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Original Article

Prosthechea karwinskii leaves extract with potential for the treatment of atherothrombosis

Extracto de hojas de Prosthechea karwinskii con potencial para el tratamiento de la aterotrombosis

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ABSTRACT

Prosthechea karwinskii is an orchid endemic to southern Mexico used in traditional medicine. It has anti-inflammatory, antioxidant and cardioprotective activity. Given the relationship between inflammation, oxidative stress, and coagulation in the development of atherothrombosis, the objective of this research was to evaluate the potential of *P. karwinskii* leaves extract as a possible alternative to reduce the risk of atherothrombosis. The compounds of the extract were identified by UHPLC-ESI-qTOF-MS. The content of total phenols and flavonoids was measured, as well as its antioxidant capacity (DPPH and DCFH-DA essays) and its effect on clotting times. The results showed the presence of phenolic and flavonoid compounds in the extract, as well as their antioxidant capacity. In addition, the extract prolonged clotting times, mainly thrombin, and activated partial thromboplastin times, i.e., it inhibited the intrinsic pathway of hemostasis and the conversion of fibrinogen to fibrin. These results and the background of the extract show its potential as a treatment to reduce atherothrombotic risk, as well as for other diseases whose pathogenesis involves oxidative stress and coagulation.

***Keywords*:** anticoagulant, cellular antioxidant, reactive oxygen species.

RESUMEN:

Prosthechea karwinskii es una orquídea endémica del sureste de México empleada en la medicina tradicional, presenta actividad antiinflamatoria, antioxidante y cardioprotección. Dada la relación que existe entre la inflamación, el estrés oxidativo y la coagulación en el desarrollo de aterotrombosis, el objetivo de esta investigación fue evaluar el potencial del extracto de hojas de *P. karwinskii* como posible alternativa para disminuir el riesgo de aterotrombosis. Se identificaron los compuestos del extracto por UHPLC-ESI-qTOF-MS, se midió el contenido de fenoles y flavonoides totales, su capacidad antioxidante (ensayos DPPH y DCFH-DA) y su efecto en los tiempos de coagulación. Los resultados mostraron la presencia de compuestos fenólicos y flavonoides en el extracto, así como su capacidad antioxidante. Además, el extracto prolongó los tiempos de coagulación, principalmente los tiempos de trombina y de tromboplastina parcial activada, es decir, inhibió la vía intrínseca de la hemostasia y la conversión del fibrinógeno en fibrina. Estos resultados y los antecedentes del extracto muestran su potencial como tratamiento para reducir el riesgo aterotrombótico, así como para otras enfermedades cuya patogénesis involucre al estrés oxidativo y la coagulación.

***Palabras clave*:** anticoagulante, antioxidante celular, especies reactivas del oxígeno.

INTRODUCTION

Thrombosis is the obstruction of blood vessels by the formation of a thrombus. It has become a worldwide health problem, being responsible for 1 in 4 deaths in humans (Yin *et al*., 2023). Atherothrombosis is the formation of a thrombus due to atherosclerosis. The site where atherosclerotic lesions develop is the vascular subendothelium, which maintains the activity of procoagulant, anticoagulant, and fibrinolytic factors in equilibrium, preventing clot formation at all times when they are not required. However, when the endothelium is dysfunctional, due to factors such as oxidative stress and inflammation, the balance is lost, giving way to the formation of atheroma plaques and pathological clots (thrombi) that occlude the vascular lumen. Therefore, it is crucial to maintain a healthy endothelium and rapidly restore its normal function to decrease the atherothrombotic risk (Nguyen *et al*., 2023).

The development of atheroma plaques is accelerated by inflammation. Inflammatory cell recruitment is accompanied by the production of proinflammatory cytokines, tumor necrosis factor (TNF-α), and C-reactive protein (CRP), among others, which in turn promote further inflammatory cell recruitment and stimulate differentiation of monocytes into macrophages and foam cells at atherosclerotic sites (Chan *et al*., 2022).

The drugs currently used for the treatment of atherothrombotic disease include, among others, anticoagulants

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and/or antiplatelet agents; however, they are not totally effective and safe, and in some cases, they are difficult to manage, with bleeding being their main complication. For this and other reasons, the discovery of new agents with antithrombotic potential continues to be important (Nguyen *et al*., 2023). Antioxidant compounds have the potential to reduce atherothrombotic risk by preventing endothelial dysfunction, and by acting as anticoagulants and antiplatelet agents. In addition, they may have beneficial effects at the vascular level by scavenging lipid peroxides and free radicals that impair vascular endothelium (Le *et al*., 2022). Because of the key role of inflammation in atherosclerosis and atherothrombosis, anti-inflammatory agents can also be considered as potential treatments for these conditions (Chan *et al*., 2022).

Prosthechea karwinskii (Mart.) J.M.H. Shaw is an orchid endemic to southeastern Mexico. It is important in the Mixtec region of the Oaxaca State due to its ornamental, ceremonial, and medicinal uses. The different parts of this orchid are used to treat diabetes, wounds and burns, cough, threatened abortion, or assist in labor (Cruz-García *et al*., 2014), all these related to inflammatory processes (Barragán-Zarate *et al*., 2022).

Leaves are part of the plant with the highest amount of total phenols and flavonoids, as well as the highest antioxidant activity *in vitro* and *ex vivo*, through ROS inhibition (Barragán-Zarate *et al*., 2022). *P. karwinskii* leaves extract obtained with ultrasound can inhibit ROS, as well as anti-inflammatory effect in models of acute inflammation induced with carrageenan (Barragán-Zarate *et al*., 2020) and chronic inflammation by decreasing TNF-α and CRP in a model of metabolic syndrome (Barragán-Zarate *et al*., 2021), both with Wistar rats. In addition, it is considered to provide cardiovascular protection (Lagunez-Rivera *et al*., 2023).

This study aimed to evaluate the potential use of *P. karwinskii* leaves extract obtained with subcritical fluids, to decrease the atherothrombotic risk through its ability to inhibit ROS and prolong clotting times.

MATERIAL AND METHODS

Reagents

RPMI-1640 reagent, 2′,7′ Dichlorodihydrofluorescein diacetate (DCFH-DA), dimethyl sulfoxide (DMSO), formic acid, acetonitrile, ethanol, phosphate-buffered saline (PBS), hydrogen peroxide (H_2O_2), trypan blue solution, DPPH, aluminum trichloride, Folin Deniss reagent, distilled water, methanol, acetonitrile, and formic acid, were purchased from Sigma Aldrich (Toluca, Mexico). The reagents for the coagulation assays: CK prest-2, Neoplastine CI+2, STa Thrombin, STA $CaCl_2$, STA System control N+P, and STA Owren-Koller were from Stago (Asnieres, France).

Plant material

Prosthechea karwinskii orchids were collected from the altar decorations of the churches after the Holy Week celebrations in Zaachila, Oaxaca, in April 2018. A specimen voucher was deposited in the OAX Herbarium of the Instituto Politécnico Nacional (Solano 4037). For this research, only the leaves of the plant were used, which were ground and sieved for the extraction of its compounds.

Obtaining the extract

The subcritical fluid-assisted extraction was performed in a high-pressure reactor (RAP-100 SEV-PRENDO) with agitation and a high-pressure HPLC pump (EX1600 PUMP Exformma Technologies). Ten g of sample were taken and 50 % ethanol in water was used as a solvent, with a sample: solvent ratio of 1:18 (18 mL of solvent is added for each gram of sample), a pressure of 50 bar and a temperature of 100 °C, for 20 min. Once the extract was obtained, it was kept frozen until analysis for preservation.

Compound identification with UHPLC-ESI-qTOF-MS

A UHPLC system (Thermo Scientific, Ultimate 3000) coupled to electrospray ionization (ESI) source to a and time-of-flight (TOF) mass spectrometer was used. The column used was an ODS Hypersil (125x4 mm, with a particle size of 3 µm). The mobile phases were A: 0.1 % formic acid in water and B: acetonitrile. The gradient system was 0 % B (0-2 min), 3 % B (2-3 min), 8 % B (3-4 min), 30 % B (4-6 min), 35 % B (6-7 min), 40 % B (7-8 min), 50 % B (8-9 min), 80 % B (9-10 min), 0 % B (10-11.5 min), 0 % B (11.5-14 min). The injection temperature was 25 °C with a flow rate of 200 µL/min. The mass spectrometer was operated in positive mode in the mass range of 50-1500 m/z. Capillary voltage ionization was 2700 V. Tentative identification was performed by comparing the m/z value with Compound Crawler and information reported in articles.

Total phenol content

The total phenol content was determined using the procedure described by Swain and Hillis (1959). For this purpose, 0.5 mL of the extract diluted to a concentration of 5 mg/mL was mixed with 4 mL of water and 0.25 mL of Folin-Denis reagent for 4 min, and then 1 mL of 1 N sodium carbonate solution was added. The mixture was allowed to stand for 2 h at room temperature, and the absorbance was determined at 760 nm in a spectrophotometer (Zeigen 1104). Gallic acid was used as a reference standard, and the results were expressed as mg gallic acid equivalent per g sample (mg GAE/g). Analyses were performed in triplicate.

Total flavonoid content

The total flavonoid content was determined according to the methodology of Chang *et al*. (2002). The extract diluted to a concentration of 5 mg/mL was taken in 0.5 mL, mixed with 1.5 mL of 95 % ethanol, 0.1 mL of 10 % aluminum trichloride ($AlCl_3$), 0.1 mL of 1 M potassium acetate (CH_3COOK) and 2.8 mL of distilled water. The mixture was left to react for 30 minutes at room temperature, and the absorbance was read at 415 nm in a spectrophotometer (Zeigen 1104). Quercetin was used as a reference standard. Results were expressed as mg quercetin equivalents per g sample (mg QueE/g). Analyses were performed in triplicate.

In vitro antioxidant capacity

The *in vitro* antioxidant activity of the extracts was determined using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging method. According to the method described by Scherer and Godoy (2009), a solution of 100 µM DPPH in methanol and the extract at different concentrations were prepared. The reaction time was 90 min in the dark at room temperature. Readings were taken at a wavelength of 517 nm in the spectrophotometer (Zeigen 1104). Analyses were performed in triplicate and expressed as % inhibition concerning the negative control (DPPH with methanol without extract). Ascorbic acid was used as a reference.

Biological evaluation in blood samples from healthy volunteers

The protocol was approved by the Research Ethics Committee of the Faculty of Dentistry of the Universidad Autónoma Benito Juárez de Oaxaca, with registration number 24PV009FO. Volunteers gave their informed consent. They were between 18 and 45 years old, of medium build, and were considered healthy as they presented blood chemistry parameters within normal ranges.

Cellular antioxidant capacity through ROS inhibition in peripheral blood mononuclear cells

The DCFH-DA assay was used to measure the inhibition of ROS in cells. This reagent is deacetylated to a non-fluorescent compound in cells, which in turn is oxidized by ROS to form the fluorescent compound 2′,7′-dichlorofluorescein (DCF), whose fluorescence is directly proportional to the content of ROS in cells (Torres-Rodríguez *et al*., 2016). The methodology used by Barragán-Zarate *et al*. (2020) was followed, where peripheral blood mononuclear cells (PBMCs) from healthy volunteers were used. Concentrations of 10, 100, 250, 250, 500, and 1000 µg/mL of 0.1 % DMSO extract were evaluated. Readings were performed on a spectrophotometer with DTX 800 multi-mode detector (Beckman Coulter) at 520 nm for emission and 485 nm for excitation. The results were expressed as % inhibition of ROS concerning the control (to which no extract was added).

Anticoagulant activity

Coagulation times were measured to evaluate the effect of *P. karwinskii* extract on the activity of plasma hemostasis factors, following the methodology of López-Pérez *et al*. (2022). For this purpose, platelet-poor plasma (PPP) was obtained from the blood of healthy volunteers (a pool obtained from ten men and ten women). Samples were collected in tubes with sodium citrate as an anticoagulant (3.2 %), maintaining a blood-anticoagulant ratio of 9:1. The PPP was obtained after centrifuging the blood at 2,500 x g for 15 min. Prothrombin time (PT), thrombin time (TT), and activated partial thromboplastin time (aPTT) were evaluated following the methodologies of the Stago brand commercial reagents (Neoplasntine CI+2, CK prest-2, and STA Thrombin) in a STart® 4 equipment (Stago, Asnieres, France) with minor modifications to evaluate *P. karwinskii* leaves extract obtained with subcritical fluid at concentrations of 1, 5, 10 and 20 mg/mL (mg extract/mL Owren Koller buffer).

Statistical analysis

The results were presented as the mean ± standard deviation. Data were subjected to a one-way analysis of variance (ANOVA) followed by a Tukey's test to compare means. Statistical analyses were performed with Graph Pad 5.0 software. A statistically significant difference was considered when the value of $P < 0.05$.

RESULTS AND DISCUSSION

Compound identification with UHPLC-ESI-qTOF-MS

Figure 1 shows the chromatogram of the leaves extract of *P. karwinskii* obtained with subcritical fluids (LEPKSU). Table 1 shows the information corresponding to the peaks of the chromatogram, which belong to the compounds present in the extract, as well as their tentative identification. A total of 16 compounds were found. The major compounds identified were: 1-O-4-Morpholinyl-d-fructose, quinic acid, adenosine, rutin, kaempferol-3-O-rutinoside, taxifolin, and histopine.

Although the UHPLC-ESI-qTOF-MS analysis performed for the extract obtained with ultrasound (Barragán-Zarate *et al*., 2021) using negative ionization, unlike the extract of the present study obtained with subcritical fluids that was analyzed with positive ionization, compounds with pronounced peaks and important biological activity (quinic acid, rutin and kaempferol-3-O-rutinoside) were identified in both extracts.

Phenol content, total flavonoids and *in vitro* antioxidant capacity

Table 2 shows the phenol and total flavonoid content and *in vitro* antioxidant capacity of the extract. The flavonoids identified with UHPLC-ESI-qTOF-MS were rutin, kaempferol-3-O-rutinoside, taxifolin, and apigenin 4′-O-β-D-glucopyranoside. Polyphenols can exert antithrombotic effects by inhibiting platelet aggregation and ameliorate endothelial dysfunction associated with various risk factors for atherosclerosis before plaque formation (Pandey and Rizvi, 2009). Flavonoids are

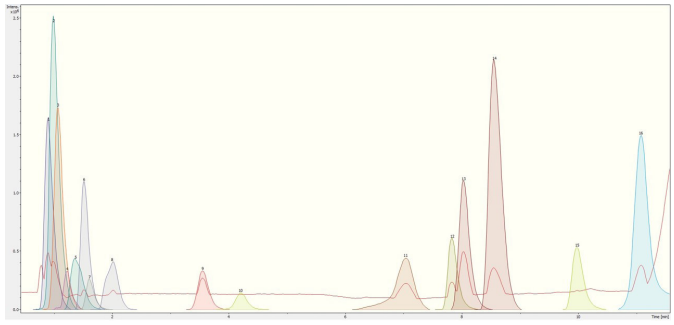

Figura 1. Cromatograma con UHPLC-ESI-qTOF-MS del extracto de hojas de *Prosthechea karwinskii* obtenido con fluidos subcríticos.
Figure 1. UHPLC-ESI-qTOF-MS chromatogram of *Prosthechea karwinskii* leaves extract obtained with subcritical fluids.

Tabla 1. Información de los compuestos identificados con UHPLC-ESI-qTOF-MS del extracto de hojas de *Prosthechea karwinskii* obtenido con fluidos subcríticos.
Table 1. Information on compounds identified with UHPLC-ESI-qTOF-MS of *Prosthechea karwinskii* leaves extract obtained with subcritical fluids.

PN	RT	m/z [M+1]	Error	msigma	Suggested formula	Compound name	Reference
1	0.9	104.1063	0.4	1.2	$C_5H_{13}NO$	Not identified	e
2	1.0	266.1235	0.4	0.8	$(C_{10}H_{19}NO_7)$	1-O-4-Morpholinyl-d-fructose	e
3	1.0	193.0697	0.3	0.9	$(C_7H_{12}O_6)$	Quinic acid	d
4	1.2	118.0856	3.1	1.5	$(C_4H_6O_4)$	Succinic acid	d
5	1.3	280.1386	0.1	2.6	$(C_{11}H_{21}NO_7)$	3-0-L-Valil-d-glucopyranose	e
6	1.5	210.0598	0.1	1.7	$(C_6H_{11}NO_7)$	2,0-aminohexuronic acid	e
7	1.6	130.0497	0.1	1.6	$(C_5H_8NO_3)$	Not identified	e
8	2.0	132.1011	- 0.8	0.3	$(C_6H_{13}NO_2)$	Aminocaproic acid	e
9	3.6	268.1025	- 1.0	5.1	$(C_{10}H_{13}N_5O_4)$	Adenosine	e
10	4.2	166.0864	- 2.4	2.1	$(C_9H_{11}NO_2)$	L-Phenylalanine	d
11	7.0	227.1744	- 0.3	3.4	$(C_{12}H_{22}N_2O_2)$	Not identified	e
12	7.8	611.1586	0.2	3.6	$(C_{27}H_{30}O_{16})$	Rutin	bd
13	8.0	595.1636	- 0.5	1.2	$(C_{27}H_{30}O_{15})$	Kaempferol-3-O-rutinoside	bd
14	8.5	305.0984	- 2.6	2.3	$(C_{15}H_{12}O_7)$	Taxifolin	a
15	9.9	433.1478	- 2.0	3.3	$(C_{21}H_{20}O_{10})$	Apigenin 4′-O-β-D-glucopyranoside	a
16	11.1	228.1943	- 2.5	2.0	$(C_9H_{14}N_3O_4)$	Histopine	c

PN: Peak Number, RT: Retention Time. [a]Tang *et al*. (2024), [b]Avula *et al*. (2022), [c]Padilla *et al*., (2021), [d]Compounds previously reported by Barragan-Zarate *et al*. (2021) for the leaf extract obtained with ultrasound, [e]Compound formula suggested by CompoundCrawler.

Tabla 2. Capacidad antioxidante *in vitro* y contenido de fenoles y flavonoides totales del extracto de hojas de *Prosthechea karwinskii* obtenido con fluidos subcríticos.
Table 2. *In vitro* antioxidant capacity and total phenol and flavonoid content of *Prosthechea karwinskii* leaves extract obtained with subcritical fluids.

Evaluation	
DPPH	**% inhibition**
Extract (mg/mL)	
0.5	5.541 ± 0.396
1.0	11.400 ± 0.762
2.0	24.192 ± 0.285
3.0	34.294 ± 0.540
4.0	44.237 ± 0.503
5.0	53.580 ± 1.890
Ascorbic acid (2 mg/mL)	49.071 ± 2.940
	mg GAE/g
Total phenols	73.318 ± 8.037
	mg QueE/g
Total Flavonoids	3.421 ± 0.020

mg GAE/g: mg gallic acid equivalent per g of sample; mg QueE/g: mg quercetin equivalent per g of sample; DPPH: 1,1-diphenyl-2-picryl-hydrazyl. Results are shown as the mean ± standard deviation.

employed in the clinical treatment of vascular disorders as they prevent capillary permeability, act as phlebotonics and improve blood rheology, although their mechanism of action remains partially unknown (Zaragozá *et al*., 2021). Flavonoids also exhibit anti-inflammatory activity, which is closely related to platelet aggregation through arachidonic acid and can inhibit thrombin generation (Leite *et al*., 2023). In addition, being anti-inflammatory, they could stop the formation of atheroma plaques by restricting the recruitment of proinflammatory cells and the production of inflammatory markers (Chan *et al*., 2022). Thus, the presence of this type of compounds in LEPKSU contributes to its potential to reduce atherothrombotic risk.

Figure 2 shows the ability of LEPKSU to inhibit ROS at different concentrations; it was observed that as the concentration of the extract increases, its ability to inhibit ROS increases. Unlike *in vitro* studies, antioxidant activity models in cells address some problems of absorption, distribution, and metabolism of the compounds evaluated (Wolfe *et al*., 2007), since they are taken to physiological pH and temperature conditions, giving us a closer overview of what happens to the compounds when administered to a living system, hence, they are more representative from the biological point of view (Shen *et al*., 2019).

Atherothrombosis can be triggered by a dysfunctional or damaged endothelium, resulting in exposure of suben-

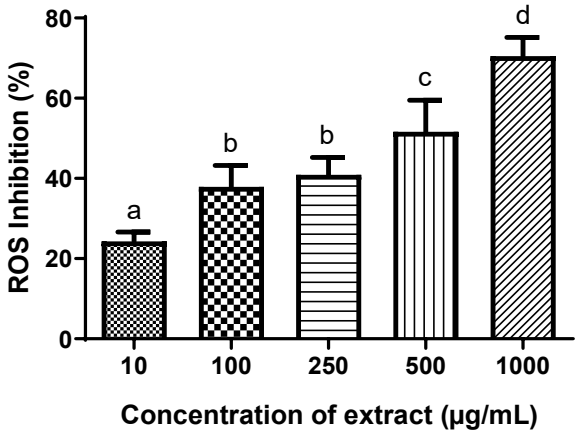

Figura 2. Inhibición de especies reactivas del oxígeno del extracto de hojas de *Prosthechea karwinskii* obtenido con fluidos subcríticos. Valores expresados como media ± desviación estándar. Superíndices distintos indican diferencia significativa (P < 0.05).
Figure 2. Inhibition of reactive oxygen species of *Prosthechea karwinskii* leaves extract obtained with subcritical fluids. Values are expressed as mean ± standard deviation. Different superscripts indicate a significant difference (P < 0.05).

dothelial plaque tissue to the bloodstream (Mastenbroek *et al*., 2015). When vascular endothelial cells are damaged, their structure and function are altered, activating procoagulant substances to activate platelets and contribute to thrombus formation. Damaged endothelium is accompanied by an inflammatory response and oxidative stress, which generates large amounts of ROS that aggravate the damage, making thrombus formation and development more likely (Wang *et al*., 2023). LEPKSU can inhibit ROS in cells, so it could decrease atherothrombotic risk by preventing aggravation of endothelial damage and thrombus development.

Of the compounds identified in the extract, those with antioxidant activity include rutin (Yeh *et al*., 2014) and kaempferol-3-O-rutinoside (Wang *et al*., 2015), which increase the activity of the antioxidant enzymes SOD, CAT, GSH-Px, and reduced GSH levels, in addition to taxifolin, which decreases malondialdehyde levels and increases total GSH levels (Bedir *et al*., 2021). These compounds may be responsible for the ability of the extract to inhibit ROS. Regarding the ROS inhibition of LEPKSU in comparison with the extract obtained with ultrasound by Barragan Zarate *et al*. (2020), a similar ROS inhibition of both extracts was observed at the 1000 mg/mL concentration; however, there were differences at the other concentrations evaluated.

Anticoagulant activity

Figure 3 shows the effect of LEPKSU on clotting times. Although a prolongation of all three clotting times was observed, the effect was greater in TT and aPTT, where these times were tripled at the 20 mg/mL concentration compared to where no extract was added. As for PT, the extract prolonged clotting time significantly (p > 0.05) only at the 20 mg/mL concentration.

The plasma phase of hemostasis comprises three stages: initiation, amplification and propagation. The study of these phases in the laboratory is based on the 1964 model of coagulation, in which fibrin formation depends on two activation pathways, the intrinsic and the extrinsic, in addition to a common one in which both pathways converge. With PT, the activity of the factors of the extrinsic pathway is evaluated, and with aPTT, the activity of the factors of the intrinsic pathway. Prolongation of one or both clotting times is indicative of a factor deficiency or the presence of an inhibitor that slows clotting (Edziri *et al*., 2020; Le *et al*., 2022). The TT represents the time it takes for fibrinogen to be converted to fibrin by addition of exogenous thrombin; a prolonged TT indicates an anticoagulant effect, while a shorter TT indicates a procoagulant effect. Platelet aggregation, as well as increased coagulation factor activity and thrombin generation induce thrombus formation and growth (Wang *et al*., 2023). The results suggest that the anticoagulant activity of the extract is given by inhibition of one or more plasma factors of the intrinsic pathway of hemostasis, or that the prolongation of aPTT is a secondary effect of thrombin inhibition by the extract.

The intrinsic coagulation pathway is triggered by contact of FXII (coagulation factor XII) with negatively charged surfaces. It has been shown that fibrillar collagen, such as that present in atherosclerotic plaques, can bind to FXII and promote its activation. Like thrombin, activated FXII activa-

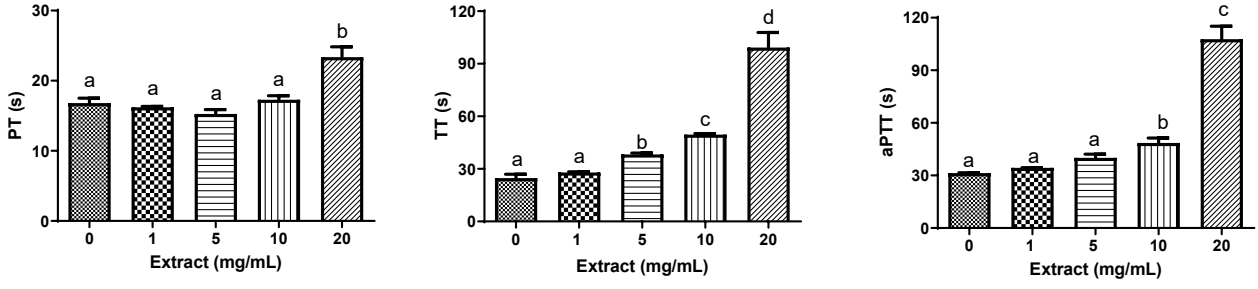

Figura 3. Tiempos de coagulación: tiempo de protrombina (PT), tiempo de trombina (TT) y tiempo de tromboplastina parcial activada (aPTT) del extracto de hojas de *Prosthechea karwinskii* obtenido con fluidos subcríticos. Valores expresados como media ± desviación estándar. Superíndices distintos indican diferencia significativa (P < 0.05).
Figure 3. Coagulation times: prothrombin time (PT), thrombin time (TT), and activated partial thromboplastin time (aPTT) of *Prosthechea karwinskii* leaves extract obtained with subcritical fluids. Values are expressed as mean ± standard deviation. Different superscripts indicate a significant difference (P < 0.05).

tes FXI, which in turn activates FIX. Given the role of the FXII pathway in atherothrombosis, but not in hemostasis, the intrinsic coagulation pathway may be an attractive target for antithrombotic drugs with low bleeding risk (Mastenbroek *et al*., 2015). Thrombin and factor Xa contribute to atherogenesis up to the point of atherothrombosis. These are not only limited to thrombus formation in unstable plaques, but play an important role in the initial development of atherosclerosis. Activation of coagulation and thrombin generation not only promote fibrin production and platelet activation, but are involved in atherogenesis (Olie *et al*., 2018).

The effect of LEPKSU in prolonging the common and intrinsic coagulation pathways, in addition to its possible inhibition of thrombin, suggests that LEPKSU has the potential to limit or slow the progression of atherogenesis to atherothrombosis. Compounds present in the extract with anticoagulant or antithrombotic activity are rutin and quinic acid. Rutin may be an antithrombotic agent due to its ability to prolong aPTT and PT, in addition to decreasing thrombin activity and inhibiting platelet aggregation (Choi *et al*., 2015). Rutin acts by blocking Glycoprotein IIb/IIIa receptors (Zaragozá *et al*., 2021), thus inhibiting platelet aggregation. Quinic acid has also shown an antithrombotic effect in a zebrafish model of thrombosis, possibly targeting the arachidonic acid pathway (Shi *et al*., 2020).

Compounds that present biological activities such as antioxidant, anti-inflammatory, antiplatelet aggregation and anticoagulant may contribute to prevent thrombotic disorders (Leite *et al*., 2023). According to the results of the present investigation, LEPKSU presents cellular antioxidant activity by inhibiting ROS, as well as anticoagulant activity, in addition, there is the antecedent of anti-inflammatory activity of *P. karwinskii* leaf extract obtained with ultrasound (Barragán-Zarate *et al*., 2020; Barragán-Zarate *et al*., 2021) and provides cardiovascular protection (Lagunez-Rivera *et al*., 2023). All this supports the potential of LEPKSU to decrease the risk of atherothrombosis.

CONCLUSIONS

The extract showed dose-dependent anticoagulant activity since it prolonged the TT and aPTT. The background of biological activity of the identified compounds in the extract and the anti-inflammatory activity of the plant, as well as the results of the present study on the phenols and flavonoids contents, the ability of the extract to inhibit ROS in mononuclear cells, and its ability to prolong the common and intrinsic pathways of coagulation, show us the potential of the extract as an alternative therapy to reduce atherothrombotic risk, as well as other conditions in whose pathogenesis oxidative stress and blood coagulation are involved.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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