydrazyl (DPPH) and 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assays were done to test the free-radical scavenging activities. Spectrophotometry assays using Folin–Ciocalteu and aluminum chloride were used to determine total flavonoid content (TFC) and total polyphenol content (TPC). The phytochemical screening was also implemented. **Results:** The n-hexane, dichloromethane, and ethyl acetate (EA) fractions from both the rhizome and aerial parts significantly inhibited ADP-, collagen-, and ristocetin-platelet aggregations and decreased significantly the area under the platelet aggregation curve and aggregation velocity in a concentration-dependent manner, but the water fractions did not. The EA fractions showed the strongest DPPH and ABTS scavenging activity and highest TPC and TFC. Organic fractions contained variable bioactive compounds: flavonoids, polyphenols, glycosides, cardiac glycosides, coumarins, steroids, emodols, tannins, and cholesterols. **Conclusion:** The plant extracts or fractions could be used in the preparation of functional foods or supplements with antioxidant and antiplatelet activity. It is also a good candidate for searching novel bioactive compounds used in pharmaceutical industries for the development of antiplatelet and antioxidant agents to prevent and/or treat heart and oxidative stress-related diseases.

Key words: Antioxidant, antiplatelet, *Canna warszewiczii* (A. Dietr) Nb. Tanaka, flavonoids, polyphenols

SUMMARY

- The n-hexane, dichloromethane, and ethyl acetate (EA) fractions from both the rhizome and aerial parts of *Canna warszewiczii* A. Dietr significantly inhibited platelet aggregation in a concentration-dependent manner
- The EA fractions showed the strongest 2,2-diphenyl-1-pycrylhydrazyl and 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) scavenging activity and highest total polyphenol and flavonoid content
- Organic fractions contained variable bioactive compounds: flavonoids, polyphenols, glycosides, cardiac glycosides, coumarins, etc.
- *C. warszewiczii* A. Dietr is a good candidate for searching antiplatelet and antioxidant agents to prevent and/or treat heart and oxidative stress-related diseases.



Abbreviations Used: EA-A: Aerial ethyl acetate fraction; EA-R: Rhizome ethyl acetate fraction; DCM-A: Aerial dichloromethane fraction; DCM-R: Rhizome dichloromethane fraction; H-A: Aerial n-hexane fraction; H-R: Rhizome n-hexane fraction; W-A: Aerial distilled water fraction; W-R: Rhizome distilled water fraction; TPC: Total polyphenol content; TFC: Total flavonoid content; GAE: Gallic acid equivalent; QE: Quercetin equivalent; DPPH: 2,2-diphenyl-1-pycrylhydrazyl; ABTS: 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid).

Correspondence:
Dr. Nguyen Thi Van Anh,
Department of Life Sciences, University of Science and Technology of Hanoi, Vietnam Academy of Science and Technology,
18 Hoang Quoc Viet Street, Cau Giay District, Hanoi, Vietnam.
E-mail: nguyen-thi-van.anh@usth.edu.vn
DOI: 10.4103/pr.pr_72_19

Access this article online
Website: www.phcogres.com
Quick Response Code:

INTRODUCTION

Platelet aggregation is the clumping together of platelets in the normal process of hemostasis to prevent further bleeding of injured blood vessels. Unusual coagulation may lead to various cardiovascular diseases (CVDs). Synthetic antiplatelet drugs are beneficial to treat and prevent thrombus formation. However, they are accompanied by limited actions, serious side effects, and therapeutic resistance.[1] The use of herbal medicines has been considered as an alternative to synthetic drugs with alleviates side effects. Searching new sources and new antiplatelet compounds from

This is an open access journal, and articles are distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 4.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.

For reprints contact: reprints@medknow.com

Cite this article as: Luyen LH, Thom VT, Huong LT, Huong DT, Anh NT. Inhibitory effect on human platelet aggregation, antioxidant activity, and phytochemicals of *Canna warszewiczii* (A. Dietr) Nb. tanaka. Phcog Res 2020;12:47-52.

potential medicinal plants for the prevention and/or treatment of CVDs is a topic of interest to the researchers.[2]

Oxidative stress is involved in the pathogenesis of atherosclerosis, and antioxidants are supposed to decrease the risk of atherothrombotic CVDs. Cardiovascular protection has been shown to be associated with dietary patterns high in antioxidants (from fruit and vegetables), and natural plant antioxidants can serve as a type of preventive medicine. Recently, the use of natural plant antioxidants has become of special interest due to their presumed safety and nutritional and therapeutic values.[3]

Canna is the only genus in the family Cannaceae, and its starchy rhizomes have long been used for food. Several species have been traditionally used for the treatment of various diseases such as fever, pain, hepatitis, cancer, gonorrhea, and heart diseases.[4] *Canna indica* and *Canna edulis* have been reported to possess bioactivities such as antioxidant activity, live protective effect,[5,6] increasing the activity of glucose transporter in muscle cells, or inhibiting the productions of NO, interleukin-1β, and prostaglandin E2 (PGE2) from lipopolysaccharide (LPS)-induced RAW 264.7 macrophages and inhibiting high glucose (HG)-induced inflammatory mediator expression.[7,8] Recently, lignin isolated from *C. edulis* was shown to inhibit α-D-glucosidase; arabinoxylan extracted from its by-product was reported to have inhibitory effects on pepsin and lipase activities.[9] Currently, no study investigates the potentials of this genus in the treatment and prevention of CVDs.

In Vietnam, *Canna warszewiczii* (A. Dietr) Nb. Tanaka, whose synonym is *C. indica* var. *warszewiczii* (A. Dietr) Nb. Tanaka, is a medicinal plant recently detected with the local name of purple arrowroot. It grows in some mountainous provinces in Vietnam, China, and South America. In our country, it is usually cultivated for medicinal purposes and traditionally used to treat gastric ulcer, chest and bone pain, and heart diseases. Despite the lack of scientific evidence, several supplement products from the plant extracts are supplied into the Vietnam market to support the treatment of heart diseases.

Given the lack of scientific evidence, this study aimed to investigate the antiplatelet aggregation activity induced by different agonists and antioxidant activity of this plant. Total flavonoid content (TFC), total polyphenol content (TPC), and the phytochemical screening were also implemented to study the main phytoconstituents of the plant. This study is of important to introduce new sources for further searching novel bioactive natural compounds to prevent and/or treat CVDs and oxidative stress-related diseases.

EXPERIMENTAL

Plant materials and chemicals

C. warszewiczii (A. Dietr) Nb. Tanaka, identified by the plant researcher Thi Thanh Huong Le – Thai Nguyen University of Sciences, was obtained from Vo Nhai district, Thai Nguyen Province, Vietnam.

The agonists (adenosine diphosphate [ADP], collagen, and ristocetin) were purchased from Chrono-Log Corporation (USA). Aspirin, heparin, 2,2-diphenyl-1-pycrylhydrazyl (DPPH), 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS; 2,2e purchased from Chrono-Log Corporation (USA acid), potassium peroxodisulfate, dimethyl sulfoxide (DMSO), gallic acid, trolox, Folin–Ciocalteu's phenol reagents, and other reagents were obtained from Sigma-Aldrich (USA). Thromborel S, Actin FS, calcium chloride, and thromboclotin reagent were purchased from Dade Behring Marburg GmbH (Marburg, Germany).

Preparation of plant fractions

The plant was separated into two parts: aerial (A) and rhizome (R) parts. The dried powder was extracted with ethanol 96% and then consecutively fractionated with n-hexane (H), dichloromethane (DCM), ethyl acetate (EA), and water (W) to obtain different fractions: aerial ethyl acetate fraction (EA-A), rhizome ethyl acetate fraction (EA-R), aerial dichloromethane fraction (DCM-A), rhizome dichloromethane fraction (DCM-R), aerial n-hexane fraction (H-A), rhizome n-hexane fraction (H-R), aerial distilled water fraction (W-A), and rhizome distilled water fraction (W-R).

The platelet aggregation assay

The study was approved by the Ethics Committee, School of Medicine and Pharmacy, Vietnam National University, Hanoi, Vietnam. Human volunteers had been given informed consent. Blood was taken from healthy volunteers who were fasting overnight and had taken no medication for 2 weeks and transferred to a 3.2% sodium citrate test tube. Then, it was centrifuged at 500 rpm for 10 min to collect the platelet-rich plasma (PRP) and at 3000 rpm for 10 min to obtain platelet-poor plasma. Platelets were counted under microscope, and the platelet count was adjusted to 250 ± 25 × 10^9/L in PRP. A complete blood count was tested before platelet aggregation experiments to ensure that volunteers had normal blood cell counts.

The platelet aggregation tests were performed in triplicate using the turbidimetric method.[10] Briefly, 450 µl PRP was preincubated at 37°C for 3 min with 50 µl fractions at final concentrations of 0.33, 0.75, and 1.5 mg/mL in DMSO 0.1%. Platelet aggregation was induced by ADP 10 µM, collagen 2 µg/mL, and ristocetin 1.25 mg/mL, respectively. The sample without fractions and aspirin was used as a negative and positive control, respectively. Results were recorded as amplitude-time curves over 6 min, including maximum aggregation, maximum slope of the curve (velocity of aggregation), and most importantly, the area under the platelet aggregation curve (AUC). The inhibition percentage of platelet aggregation was calculated as % Inhibition = $\frac{X-Y}{X} \times 100\%$, X is maximum aggregation of the negative control and Y is maximum aggregation of tested fractions.

2,2-diphenyl-1-pycrylhydrazyl antiradical activity

The DPPH assay was carried out in triplicate according to the method described previously.[11] The absorbance was read at 517 nm. Ascorbic acid (10, 25, and 50 µg/mL) was used as the positive control. The percentage of radical inhibition was calculated as % Inhibitio n = 100 – $\left(\frac{OD_s}{OD_c} \times 100\%\right)$, OD_s is an average optical density of the sample and OD_c is an average optical density of the control. Half-maximal inhibitory concentration (IC_{50}) values were determined as the concentrations needed for 50% inhibition of the radicals.

2,2'-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) antiradical activity

This experiment was done in triplicate following the method described by Re *et al*. with some modifications.[12] The radicals were generated by mixing potassium persulfate (2.45 mM) and ABTS (7 mM) and incubating the mixture in the dark at room temperature for 12–16 h. The working solution of $ABTS^+$ was further diluted in ethanol to give an absorbance of 0.70 ± 0.02 at 734 nm. Then, 190 µL of the diluted solution of $ABTS^+$ was mixed with a 10 µL sample at room temperature for 10 min in the dark, and the absorbance was

measured at 734 nm. Trolox (50, 100, and 200 μg/mL) was used as the positive control. The capacity to inhibit the radical and IC_{50} was calculated as described above.

Analysis of total phenolic content

The TPC of plant fractions was performed using the Folin–Ciocalteu assay as described previously.[13] The absorbance was read at 765 nm. A standard curve of gallic acid at 0, 100, 250, 500, 1000, and 2000 μg/mL was used to measure the TPC in fractions of interest. This was expressed as grams of gallic acid equivalent per 100 g of the sample (g GAE/100 g sample).

Analysis of total flavonoid content

The TFC of plant extracts was determined using the method described by Mishra *et al.* with some modifications.[14] Fractions were prepared at concentrations from 1000 to 5000 μg/mL in methanol and then 240 μL of each solution was mixed with 40 μL of 5% $NaNO_2$, and the mixture was incubated for 6 min at 25°C and then 40 μL of 10% $AlCl_3$ was added before the same incubation. The reaction mixtures were treated with 400 μL of 1 M NaOH and 280 μL of 30% ethanol and incubated at room temperature for 15 min. The absorbance was measured at 510 nm. A standard curve of quercetin at 0, 10, 50, 100, and 200 μg/mL was used to estimate the TFC in the fractions. This was expressed as grams of quercetin equivalent per 100 g of the sample.

Phytochemical screening for secondary metabolites

Each fraction was screened for the following phytochemicals: glycosides, cardiac glycosides, flavonoids, saponins, coumarins, emodols, steroids, cholesterols, and tannins using standard phytochemical methods described by Harbourne.[15]

Statistical analysis

Data were expressed as mean ± standard deviation and analyzed by the SPSS 23.0 (IBM corporation, Armonk, New York, USA) software using one-way ANOVA and independent sample *t*-test. Pearson's correlation coefficient (r) was calculated to determine the relationship between variables. $P < 0.05$ was considered as statistically significant.

RESULTS

Antiplatelet aggregation activity of *Canna warszewiczii* A. Dietr fractions

Percentage inhibition of platelet aggregation

The inhibitory effect on platelet aggregation induced by ADP, collagen, or ristocetin of EA, DCM, and H fractions of both the aerial and rhizome parts was positively correlated with fraction concentration ($r > 0.8$ and $P < 0.05$ for all fractions). However, the water fractions of both plant parts showed very low percentage inhibition of platelet aggregation at all concentrations tested compared to others ($P < 0.05$). At 1.5 mg/mL, all EA, DCM, and H fractions inhibited strongly platelet aggregation (percentage inhibition ranged from 74.6% to 100%) and showed significantly higher percentage inhibition than the positive control ($P < 0.05$) [Figure 1].

Area under the platelet aggregation curve and aggregation slope

When different platelet activators (ADP, collagen, and ristocetin) were used, the AUC of all EA, DCM, and H fractions of both the aerial and rhizome parts at 0.75 and 1.5 mg/mL decreased significantly compared to the negative control ($P < 0.05$). Especially, at 1.5 mg/mL, all these organic factions yielded significantly lower AUC than the positive control ($P < 0.05$). In general, a negative association between AUC and fraction concentrations was observed in these fractions ($r < -0.8$, $P < 0.05$). The water fractions (W-A and W-R), however, had much higher AUC than the others ($P < 0.05$) and showed no difference from the negative control ($P > 0.05$) in all cases of agonists used [Figure 2].

Similarly, the aggregation slope of EA, DCM, and H fractions of both the aerial and rhizome parts at all concentrations, except for the EA-A, EA-R, and DCM-R faction at 0.33 mg/mL, decreased remarkably compared to the negative control ($P < 0.05$) when any of the agonists ADP, collagen, or ristocetin was used. The slopes produced by these fractions at 1.5 mg/mL were even significantly lower than that of aspirin ($P < 0.05$). This parameter also showed a concentration-dependent manner ($r < -0.7$, $P < 0.05$). However, the water fractions did not show any difference in slope compared to the negative control, except for the W-R fraction in the case of ristocetin ($P > 0.05$) [Figure 3].

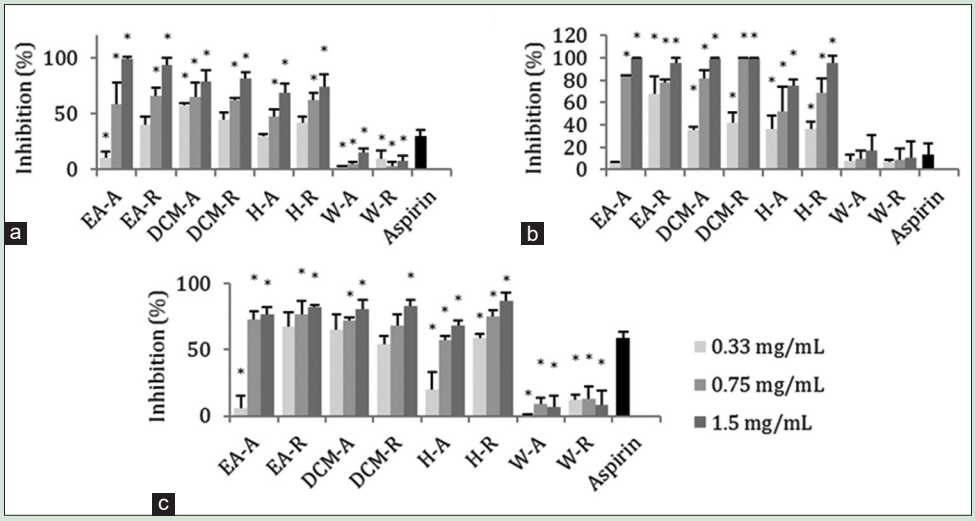


Figure 1: Percentage inhibition of platelet aggregation induced by (a) ADP, (b) collagen, or (c) ristocetin of fractions (*$P < 0.05$ compared to aspirin 0.01 mg/mL (collagen) or aspirin 0.1 mg/mL [ADP or ristocetin]). EA-A: Aerial ethyl acetate fraction; EA-R: Rhizome ethyl acetate fraction; DCM-A: Aerial dichloromethane fraction; DCM-R: Rhizome dichloromethane fraction; H-A: Aerial n-hexane fraction; H-R: Rhizome n-hexane fraction; W-A: Aerial distilled water fraction; W-R: Rhizome distilled water fraction; ADP: Adenosine diphosphate

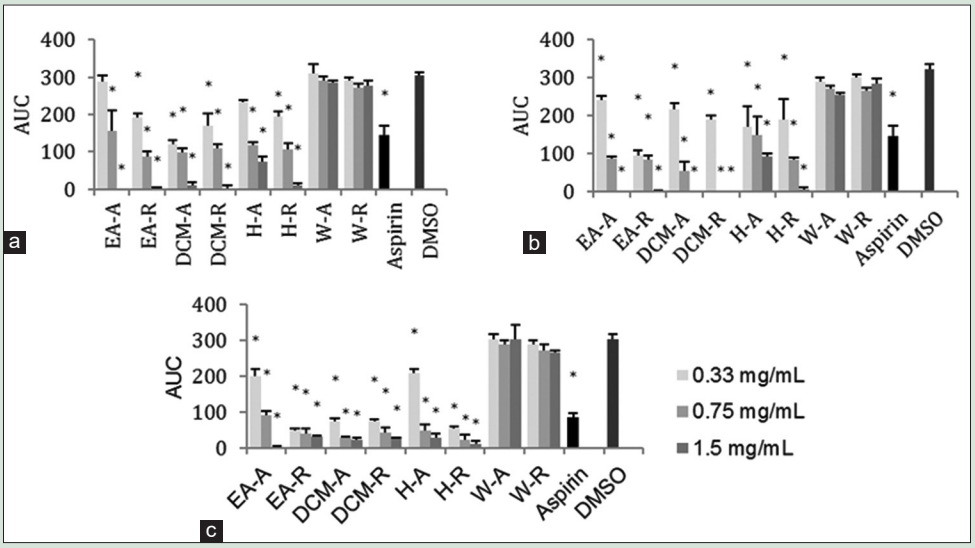


Figure 2: Area under the platelet aggregation curve induced by (a) ADP, (b) collagen, or (c) ristocetin of fractions (*$P < 0.05$ compared to DMSO). EA-A: Aerial ethyl acetate fraction; EA-R: Rhizome ethyl acetate fraction; DCM-A: Aerial dichloromethane fraction; DCM-R: Rhizome dichloromethane fraction; H-A: Aerial n-hexane fraction; H-R: Rhizome n-hexane fraction; W-A: Aerial distilled water fraction; W-R: Rhizome distilled water fraction; DMSO: Dimethyl sulfoxide; ADP: Adenosine diphosphate; AUC: Area under the platelet aggregation curve

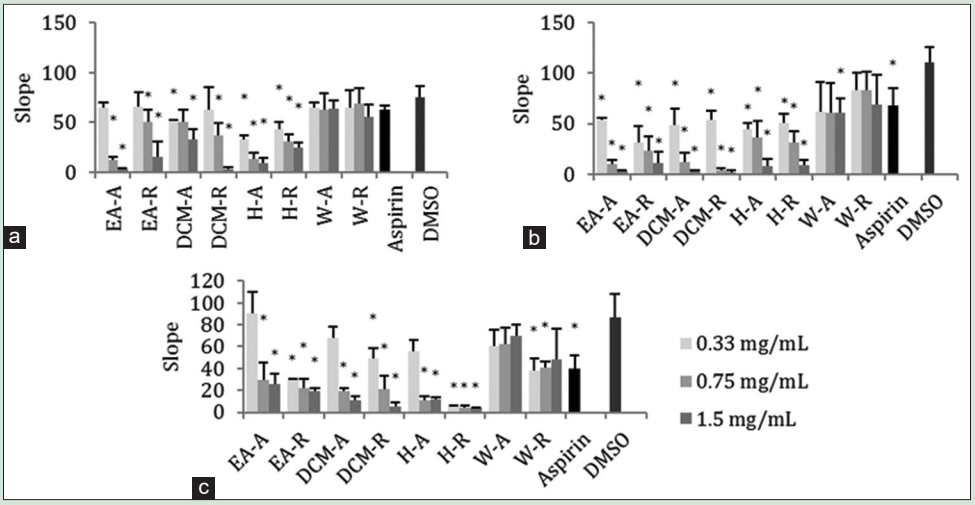


Figure 3: Aggregation slope induced by (a) ADP, (b) collagen, or (c) ristocetin of *Canna warszewiczii* fractions (*$P < 0.05$ compared to DMSO). EA-A: Aerial ethyl acetate fraction; EA-R: Rhizome ethyl acetate fraction; DCM-A: Aerial dichloromethane fraction; DCM-R: Rhizome dichloromethane fraction; H-A: Aerial n-hexane fraction; H-R: Rhizome n-hexane fraction; W-A: Aerial distilled water fraction; W-R: Rhizome distilled water fraction; DMSO: Dimethyl sulfoxide; ADP: Adenosine diphosphate

Capacity of radical scavenging of Canna warszewiczii A. Dietr fractions

For the aerial part, the EA fraction (EA-A) expressed the highest capacity to scavenge both DPPH and ABTS radicals (IC_{50} = 1.43 ± 0.13 and 1.87 ± 0.28 mg/mL, respectively) ($P < 0.01$). The DCM-A and W-A fractions showed the same scavenging effect, followed by the H fraction with the lowest activity (IC_{50} = 22.77 ± 0.12 mg/mL) against DPPH radical ($P < 0.01$) [Table 1].

For the rhizome part, the EA fraction was also the strongest scavenger against DPPH (IC_{50} = 0.14 ± 0.02 mg/mL) and ABTS (IC_{50} = 0.36 ± 0.08 mg/mL) ($P < 0.01$). The other fractions were in a decreasing order of ability as follows: DCM-R, H-R, and W-R [Table 1].

Total polyphenol and flavonoid content

The highest TPC and TFC were observed in the EA fractions compared with others from the same plant part ($P < 0.01$). The EA-R fraction significantly exhibited maximum phenolic and flavonoid content with 59.25% ± 7.56% and 60.00% ± 5.37%, respectively ($P < 0.05$). The DCM-R fraction approximately had two times higher in TPC and TFC than the DCM-A fraction. However, the H-A fraction had about two times higher in TFC than the H-R fraction (34.87% ± 3.77% vs. 17.57% ± 2.15%). Finally, the water fractions contained the lowest TPC and TFC in both the aerial and rhizome parts of the plant ($P < 0.05$) [Table 1].

Chemical screening of Canna warszewiczii A. Dietr fractions

It was evident that all the organic fractions were found to contain selected analyzed phytochemicals. Both the aerial and rhizome parts contained

Table 1: IC_{50} (mg/mL) values for 2,2-diphenyl-1-pycrylhydrazyl and 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) assays, total phenolic content, and total flavonoid content of fractions from *Canna warszewiczii* A. Dietr

Fractions/ compounds	DPPH assay (IC_{50})	ABTS assay (IC_{50})	TPC (g GAE/100 g dried sample or %)	TFC (g QE/100 g dried sample or %)
Aerial part				
H-A	22.77±0.12	10.80±0.89	1.33±0.13	34.87±3.77
DCM-A	9.05±0.23	10.33±1.93	3.99±0.40	17.32±0.34
EA-A	1.43±0.13	1.87±0.28	10.57±1.29	47.15±0.86
W-A	9.52±1.48	10.82±1.43	1.34±0.05	1.57±0.17
Rhizome part				
H-R	4.83±0.15	8.05±0.52	1.82±0.15	17.57±2.15
DCM-R	1.05±0.02	1.92±0.17	9.75±0.49	33.17±1.48
EA-R	0.14±0.02	0.36±0.08	59.25±7.56	60.00±5.37
W-R	37.55±1.78	44.57±2.71	0.43±0.09	0.42±0.08
Ascorbic acid	0.072±0.00			
Trolox		0.11±0.01		

EA-A: Aerial ethyl acetate fraction; EA-R: Rhizome ethyl acetate fraction; DCM-A: Aerial dichloromethane fraction; DCM-R: Rhizome dichloromethane fraction; H-A: Aerial n-hexane fraction; H-R: Rhizome n-hexane fraction; W-A: Aerial distilled water fraction; W-R: Rhizome distilled water fraction; TPC: Total polyphenol content; TFC: Total flavonoid content; g GAE/100 g dried sample: Grams of gallic acid equivalent per 100 g of the sample; g QE/100 g dried sample: Grams of quercetin equivalent per 100 g of the sample; DPPH: 2,2-diphenyl-1-pycrylhydrazyl; ABTS: 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid); IC_{50}: Half-maximal inhibitory concentration

Table 2: Phytochemical screening of different fractions of the aerial and rhizome parts of *Canna warszewiczii* A. Dietr

Fractions	EA-A	EA-R	DCM-A	DCM-R	H-A	H-R	W-A	W-R
Glycosides	++	++	+	+	−	−	−	−
Cardiac glycosides	++	++	++	++	++	++	+	+
Flavonoids	++	++	+	+	−	−	−	−
Polyphenols	++	++	++	++	+	++	−	−
Saponins	−	−	−	−	−	+	+	+
Coumarins	−	−	++	++	+	+	−	−
Emodols	+	+	−	++	−	+	−	−
Tannins	−	−	++	++	+	+	−	−
Steroids	+	+	+	+	++	++	−	−
Cholesterol	+	+	+	+	+	+	++	++

EA-A: Aerial ethyl acetate fraction; EA-R: Rhizome ethyl acetate fraction; DCM-A: Aerial dichloromethane fraction; DCM-R: Rhizome dichloromethane fraction; H-A: Aerial n-hexane fraction; H-R: Rhizome n-hexane fraction; W-A: Aerial distilled water fraction; W-R: Rhizome distilled water fraction; −: Nondetected; +: Detected; ++: Strongly detected

the major active phytoconstituents such as flavonoids, polyphenols, glycosides, cardiac glycosides, coumarins, steroids, emodols, tannins, and cholesterols. The water fractions showed few selective phytochemicals in comparison to the others [Table 2].

DISCUSSION

We demonstrated for the first time the antiplatelet aggregation of *C. warszewiczii* A. Dietr's organic fractions induced by different agonists through evaluation of three valuable parameters in assessing platelet function: percentage inhibition of platelet aggregation that represents the ability to inhibit platelet aggregation, AUC which is considered the best parameter to indicate the overall platelet aggregation, and the aggregation slope that indicates the aggregation velocity per min, whereas many previous studies only reported the maximum platelet aggregation or percentage inhibition of platelet aggregation yielded by plant extracts.[16-18] The study showed that the organic fractions from both the rhizome and aerial parts significantly inhibited ADP, collagen, and ristocetin-platelet aggregation and decreased significantly AUC and aggregation velocity in a concentration-dependent manner compared to both negative and positive controls. Especially, the percentage inhibition of EA-A and EA-R was over 90% at 1.5 mg/mL, indicating the strong platelet aggregation inhibition. In the study by Wu *et al.*[19] the n-hexane and the chloroform layer of the aerial part and the chloroform layer of the root of *Ruta graveolens* showed antiplatelet aggregation activity. Then, subsequent bioguided fractionation resulted in the isolation of 19 known compounds and 6 compounds showed a significant antiplatelet aggregation effect.[19] In this study, the EA, DCM, and H fractions had a significant antiplatelet aggregation activity, whereas the W fractions did not. This novel finding would be useful to bioguide the isolation of antiplatelet components from this plant in further studies.

Collagen, ADP, and ristocetin are used as agonists in routine platelet aggregation studies.[20] ADP initiates platelet aggregation through the activation of two major ADP receptors, $P2Y_1$ and $P2Y_{12}$. Collagen binds to the Glycoprotein VI (GpVI) and GpIa/IIa receptors and induces granule release and thromboxane A2 (TXA2) generation and then supports GPIIb-IIIa activation. Ristocetin produces platelet agglutination through von Willebrand factor (vWF) and GPIb-IX-V complex. The observed significant antiplatelet activity suggests that the plant organic fractions present several compounds with antiplatelet activity with different mechanisms of action, which could be either the inhibition of thromboxane pathway, or the activation of $P2Y_1$ and/or $P2Y_{12}$ receptor, or the interaction with GPIb and vWF. Further investigations are required to better understand the mode of action of these fractions.

Medicinal plants are potential sources of lead compounds for further discovery and optimization as new drugs. Phytochemical analysis of *C. warszewiczii* A. Dietr revealed the presence of variable compounds in the organic fractions including flavonoids, polyphenols, glycosides, cardiac glycosides, coumarins, steroids, tannins, emodols, and cholesterols. Polyphenols are an important family found abundantly in plants with powerful antioxidant activity. Polyphenols and coumarins

have been identified to inhibit platelet aggregation.[21] Cardiac glycosides are a unique group of secondary metabolites used during treatment of heart failure.[22] Flavonoids have been reported to inhibit atherosclerosis, platelet adhesion, and platelet aggregation through inhibition of TXA2 mechanisms.[23] This plant thus could be a potential source to explore active components with new pharmacophores and novel drug targets for the treatment and prevention of CVDs and oxidative stress-related diseases.

Recently, antioxidant activity has been proven to be involved in the mechanism of antiplatelet effect.[24] An antioxidant agent, theoretically, may have antiplatelet aggregation effect. In this study, the EA fractions revealed a strong inhibitory effect of platelet aggregation and antioxidant activity and contained the highest polyphenol and flavonoid content. Several studies showed a high amount of phenolics in *C. edulis*.[14,25] In *C. edulis* rhizome growing in China, 17.47 g GAE/100 g sample was found.[5] *C. indica* L. was also a good source of anthocyanins.[26,27] Diets rich in antioxidants can be effective for the treatment and protection of CVDs. Our previous study proved the anticoagulant activity of the EA fractions of *C. warszewiczii* A. Dietr.[28] Thus, the EA fractions might be utilized as real sources of the valuable phytochemicals used in pharmaceutical industries for the development of anticoagulant, antiplatelet, and antioxidant agents.

Moreover, since extracts sometimes have more powerful pharmacological activities than pure molecules, there is a growing attention for the use of plant extracts as a food supplement.[29] This study also provided scientific information that *C. warszewiczii* A. Dietr might be a good source of a dietary supplement and functional food to protect and treat CVDs and certain diseases in which free radicals are implicated.

CONCLUSION

This is the first experimental study to demonstrate that the organic fractions of both the aerial and rhizome parts of *C. warszewiczii* A. Dietr. showed dose-dependent effective antiplatelet aggregation and antioxidant activity. Particularly, the EA fractions possessed the strongest antioxidant properties in scavenging free radicals and contained the highest TPC and TFC. The knowledge of antiplatelet and antioxidant activity of this plant could contribute to the prevention of CVDs and oxidative stress-related diseases. Its extracts or fractions could be used in the preparation of functional foods or supplements with antioxidant and antiplatelet aggregation activity. It is also a good candidate for further *in vitro* and *in vivo* studies to identify the active molecules with antioxidant and platelet antiaggregating activity and understand the mechanisms for these actions.

Acknowledgements

The authors would like to thank the University of Science and Technology of Hanoi for the financial support of this research with Grant Number USTH.BIO.02/18-19.

Financial support and sponsorship

This study was financially supported by the University of Science and Technology of Hanoi.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Angiolillo DJ. Variability in responsiveness to oral antiplatelet therapy. Am J Cardiol 2009;103:27A-34.
2. Tachjian A, Maria V, Jahangir A. Use of herbal products and potential interactions in patients with cardiovascular diseases. J Am Coll Cardiol 2010;55:515-25.
3. Leopold JA. Antioxidants and coronary artery disease: From pathophysiology to preventive therapy. Coron Artery Dis 2015;26:176-83.
4. Duke JA, Ayensu ES. Medicinal plants in China. Algonac: Reference publications; 1985.
5. Zhang J, Wang ZW, Mi Q. Phenolic compounds from *Canna edulis* Ker residue and their antioxidant activity. Food Sci Technol 2011;44:2091-6.
6. Joshi YM, Kadam VJ, Patil YV, Kaldhone PR. Investigation of hepatoprotective activity of aerial parts of *Canna indica* L. on carbon tetrachloride treated rats. J Pharm Res 2009;2:1879-82.
7. Purintrapiban J, Suttajit M, Forsberg NE. Differential activation of glucose transport in cultured muscle cells by polyphenolic compounds from *Canna indica* L. Root. Biol Pharm Bull 2006;29:1995-8.
8. Chen HJ, Chen CN, Sung ML, Wu YC, Ko PL, Tso TK. *Canna indica L*. attenuates high-glucose-and lipopolysaccharide-induced inflammatory mediators in monocyte/macrophage. J Ethnopharmacol 2013;148:317-21.
9. Xie F, Gong S, Zhang W, Wu J, Wang Z. Potential of lignin from *Canna edulis* Ker residue in the inhibition of α-d-glucosidase: Kinetics and interaction mechanism merging with docking simulation. Int J Biol Macromol 2017;95:592-602.
10. Mustard JF, Perry DW, Ardlie NG, Packham MA. Preparation of suspensions of washed platelets from humans. Br J Haematol 1972;22:193-204.
11. Chang ST, Wu JH, Wang SY, Kang PL, Yang NS, Shyur LF. Antioxidant activity of extracts from Acacia confusa bark and heartwood. J Agric Food Chem 2001;49:3420-4.
12. Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radic Biol Med 1999;26:1231-7.
13. Soong YY, Barlow PJ. Antioxidant activity and phenolic content of selected fruit seeds. Food Chem 2004;88:411-7.
14. Mishra T, Goyal AK, Middha SK, Sen A. Antioxidative properties of *Canna edulis* Ker-Gawl. Indian J Nat Prod Res 2011;2:315-21.
15. Harbourne JB. Phytochemical Methods, a Guide to Modern Techniques of Plant Analysis, 2nd ed. New York: Chapman and Hall; 1984.
16. Tóth O, Calatzis A, Penz S, Losonczy H, Siess W. Multiple electrode aggregometry: A new device to measure platelet aggregation in whole blood. Thromb Haemost 2006;96:781-8.
17. Pierre S, Crosbie L, Duttaroy AK. Inhibitory effect of aqueous extracts of some herbs on human platelet aggregation *in vitro*. Platelets 2005;16:469-73.
18. Li L, Shen YM, Yang XS, Zuo GY, Shen ZQ, Chen ZH, *et al*. Antiplatelet aggregation activity of diterpene alkaloids from *Spiraea japonica*. Eur J Pharmacol 2002;449:23-8.
19. Wu TS, Shi LS, Wang JJ, Iou SC, Chang HC, Chen YP, *et al*. Cytotoxic and antiplatelet aggregation principles of *Ruta graveolens*. J Chinese Chem Soc 2003;50:171-8.
20. Zhou L, Schmaier AH. Platelet aggregation testing in platelet-rich plasma: Description of procedures with the aim to develop standards in the field. Am J Clin Pathol 2005;123:172-83.
21. Costa AG, Garcia-Diaz DF, Jimenez P, Silva PI. Bioactive compounds and health benefits of exotic tropical red-black berries. J Funct Foods 2013;5:539-49.
22. Morsy N. Cardiac glycosides in medicinal plants. In: El-Shemy H, editor. Aromatic and Medicinal Plants – Back to Nature. London: Intechopen; 2017. p. 29-45.
23. Koshy AS, Anila L, Vijayalakshmi NR. Flavonoids from *Garcinia cambogia* lower lipid levels in hypercholesterolemic rats. Food Chem 2001;72:289-94.
24. Singh I, Mok M, Christensen AM, Turner AH, Hawley JA. The effects of polyphenols in olive leaves on platelet function. Nutr Metab Cardiovasc Dis 2008;18:127-32.
25. Mishra T, Das AP, Sen A. Phytochemical screening and *in-vitro* antioxidant profiling of solvent fractions of *Canna edulis* Ker Gawler. Free Rad Antiox 2012;2:13-20.
26. Kumbhar ST, Patil SP, Une HD. Phytochemical analysis of *Canna indica* roots and rhizomes extract. Biochem Biophys Rep 2018;16:50-5.
27. Srivastava J, Vankar PS. *Canna indica* flower: New source of anthocyanins. Plant Physiol Biochem 2010;48:1015-9.
28. Nguyen TV, Duong TL, Vu TT, Nguyen TT, Le HL. Novel finding on anticoagulant activity of *Canna warszewiczii* extracts. Asia J Pharmacog 2018;2:5-10.
29. Calliste CA, Trouillas P, Allais DP, Duroux JL. *Castanea sativa* mill. Leaves as new sources of natural antioxidant: An electronic spin resonance study. J Agric Food Chem 2005;53:282-8.