Botanical Sciences 103 (3): 644-657. 2025
DOI: 10.17129/botsci.3645

Received: January 13, 2025, Accepted: May 1, 2025
On line first: June 12, 2025

Phytochemistry / Fitoquímica

The hydroethanolic extract of leaves of *Laelia furfuracea* inhibits blood coagulation and platelet aggregation

Abimael López-Pérez[1], Luicita Lagunez-Rivera[1]*, Rodolfo Solano[1], Edgar García-Sánchez[2], Gabriela Soledad Barragán-Zárate[1], Berenice Fernández-Rojas[3], Abraham Majluf-Cruz[4], Jesús Hernández-Juárez[2]*

[1] Laboratorio de Extracción y Análisis de Productos Naturales Vegetales, Centro Interdisciplinario de Investigación para el Desarrollo Integral Regional Unidad Oaxaca, Instituto Politécnico Nacional, Oaxaca, México.
[2] SECIHTI-Centro Interdisciplinario de Investigación para el Desarrollo Integral Regional Unidad Oaxaca, Instituto Politécnico Nacional, Oaxaca, México.
[3] Departamento de Fisiología, Instituto Nacional de Cardiología Ignacio Chávez, Ciudad de México, México.
[4] Unidad de Investigación Médica en Trombosis, Hemostasia y Aterogénesis, Instituto Mexicano del Seguro Social, Ciudad de México, México.
*Corresponding authors: jehernandezju@ipn.mx; llagunez@ipn.mx

Abstract
Background: *Laelia furfuracea* is an orchid with anticoagulant properties; however, it is unknown which coagulation factors are inhibited and whether the plant has antiplatelet activity.
Questions: Does the hydroethanolic extract of leaves of *L. furfuracea* inhibit coagulation factor activity and platelet aggregation?
Studied species: *Laelia furfuracea.*
Study site and dates: Plant material was collected in April 2023 in Santo Domingo Yanhuitlán, Oaxaca, Mexico.
Methods: The anticoagulant and antiplatelet effects of the extracts of *L. furfuracea* were evaluated in samples from patients with thrombosis through coagulometric assays and light transmission aggregometry.
Results: The extract of *L. furfuracea* inhibited the activity of factors II, V, VIII, and XI in a concentration-dependent manner. The highest extract concentration, 14.0 mg/mL, inhibited about 60 % of factors II and V activity, but > 95 % of factors VIII and XI. Moreover, at a 1.0 mg/mL concentration, the extract inhibited platelet aggregation induced by ADP, thrombin, and epinephrine in 85, 97, and 83 %, respectively.
Conclusions: It was demonstrated that the orchid extract inhibits both the activity of factors II, V, VIII, XI, and thrombin-, ADP-, and epinephrine-induced platelet aggregation. Polyphenols stand out among the possible bioactive compounds of the orchid with anticoagulant and/or antiplatelet activity.
Keywords: coagulation factors, antiplatelet, polyphenols, orchid, thrombosis.

Resumen
Antecedentes: *Laelia furfuracea* es una orquídea con propiedades anticoagulantes; sin embargo, se desconoce qué factores de coagulación inhibe y si la planta tiene actividad antiplaquetaria.
Preguntas: ¿El extracto hidroetanólico de hojas de *L. furfuracea* inhibe la actividad de los factores de coagulación y la agregación plaquetaria?
Especie estudiada: *Laelia furfuracea*.
Sitio y fechas de estudio: El material vegetal fue colectado en abril de 2023, en Santo Domingo Yanhuitlán, Oaxaca, México.
Métodos: El efecto anticoagulante y antiplaquetario de los extractos de *L. furfuracea* fueron evaluados en muestras de pacientes con trombosis mediante ensayos coagulométricos y de agregación plaquetaria.
Resultados: El extracto de *L. furfuracea* inhibió la actividad de los factores II, V, VIII y XI de manera dependiente de la concentración. A la concentración de 14.0 mg/mL, el extracto inhibió aproximadamente el 60 % de la actividad de los factores II y V, pero > 95 % la de los factores VIII y XI. Además, a la concentración de 1.0 mg/mL, el extracto inhibió la agregación plaquetaria inducida por ADP, trombina y epinefrina en 85, 97 y 83 %, respectivamente.
Conclusiones: Se demostró que el extracto de la orquídea inhibe la actividad de los factores II, V, VIII y XI, y la agregación plaquetaria inducida con trombina, ADP y epinefrina. Los polifenoles destacan entre los posibles compuestos bioactivos de la orquídea con actividad anticoagulante y/o antiplaquetaria.
Palabras clave: factores de coagulación, antiplaquetarios, polifenoles, orquídea, trombosis.

Hemostasis is a physiological response to vessel damage that comprises the sequential activation of the blood coagulation factors on the cell membrane of activated platelets. In contrast, thrombosis is a pathological response of the coagulation system in which an obstruction of the blood flow in veins, arteries, or cardiac cavities occurs. Because thrombotic diseases are responsible for the largest number of deaths worldwide (Raskob *et al*. 2014, Wendelboe & Raskob 2016), identifying and developing new antithrombotic agents is highly desirable.

Current anticoagulant drugs include vitamin K antagonists (VKA), heparins, fondaparinux, and the direct inhibitors of activated factor X and thrombin. Despite this variety of anticoagulant agents, none is entirely effective and safe for patients (van der Hulle *et al*. 2014, Li *et al.* 2023). On the other hand, due to the role of platelets in the pathophysiology of arterial thrombosis (AT) and its main clinical manifestations, acute myocardial infarction and stroke (Huseynov *et al*. 2023), it is also essential to search for better antiplatelet treatments. Moreover, in some cases, anticoagulant treatment (Hand & Hale 2021, Rivera-Caravaca *et al*. 2021) requires antiplatelet drugs.

Under this scenario, it would be desirable to have agents with a dual effect on hemostasis, in order to regulate both thrombin generation and platelet aggregation in a controlled manner.

Bioactive compounds derived from plants represent an alternative for treating and preventing thrombosis. Today, it is possible to identify and purify compounds with anticoagulant, antiplatelet, and/or fibrinolytic effects derived from plants (Yoon *et al*. 2002, Pawlaczyk *et al*. 2009, 2011, Pawlaczyk-Graja *et al*. 2016, 2019, Sadeghi *et al*. 2017, Silva *et al.* 2018, Singh *et al*. 2021). These compounds have potential to treat or prevent thrombotic episodes after improving their bioactive properties through chemical processes (Shi *et al*. 2012). Polyphenols are just one example among many plant-derived natural compounds with inhibitory properties on hemostasis (Bijak *et al*. 2014a,b).

Orchids are plants with antithrombotic properties *in vitro* and *in vivo* assays. In fact, some studies have shown that these plants mainly contain antiplatelet compounds (Chen *et al*. 1994, Paik *et al*. 1995, Chen *et al.* 2000, Fan *et al*. 2001, Pyo *et al*. 2004, Lin *et al*. 2006, Ding *et al*. 2007, Hu *et al*. 2008a,b, Lee *et al*. 2014, Jeon *et al*. 2016, Sharma *et al*. 2018, Swe *et al*. 2021, Rahman *et al*. 2022), however, the evidence is scare regarding their mechanism of action on the hemostasis pathways (Ding *et al.* 2007, López-Pérez *et al*. 2022). *Laelia furfuracea* Lindl. is an endemic orchid from Mexico. In a previous study, it was demonstrated that hydroethanolic extract and organic fractions of *L. furfuracea* leaves induced a concentration-dependent prolongation of prothrombin (PT), activated partial thromboplastin (APTT), and thrombin (TT) times (López-Pérez *et al*. 2022). These findings motivated us to investigate which coagulation factors may be inhibited by the hydroethanolic extract of *L. furfuracea* and to explore its mechanisms of action and whether the extract also exhibited an antiplatelet effect. Blood coagulation factors are plasma proteins synthesized in the liver which, after being sequentially activated, promote fibrin formation on the membrane of activated platelets. Factors II, VII, IX, X, XI, and XII are serine proteases; factors V and VIII act as cofactors; and factor I acts as a substrate for activated factor II (Yong & Toh 2023). It must be emphasized that today, knowledge regarding the inhibitory effects of the extract on blood coagulation factors that the extract may inhibit is imperative in the current era due to a global trend that seeks the development of a new generation of antithrombotic drugs. Today, may anticoagulants focus on blood coagulation factor XI (Barnes 2025) and this is important because the inhibitory activity of natural compounds against activated factors II and X is well known (Pawlaczyk *et al*. 2011, Bijak *et al*. 2014a,b, Choi *et al*. 2016, Olas *et al*. 2018, Lamponi *et al*. 2021). Although the pharmacological inhibition of blood coagulation factors II and X is associated with evident benefits for patients with thrombosis (Pollack *et al*. 2020), the chemistry of natural products should focus on searching for new anticoagulant compounds with other specific action targets. Therefore, the study aimed to evaluate further the effects of the hydroethanolic extract of *L. furfuracea* leaves on the activity of coagulation factors and platelet aggregation.

Materials and methods

Plant material and extraction. Collection of vegetable material and extraction from *L. furfuracea* leaves was carried out as described previously in López-Pérez *et al*. (2022). Rodolfo Solano identified the plant and a voucher speci-

men was herborized and deposited at the OAX Herbarium (OAX-FLO-129-0402) of the Centro Interdisciplinario de Investigación para el Desarrollo Integral Regional Oaxaca, Instituto Politécnico Nacional, (R. Solano 4244). Briefly, orchid leaves were dehydrated at 40 °C until constant weight and pulverized in a mill (IKA® M20, Northchase Parkway SE, Wilmington, NC, USA), before being subjected to solid-liquid extraction. The extraction was performed using Soxhlet equipment, with 5 g of leaf material added to a water-ethanol solution in a 1:1 ratio at 78.2 °C for 2 h. The extract was dried on a rotary evaporator (BÜCHI, R-210, Darmstadt, Germany) and stored at 5-10 °C until processing.

Preparation of blood samples. Platelet-poor plasma (PPP) and platelet-rich plasma (PRP) samples were obtained from patients with venous thromboembolism (VTE) and arterial thrombosis (AT) at Unidad de Investigacion Médica en Trombosis Hemostasia y Aterogénesis, of the Instituto Mexicano del Seguro Social. PPP was obtained from VTE patients (n = 10) after centrifuging 5.4 mL of blood collected in vacuum plastic tubes (BD Vacutainer®, Franklin Lakes, NJ, USA) with sodium citrate (3.2 %) at 2,500 g for 15 min. Sodium citrate in vacuum plastic tubes acted as a reversible anticoagulant by binding to calcium ions and subsequently interrupting the activation of coagulation factors, allowing us to obtain PPP after centrifuging whole blood. PPP was preserved at -80 °C and thawed at 37 °C for 6 min before processing. Samples with prolonged clotting times as compared with control plasma ≥ 3 s for prothrombin time (PT) and thrombin time (TT) and ≥ 5 s for activated partial thromboplastin time (APTT) were discharged. For PRP, blood samples were drawn by venipuncture from AT patients (n = 10) and were collected in vacuum plastic tubes (BD Vacutainer®, Franklin Lakes, NJ, USA) with sodium citrate (3.2 %). Subsequently, the tubes were centrifuged at 800 g for 10 min to obtain PRP. PPP was used as a blank in platelet aggregation assays. Platelet aggregation assays were performed immediately after PRP was obtained.

For thrombin assays, platelets were washed and quantified in a Cell-Dyn® 3700 automated blood cell counter (Abbott Park, IL, USA); platelet concentrations ranged from 200,000 to 300,000 platelets/µL. We also obtained serum for blood chemical analysis (Table 1) in the Synchron LX 20 automated analyzer (Beckman Coulter, Fullerton, CA, USA). Serum samples were obtained after centrifuging whole blood collected in plastic tubes without anticoagulant at 2,000 g for 10 min.

Table 1. Screening blood tests in patients with thrombosis.

Parameters	Mean ± SEM	Parameters	Mean ± SEM
Glucose (mg/dL)	82.8 ± 8.8	AP (IU/L)	91.0 ± 45.7
Urea (mg/dL)	33.2 ± 11.2	LDH (IU/L)	162.5 ± 28.2
Creatinine (mg/dL)	0.8 ± 0.2	GGT (g/L)	29.3 ± 16.2
Triglycerides (mg/dL)	130.3 ± 68.3	PT (s)	171.8 ± 30.7
Total cholesterol (mg/dL)	171.8 ± 30.7	APTT (s)	53.1 ± 11.3
HDL-C (mg/dL)	53.1 ± 11.3	TT (s)	92.4 ± 36.8
LDL-C (mg/dL)	92.4 ± 36.8	TI-PA (%)	81.0 (65 – 87)
VLDL-C (mg/dL)	91.0 ± 45.7	ADPI-PA (%)	86.0 (72 – 92)
sGOT (IU/L)	22.5 ± 8.2	EI-PA (%)	87.0 (78 – 95)
sGPT (IU/L)	18.8 ± 5.5		

Results are expressed as the mean ± SEM. HDL-C: high-density lipoprotein cholesterol: LDL-C: low-density lipoprotein cholesterol; VLDL-C: very low-density lipoprotein cholesterol; sGOT: serum glutamic oxaloacetic transaminase; sGPT: serum glutamic pyruvic transaminase; AP: alkaline phosphatase; LDH: lactic dehydrogenase; GGT: gamma glutamyl transferase. TI-PA: thrombin-induced platelet aggregation; ADPI-PA: ADP-induced platelet aggregation; EI-PA: epinephrine-induced platelet aggregation; n = 20.

Anticoagulant assays. Stago brand reagents (Neoplastin Plus, Diagnostica Stago, Asnieres, France and STA-aPTT, Diagnostica Stago) were used to measure TT, PT, and APTT in a STA-COMPAQ equipment (Diagnostica STAGO), according to the procedure described in López-Pérez *et al*. (2022). Briefly, the clotting time was measured after incubating the plasma with the orchid extract. To determine PT, 50 µL the patient's PPP was mixed with either Owren's saline buffer (OSB; basal) or with orchid extract in a 2:1 ratio of each mixture to obtain a final concentration of 0.01, 0.03, 0.06, 0.12, 0.25, 0.50, 0.75, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, 7.0, 10.0, and 15.0 mg/mL. Mixtures were incubated at 37 °C for 180 s, and then, 100 µL of the PT reagent (calcium thromboplastin at 37 °C) was added. Time required for fibrin formation was registered. Similarly, TT was determined by incubating 100 µL of PPP (basal or post-extract) at 37 °C for 180 s with 100 µL of thrombin (2 IU/mL; 37 °C). Finally, APTT was determined by mixing 50 µL of PPP (basal or post-extract) with 50 µL of partial thromboplastin reagent. The mixture was incubated for 180 s at 37 °C, and immediately after, 50 µL of $CaCl_2$ (0.25 M; 37 °C) was added, and the clotting time was recorded. Unfractionated heparin (UFH: 1,000 IU/mL; Inhepar, PiSA, Mexico) was a positive anticoagulant control. Clotting times were measured at a final concentration of 0.01 mg/mL UFH. Ten samples from VTE patients were analyzed.

One-stage coagulation factor activity assays. A calibration curve was constructed for each coagulation factor assay (Diagnostica Stago, Asnieres, France). A one-step coagulometric assay based on the measurement of clotting time was used with some modifications. The activity of factors II, V, VII, and X was evaluated using PT, while APTT was used to assay the activity of factors VIII, IX, XI, and XII. First, 25 µL of the patient's PPP diluted (1:10, 1:20, 1:40, or 1:80) with OSB was mixed with 25 µL of factors II, V, VII, VIII, IX, X, XI, or XII deficient plasma. Then, 25 µL of OSB (basal) or 25 µL of extract were added to the mixture to obtain the final concentrations (1.0, 3.5, 7.0, 10.0, and 15.0 mg/mL) used for PT and APTT; mixtures were incubated for 1 min at 37 °C and, subsequently, PT or APTT were performed. For the calibration curve, the standard plasma was diluted 1:10, 1:20, 1:40, and 1:80 with OSB; each dilution was mixed with an equal volume of factor-deficient plasma to evaluate the coagulation time. PT or APTT from standard plasma dilution 1:10 corresponded to the maximum activity of each coagulation factor (usually equal to or >100 IU/dL or 100 % of activity), while the dilutions 1:20, 1:40, and 1:80 correspond to 50, 25, and 12.5 %, respectively. The activity of each coagulation factor in the tested plasmas was obtained by interpolating the result of PT or APTT in the calibration curve. Straight lines, parallel to each other, were obtained between tested plasmas incubated with the extract and standard plasma; otherwise, the results were discarded because of possible interference in the test. Considering a standard plasma concentration of 99, 113, 118, 114, 103, 105, 95, and 99 IU/dL for factors II, V, VII, VIII, IX, X, XI and XII, respectively. The activity values expressed in percentage of activation for each coagulation factor were converted into IU/dL as follows: [(factor activity (%) / 100 %) × factor activity (IU/dL)].

UFH (0.01 mg/mL) was used as a positive control of inhibition of coagulation factors. The percentage of inhibition for each coagulation factor was calculated as follows: % inhibition = 100 – [((% activity with extract or UFH) / (% activity basal)) × 100 %].

These assays were performed in samples from VTE patients (n = 10).

Antiplatelet aggregation assays. The effect of the *L. furfuracea* extracts on platelets was evaluated with a turbidimetric method using a Chrono-Log 700-2 platelet aggregometer (Chrono-log Corp., Havertown, PA, USA). For these assays, platelets from AT patients (n = 10) were activated with thrombin (1.0 U/mL), adenosine diphosphate (ADP) (2.5 µM), or epinephrine (10.0 µM), the three reagents from Chrono-log brand (Chrono-log Corp., Havertown, PA, USA). For each assay, before adding the agonists, either 20 µL of phosphate buffer (PBS) or 20 µL of orchid extract (0.01, 0.03, 0.06, 0.12, 0.25, 0.50, 0.75, and 1.0 mg/mL) were added to PRP and incubated for 1 min at 37 °C. Higher concentrations were not evaluated because they interfered with the test. Then, platelets were activated with thrombin, ADP, or epinephrine. As a positive inhibition control, we used PRP from patients under treatment with acetylsalicylic acid (ASA) (100 mg/d) or clopidogrel (75 mg/d). A platelet aggregation test was performed for 10 min after adding the agonist or until aggregation was stable. Maximal platelet aggregation was expressed as a percentage corresponding to the maximum light

transmission in the PRP (with and without extract) as a function of time. The percentage of inhibition of platelet aggregation was calculated as follows: % inhibition = 100 – [((% aggregation with extract) / (% aggregation basal)) × 100%].

The extract (0.01 - 1.0 mg/mL) underwent a comprehensive cytotoxicity assay before platelet aggregation was evaluated, ensuring the extract´s safety for further research and potential use.

Statistical analysis. It was performed using GraphPad Prism 8 software. Results are expressed as means ± standard error of the mean (SEM). Variance analysis (ANOVA) and Tukey tests were used to compare the results between different concentrations of the orchid extract. Results were considered statistically significant if $p < 0.05$.

Results

Demographics and blood analysis in samples from thrombotic patients. Blood samples were obtained from 20 patients with thrombosis (VTE, n = 10; and AT, n = 10). Median age for VTE patients was 41 years old (ranges 19-59) and 55 years old (ranges 36-66) for AT patients ($p > 0.05$). The results of blood chemistry analysis and the screening blood coagulation tests in samples of patients with thrombosis are shown in Table 1.

The anticoagulant effect of the hydroethanolic extract of L. furfuracea *leaves.* As previously published (López-Pérez *et al*. 2022), *L. furfuracea* extracts significantly prolonged PT, APTT, and TT assays. For the PT and APTT, prolongation was observed from 2.5 and 1.0 mg/mL, respectively, while for TT, the effect started at 3.0 mg/mL (Table 2). Results for the positive control were 17.4 ± 1.3 s, 293.7 ± 2.5 s, and 20.6 ± 1.4 s for PT, APTT, and TT, respectively. On the other hand, the extract of *L. furfuracea* inhibited the activity of factors II, V, VIII, and XI in a concentration-dependent manner (Table 3). The greatest inhibitory effect was observed at 15.0 mg/mL, decreasing the activity of factors VIII and XI by > 95 % as compared to the basal activity. Moreover, the extract decreased the activity of factors II and V by almost 60 % (Figure 1). On the contrary, the extract increased the activity of factors VII, IX, and XII concerning the baseline values, while factor X remained unchanged (Table 3). UFH decreased the activity of coagulation factors, mainly factors II, V, VIII, X, and XI (Table 3).

Antiplatelet effect of hydroethanolic extract of L. furfuracea *leaves.* The orchid extract decreased platelet aggregation concentration-dependently manner when platelets were activated with thrombin, ADP, and epinephrine (Table 4). Thrombin-induced platelet aggregation decreased 64.3 ± 4.2 % at 0.06 mg/mL of the extract, but the inhibition was up to 97.0 % using the highest concentration (1.0 mg/mL) (Figure 2). On the other hand, the extract inhibited ADP-induced (29.2 ± 4.6 %), as well as epinephrine-induced platelet aggregation (23.8 ± 6.7 %) since the concentration of 0.25 mg/mL (Figure 2).

Discussion

The leaf extract of *L. furfuracea* inhibited the *in vitro* activity of coagulation factors II, V, VIII, and XI, with a more prominent effect on factors VIII and XI; these findings were not observed in other orchids, and, to our knowledge, there are no other reports of natural extracts or compounds that exert inhibitory activity against FXI. However, an overall comprehensive analysis, including the results of blood coagulation tests and the coagulation factors' activity, suggests that the orchid's anticoagulant mechanism relies on thrombin inhibition. Thus, thrombin inhibition may be responsible for the decreased activation of factors II, V, VIII, and XI, as well as for the lower conversion of fibrinogen into fibrin (Hoffman 2003, Choi *et al*. 2011, Mann 2011). In addition, the profiles of the blood coagulation test between the orchid extract and those observed with well-known anticoagulant drugs support a likely inhibition of thrombin. Indeed, the orchid extract induces a different profile of coagulation tests compared to VKA or direct oral factor X- activated inhibitors but a similar profile to those induced by direct thrombin inhibitors or UFH. VKA exert their anticoagulant effect by inhibiting the liver enzyme vitamin K oxide reductase, affecting the carboxylation of vitamin K-dependent factors (mainly factor VII). Depending on the dose, VKA prolong PT (Gómez-Rosas *et al*. 2021) and occasionally APTT, but not TT. Similarly, FXa inhibitors may prolong PT and APTT but not TT (Dunois 2021, Margetić *et al.* 2022).

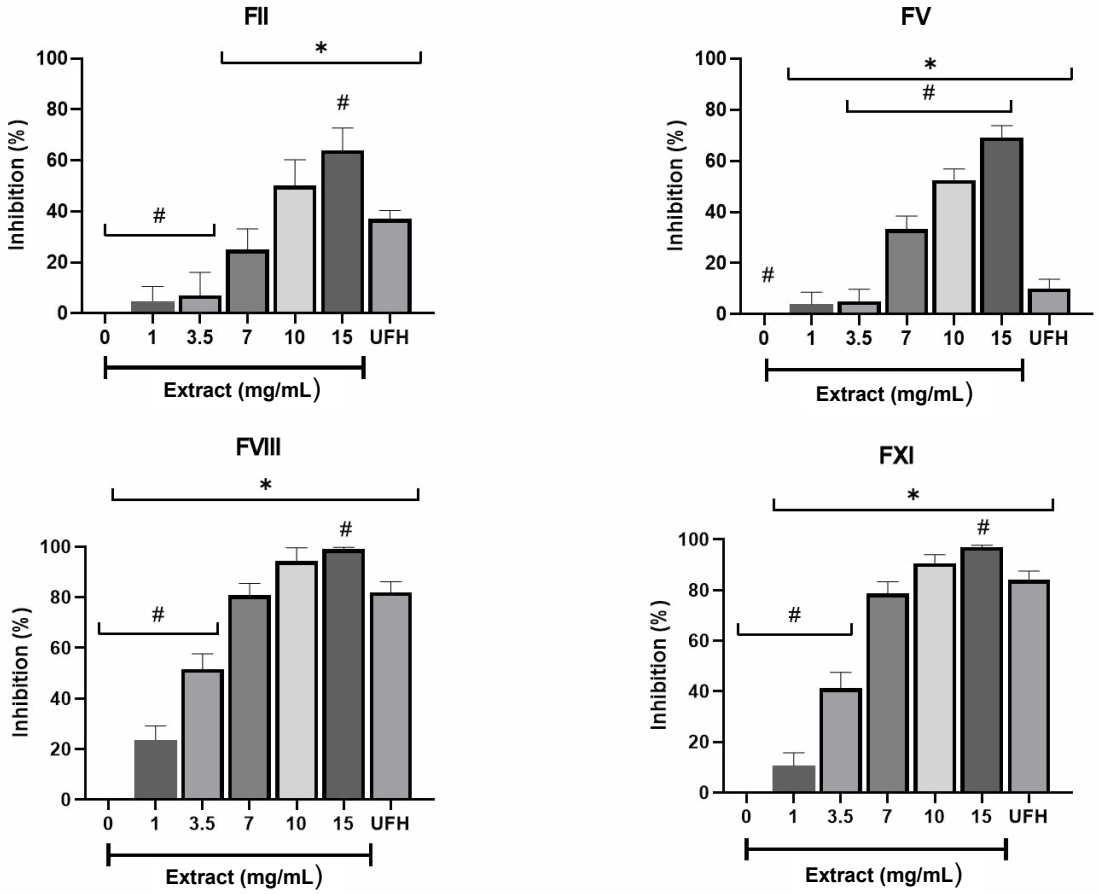

Figure 1. Percentage of inhibition of the hydroethanolic extract of *Laelia furfuracea* leaves on the activity of blood coagulation factors. The results are expressed as mean ± SEM. At 15 mg/mL of extract, the activity of factors II, V, VIII, and XI decreased 64 ± 8.8 %, 68 ± 4.8 %, 99 ± 0.9 %, and 97 ± 0.8 %, respectively, compared with basal activity. For the positive control (UFH 10.0 µg/mL), the activity of factors II, V, VIII, and XI decreased by 37 ± 3.4 %, 10 ± 4.1 %, 82 ± 4.5 %, and 83 ± 3.9 %, respectively. *: Significant difference between experimental conditions (Extract 1, 3.5, 7, 10, and 15 mg/mL) and basal determination (0 mg/mL). #: Significant difference between experimental conditions and UFH; n = 10.

In contrast, direct thrombin inhibitors prolong these clotting assays in a dose-dependent manner, although TT assay best assesses the anticoagulant effect (Dunois 2021, Margetić *et al*. 2022). On the other hand, UFH potentiates the anticoagulant effect of antithrombin, which mainly inhibits FXa and thrombin. Therefore, the clotting assays could be prolonged (Apipongrat *et al*. 2022), although APTT is more sensitive to UFH. However, the extract of *L. furfuracea* did not affect FX activity, suggesting another possible mechanism. Although the *L. furfuracea* extract showed an anticoagulant effect on coagulation factors (II, V, VIII, and XI) and coagulation times (PT, APTT, and TT), this appears to be very weak compared to UFH (positive control) and other drugs used in clinical practice. However, these results should not be misinterpreted. The study aimed not to prove that the leaf extract has a more potent anticoagulant effect than UFH but to demonstrate that the orchid contains compounds with this activity. Once we know the mechanism of action, the potency of the anticoagulant effect will be established. However, we must point out that the efficacy of an anticoagulant drug is not directly related to the magnitude of its inhibitory effect (May & Moll 2024). In fact, the current investigation looks for the development of new anticoagulants with specific mechanisms of action which increase their safety, namely, less bleeding side effects. Direct oral anticoagulants brought benefits to antithrombotic treatment because of their ease of use, favorable pharmacological profile, and lack of monitoring requirements. However, despite having a better safety profile than other anticoagulants, such as VKA, their bleeding risk persists (Pasqualotto *et al*. 2024). A new strategy to reduce the risk of bleeding is to act on the pre-

viously known intrinsic pathway of hemostasis, particularly FXI, to prevent thrombosis without harming hemostasis (Bentounes *et al*. 2023). It is known that patients with FXI deficiency have a reduced tendency toward thrombosis and bleeding, suggesting that FXI plays a crucial role in thrombosis but not in hemostasis (Barnes 2025). In other words, FXI inhibition could alter thrombus formation and growth without significantly affecting clot formation. This unique feature makes FXI inhibitors effective (less thrombosis) and safer (less bleeding) than current anticoagulants (Cave & Shah 2021). The extract under study in our research inhibited FXI, directly or indirectly. Of course, one important aim for our group is to identify the natural compound in the *L. furfuracea* extract acting as a FXI inhibitor. Therefore, regarding anticoagulant therapy, searching for a potential FXI inhibitor in the studied extract undoubtedly represents the beginning of a new line of research.

In an opposite scenario, we must highlight the likely presence of procoagulant compounds in the leaves of *L. furfuracea,* which could increase the activity of factors VII, IX, and XII. It is possible that the increased activity of these factors affected the overall anticoagulant effect of the extract. The procoagulant effect of the extract was most evident when measuring the activity of FVII, which increased up to 148 IU/dL at the highest concentration tested, probably reducing the anticoagulant effect of the extract on PT. Similarly, we assume that the increased activity of factors IX and XII decreased the anticoagulant effect of the extract on APTT, even though factors VIII and XI were strongly inhibited. We also assume that the lack of anticoagulant effect of the extract at the lowest concentrations tested (0.01 to 3.0 mg/mL) may be due to procoagulant compounds. In our previous study (López-Pérez *et al*. 2022), the results showed that hexane and chloroform partitions (1.0, 2.5, and 5.0 mg/mL) from the hydroethanolic extract

Table 2. Concentration-dependent effects of hydroethanolic extract of *Laelia furfuracea* leaves on PT, APTT, and TT

Extract (mg/mL)	PT (s)	APTT (s)	TT (s)
0	14.4 ± 0.4	33.7 ± 1.3	16.1 ± 0.6
0.01	14.2 ± 0.5	33.2 ± 0.4	16.3 ± 0.6
0.03	14.4 ± 0.8	33.3 ± 0.2	17.8 ± 0.3
0.06	14.0 ± 0.1	33.6 ± 0.2	17.0 ± 0.2
0.12	14.8 ± 0.7	33.8 ± 0.5	16.9 ± 0.1
0.25	15.1 ± 0.3	34.7 ± 0.4	17.3 ±0.2
0.50	15.4 ± 0.8	35.2 ± 0.4	17.4 ± 0.2
0.75	15.6 ± 0.4	37.2 ± 0.4	17.9 ± 0.1
1.0	15.7 ± 0.8	38.3 ± 0.3*	18.1 ± 0.2
1.5	16.2 ± 1.5	40.0 ± 2.4*	17.5 ± 0.6
2.0	17.0 ± 0.8	42.6 ± 1.9 *	17.8 ± 0.4
2.5	17.4 ± 2.2*	43.4 ± 2.6*	18.2 ± 0.5
3.0	18.2 ± 1.4*	43.9 ± 1.7*	18.5 ± 0.8*
3.5	18.4 ± 0.8*	44.4 ± 0.7*	19.0 ± 0.7*
4.0	23.4 ± 0.4*	58.9 ± 1.3*	23.0 ± 0.9*
4.5	26.3 ± 1.1*	66.3 ± 0.9*	25.9 ± 0.3*
5.0	30.3 ± 0.9*	75.7 ± 1.7*	28.8 ± 1.2*
7.0	43.5 ± 0.6*	133.9 ± 1.9*	35.5 ± 0.8*
10.0	87.1 ± 1.5*	205.2 ± 3.1*	83.4 ± 1.4*
15.0	141.0 ± 2.1*	298.1 ± 2.8*	137.9 ± 2.3*
UFH (0.01 mg/mL)	17.4 ± 1.3	293.7 ± 2.1*	20.6 ± 1.4*

Results are expressed as the mean ± SEM. PT, APTT, and TT are expressed in seconds (s). CT: clotting times. *: significant comparisons to the baseline determination (0 mg/mL); n = 10.

Table 3. Anticoagulant effects of the hydroethanolic extract of *Laelia furfuracea* leaves.

Factor	Hydroethanolic extract (mg/mL)						UFH (mg/mL)
	0	1.0	3.5	7.0	10.0	15.0	0.01
II	84 ± 6.7	80 ± 5.8	78 ± 9.0	63 ± 8.1*	42 ± 10.3*	31 ± 8.2*	52 ± 2.5*
V	78 ± 3.5	75 ± 4.8	74 ± 4.7	52 ± 5.1*	37 ± 4.3*	24 ± 4.6*	70 ± 3.7
VII	94 ± 3.2	109 ± 6.5	133 ± 10.2*	139 ± 8.6*	143 ± 5.8*	148 ± 5.6*	88 ± 2.5
VIII	89 ± 7.7	68 ± 5.6*	43 ± 6.1*	17 ± 4.6*	5 ± 5.3*	0.8 ± 0.7*	16 ± 4.2*
IX	96 ± 3.8	159 ± 4.6*	150 ± 4.8*	137 ± 5.2*	124 ± 4.7*	113 ± 4.9*	82 ± 3.8*
X	90 ± 3.6	87 ± 4.2	83 ± 3.8	89 ± 5.5	87 ± 2.7	88 ± 4.7	75 ± 4.1*
XI	75 ± 3.9	67 ± 5.1	44 ± 6.2*	16 ± 4.7*	7 ± 3.4*	2 ± 0.5*	12 ± 3.5*
XII	63 ± 4.4	152 ± 4.8*	144 ± 5.0*	140 ± 4.1*	117 ± 4.5 *	100 ± 5.1*	48 ± 4.3*

Results are expressed as the mean ± SEM. PT, APTT, and TT are expressed in seconds (s), while the activity of blood coagulation factors is expressed in international units/deciliter (IU/dL). Normal ranges for all factors are as follows: II (78 – 133), V (53 – 125), VII (62 – 123), VIII (51 – 209), IX (67 – 209), X (78 – 138), XI (51 – 158), and XII (36 – 159). CT: clotting times. *: significant comparisons to the baseline determination (0 mg/mL); n = 10.

Table 4. Concentration-dependent effects of hydroethanolic extract of *Laelia furfuracea* leaves on thrombin-, ADP-, and epinephrine-induced platelet aggregation.

Extract (mg/mL)	Thrombin	ADP	Epinephrine
	PA (%)	PA (%)	PA (%)
0	77.0 ± 3.4	84.8 ± 3.6	86.5 ± 3.3
0.01	62.2 ± 4.7*	82.5 ± 3.2	86.0 ± 2.2
0.03	45.7 ± 6.1*	84.5 ± 2.4	85.0 ± 2.5
0.06	33.2 ± 5.8*	78.0 ± 2.8	80.7 ± 2.5
0.12	23.3 ± 4.3*	67.5 ± 3.9*	74.6 ± 2.8*
0.25	14.7 ± 3.1*	59.0 ± 4.9*	69.5 ± 4.2*
0.50	8.0 ± 2.2*	15.5 ± 2.9*	51.0 ± 3.8*
0.75	4.5 ± 1.5*	13.0 ± 2.2*	37.0 ± 3.1*
1.0	2.0 ± 1.0*	9.3 ± 1.8*	16.7 ± 1.2*
ASA	52.0 ± 5.2*	66.2 ± 4.5*	22.5 ± 3.7*
Clopidogrel	68.5 ± 4.2	7.4 ± 3.4*	10.4 ± 3.1*

Results are expressed as mean ± SEM. ASA: acetylsalicylic acid; PA: platelet aggregation. *: significant comparisons about the baseline determination (0 mg/mL); n = 10.

of *L. furfuracea* were able to shorten PT and TT tests up to 5.0 s as compared to the basal condition, suggesting the presence of procoagulant compounds. Of course, all these hypotheses derived from our studies require to be clarified in subsequent investigations. Meanwhile, given the effect of the polarity of orchid extract, it seems mandatory to identify, isolate, and purify the procoagulant and anticoagulant compounds in the leaves of *L. furfuracea*. These efforts could elucidate the mechanism of action of the bioactive compounds and evaluate their *in vivo* effects.

Accordingly, a previous chemical analysis by UPLC-ESI-MS/MS-qTOF (López-Pérez *et al*. 2022) revealed that the extract of *L. furfuracea* contains terpene, and polyphenolic compounds, which protocatechuic acid is the only one with a known effect on the secondary hemostasis (Huang *et al*. 2010, Cao *et al*. 2017). However, this phenol acts as a procoagulant rather than an anticoagulant, and other orchid compounds may determine the anticoagulant activity. The fact that the orchid extract contains procoagulant compounds should not be surprising because, in addition to protocatechuic acid, other phenols also possess this activity, such as vanillic acid, p-coumaric acid, syringic acid, and astragalin

(Huang *et al*. 2010, Kakkar & Bais 2014, Li *et al*. 2020, Mu *et al*. 2023), polycephalum A (saponin) and polycephalum B (flavanone glycoside) of *Clinopodium polycephalum* (Vaniot) C.Y Wu & S. J. Hsuan have procoagulant properties by shortening the APTT and PT, respectively (Tao *et al*. 2023). Besides, a more recent study about integrated metabolomics and network pharmacology suggests that other natural compounds (naringenin, guanine, 2,4-di-tert-butylphenol, calycosin-7-O-beta-D-glucoside, flavone, vitexin, and tiliroside) may exert this activity (Yang *et al*. 2024). According to this study, vitexin another compound found in the extract of *L. furfuracea* may contribute to the procoagulant activity.

Regarding the antiplatelet effect, we showed that the extract contains compounds that inhibit platelet activation induced by thrombin, ADP, and epinephrine. The PRP from patients treated with ASA (COX-1 enzyme inhibitor) or clopidogrel (ADP receptor inhibitor) was used as positive controls for these assays. The epinephrine-induced platelet aggregation was mainly inhibited with ASA, while clopidogrel inhibited that with ADP. A different profile was seen when the extract was tested. This exerted an inhibitory effect almost on the same magnitude for the three agonists. The mechanism is unknown. However, we believe that the bioactive compounds in the leaves of *L. furfuracea* have a final common effect, decreasing the activation of the $\alpha_{IIb}\beta_3$ receptor required for platelet aggregation (Estevez & Du 2017). Unlike coagulation factors, protocatechuic acid might contribute to the antiplatelet effect of the orchid extract. Protocatechuic acid inhibits platelet aggregation through the decrease in the mobilization of intracellular calcium and granular secretion mediated by the interaction between von Willebrand factor and glycoprotein Iba on the platelets (Kim *et al*. 2012). Despite this, it should be highlighted that the influence of protocatechuic acid on the activity of the *L. furfuracea* extract is probably not very significant because the extract seems to contain only small amounts of this compound. Therefore, we do not rule out that other compounds in the extract exert antiplatelet activity.

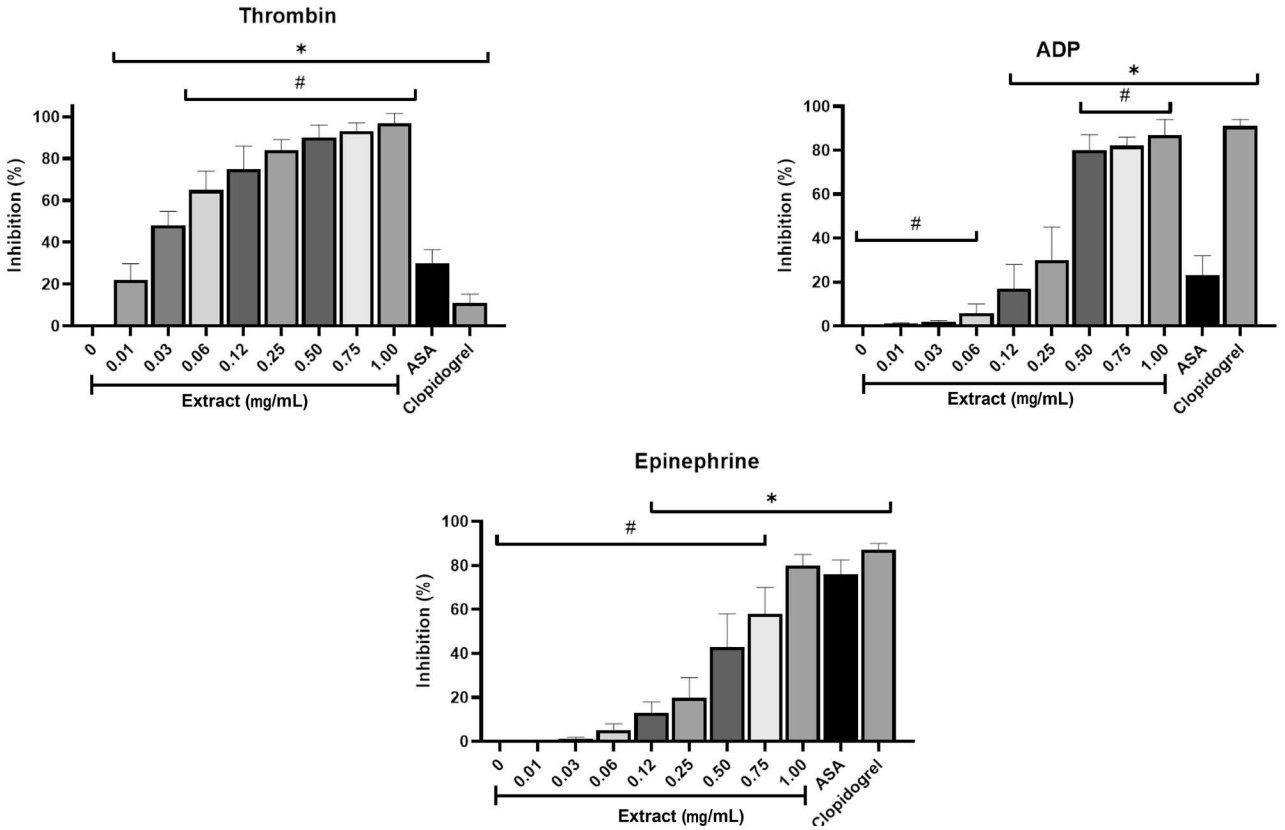

Figure 2. Inhibition of platelet aggregation by the hydroethanolic extract of *Laelia furfuracea* leaves. The results are expressed as mean ± SEM. For each experimental condition, samples from 10 patients with thrombosis were analyzed. For ASA, the percentage of inhibition for thrombin-, clopidogrel, the percentage of inhibition for ADP-, and epinephrine-induced platelet aggregation was 30.0 ± 6.4 %, 23.4 ± 9.0 %, and 76.2 ± 6.5 %, respectively. *Shows a significant difference between experimental conditions and basal determination (0 µg/mL). #: Significant difference between experimental conditions and ASA; n = 10.

As stated above, the study did not aim to compare the antiplatelet effect of the leaf extract versus current drugs or purified natural compounds. Although the concentration used seems high (0.01 to 1.0 mg/mL), they are not. In fact, other studies with orchid extracts have used concentrations in a similar range or even higher (Paik *et al*. 1995, Lin *et al*. 2006). Although the concentrations of the extracts did not appear cytotoxic for platelets, we evaluated the viability of these cells before performing the aggregation assays. It was found that under the experimental conditions of concentration (0.01 to 1.0 mg/mL) and incubation time (10 min) with the extract, the viability of platelets remained unchanged (data not shown).

In comparison to other orchid extracts and their effects, we found, for example, that the antiplatelet effect of the leaves extract of *L. furfuracea* is apparently more potent than *Gastrodia elata* Blume (Paik *et al*. 1995, Lin *et al*. 2006). The maximum concentration (2.4 mg/mL) of G2 fraction of *G. elata* decreased ADP-induced platelet aggregation from 49.74 ± 7.36 to 11.21 ± 6.47 % (Lin *et al*. 2006), while 1.0 mg/mL of the extract of *L. furfuracea* decreased platelet aggregation from 84.8 ± 3.6 to 9.3 ± 1.8 %. Moreover, the fractions II, III, and IV from the hydroethanolic extract of *G. elata* required a concentration of 1.0 mg/mL to exert an inhibitory effect on ADP-induced platelet aggregation (Paik *et al*. 1995), while 0.5 mg/mL of the extract of *L. furfuracea* inhibited platelet aggregation about 80 %. Notably, in these two previous studies, the inhibitory effect of *G. elata* fractions was achieved using higher concentrations than those used for the *L. furfuracea* extract. Nevertheless, we recognize that the antiplatelet potency of *L. furfuracea* extract is too low in comparison to the purified bioactive compounds in *G. elata* (Pyo *et al*. 2004) and other orchids with antiplatelet activity, such as *Dendrobium loddigesii* Rolfe., *D. densiflorum* Lindl., *D. longicornu* Lindl., *D. trigonopus* Rchb.f., *D. venustum* Teijsm. & Binn., *Calanthe arisanensis* Hayata, *Cephalanceropsis gracilis* (Lindl.) S.Y.Hu., and *Ephemerantha lonchophylla* (Hook. f.) P.F. Hunt & Summerh. (Chen *et al*. 1994, 2000, Fan *et al*. 2001, Hu *et al*. 2008a,b, Lee *et al*. 2014, Sharma *et al*. 2018, Swe *et al*. 2021) that exert their effects at the range of micromolar concentration.

On the other hand, the possibility of finding an extract with a dual inhibitory effect on hemostasis (anticoagulant and antiplatelet) was demonstrated, but not as we expected. Although the extract showed both effects, we assume that different compounds exert these because platelet aggregation was inhibited at concentrations less than 1.0 mg/mL, while the anticoagulant effect required more than 1.0 mg/mL. Although the extract did not show the expected dual activity, it is still possible that the anticoagulant bioactive compounds in the orchid also behave as antiplatelet agents. This could become very relevant for the treatment of thrombosis because the anticoagulant and antiplatelet effects might benefit patients with acute coronary syndrome, atrial fibrillation, or transient cerebral ischemia in whom the combined use of anticoagulants and antiplatelet drugs is simultaneously indicated (Espinola-Klein 2022). To date, no drug in medical practice has both effects. However, the significance of these findings depends on identifying the orchid bioactive compounds.

In conclusion, this study demonstrated for the first time that the hydroethanolic extract of *L. furfuracea* leaves inhibits *in vitro* coagulation factors II, V, VIII, and XI as well as a significant inhibition of platelet aggregation induced by thrombin, ADP, and epinephrine. In addition, the results suggest that some compounds in the extract may also act as procoagulants. The polarity of these findings makes the identification of the bioactive compounds unavoidable.

Acknowledgments

The authors thank the Santo Domingo Yanhuitlán, Oaxaca communal authority for donating the plant material of orchid *L. furfuracea*. The authors especially thank the reviewers and the editor section for their feedback and comments.

Literature cited

Apipongrat D, Police P, Lamool R, Butthep P, Chantkran W. 2022. Validation of high concentrated thrombin time assay for unfractionated heparin monitoring. *Journal of Clinical Laboratory Analysis* **36**: e24695. DOI: https://doi.org/10.1002/jcla.24695

Barnes GD. 2025. New targets for antithrombotic medications: seeking to decouple thrombosis from hemostasis. *Journal of Thrombosis and Haemostasis* **23**: 1146-1159. DOI: https://doi.org/10.1016/j.jtha.2024.12.003

Bentounes NK, Melicine S, Martin AC, Smadja DM, Gendron N. 2023. Development of new anticoagulant in 2023: Prime time for anti-factor XI and XIa inhibitors. *Journal de Medecine Vasculaire* **48**: 69-80. DOI: https://doi.org/10.1016/j.jdmv.2023.04.002

Bijak M, Ponczek MB, Nowak P. 2014a. Polyphenol compounds belonging to flavonoids inhibit activity of coagulation factor X. *International Journal of Biological Macromolecules* **65**: 129-135. DOI: https://doi.org/10.1016/j.ijbiomac.2014.01.023

Bijak M, Ziewiecki R, Saluk J, Ponczek M, Pawlaczyk I, Krotkiewski H, Wachowicz B, Nowak P. 2014b. Thrombin inhibitory activity of some polyphenolic compounds. *Medicinal Chemistry Research* **23**: 2324-2337. DOI: https://doi.org/10.1007/s00044-013-0829-4

Cao P, Xi P, Wang X, Wang J, Wei J, Kang WY. 2017. Chemical constituents and coagulation activity of *Agastache rugosa*. *BMC Complementary and Alternative Medicine* **17**: 93-98. DOI: https://doi.org/10.1186/s12906-017-1592-8

Cave BE, Shah SP. 2021. Turning up to eleven: Factor XI inhibitors as novel agents to maximize safety and maintain efficacy in thromboembolic disease. *Current Problems in Cardiology* **46**: 100696. DOI: https://doi.org/10.1016/j.cpcardiol.2020.100696

Chen CC, Huang YL, Teng CM. 2000. Antiplatelet aggregation principles from *Ephemerantha lonchophylla*. *Planta Medica* **66**: 372-374. DOI: https://doi.org/10.1055/s-2000-8553

Chen CC, Wu LG, Ko FN, Teng CM. 1994. Antiplatelet aggregation principles of *Dendrobium loddigesii*. *Journal of Natural Products* **57**: 1271-1274. DOI: https://doi.org/10.1021/np50111a014

Choi JH, Kim KJ, Kim S. 2016. Comparative Effect of Quercetin and Quercetin-3-O-β-d-Glucoside on Fibrin Polymers, Blood Clots, and in Rodent Models. *Journal of Biochemical and Molecular Toxicology* **30:** 548-558. DOI: https://doi.org/10.1002/jbt.21822

Choi SH, Smith SA, Morrissey JH. 2011. Polyphosphate is a cofactor for the activation of factor XI by thrombin. *Blood* **118**: 6963-6970. DOI: https://doi.org/10.1182/blood-2011-07-368811

Ding CS, Shen YS, Li G, Wei Z, Wei F. 2007. Study of a glycoprotein from *Gastrodia elata*: its effects of anticoagulation and antithrombosis. *Zhongguo Zhong Yao Za Zhi* **32**: 1060-4.

Dunois C. 2021. Laboratory monitoring of direct oral anticoagulants (DOACs). *Biomedicines* **9**: 445. DOI https://doi.org/10.3390/biomedicines9050445

Espinola-Klein C. 2022. When and how to combine antiplatelet and anticoagulant drugs? *Hamostaseologie* **42**: 73-79. DOI: https://doi.org/10.1055/a-1724-4922

Estevez B, Du X. 2017. New concepts and mechanisms of platelet activation signaling. *Physiology (Bethesda)* **32:** 162-177. DOI: https://doi.org/10.1152/physiol.00020.2016

Fan C, Wang W, Wang Y, Qin G, Zhao W. 2001. Chemical constituents from *Dendrobium densiflorum*. *Phytochemistry* **57**: 1255-1258. DOI: https://doi.org/10.1016/s0031-9422(01)00168-6

Gómez-Rosas P, Sosa-Velázquez MÁ, Yáñez-Funes LA, González-Trejo JJ, Majluf-Cruz K, Isordia-Salas I, Ignacio-Ibarra G, Almaraz-Delgado M, Moreno-Ruiz LA, Valencia-Chávez JS, Hernández-Juárez J, Vázquez-Rubiel D, Domínguez-Reyes V, Arreola-Díaz R, García-Lee MT, Vela-Ojeda J, Majluf-Cruz A. 2021. Analysis of the quality of anticoagulation with vitamin K antagonists in three clinical scenarios in Mexico. Análisis de la calidad de la anticoagulación con antagonistas de la vitamina K en tres escenarios clínicos en México. *Gaceta Médica de México* **157**: 284-292. DOI: https://doi.org/10.24875/GMM.M21000559

Hand KR, Hale GM. 2021. Antithrombotic therapy in peripheral artery disease: Stepping in the right direction. *American Journal of Cardiovascular Drugs* **21**: 523-534. DOI: https://doi.org/10.1007/s40256-021-00465-x

Hoffman M. 2003. A cell-based model of coagulation and the role of factor VIIa. *Blood Reviews* **17**: S1-S5. DOI: https://doi.org/10.1016/s0268-960x(03)90000-2

Hu JM, Chen JJ, Yu H, Zhao YX, Zhou J. 2008a. Five new compounds from *Dendrobium longicornu*. *Planta Medica* **74**: 535-539.DOI: https://doi.org/10.1055/s-2008-1074492

Hu JM, Chen JJ, Yu H, Zhao YX, Zhou J. 2008b. Two novel bibenzyls from *Dendrobium trigonopus*. *Journal of Asian Natural Products Research* **10**: 653-657. DOI: https://doi.org/10.1080/10286020802133605

Huang L, Lin C, Li A, Wei B, Teng J, Li L. 2010. Pro-coagulant activity of phenolic acids isolated from *Blumea riparia*. *Natural Product Communications* **5**: 1263-1266.

Huseynov A, Reinhardt J, Chandra L, Dürschmied D, Langer HF. 2023. Novel aspects targeting platelets in atherosclerotic cardiovascular disease- A Translational Perspective. *International Journal of Molecular Sciences* **24**: 6280. DOI: https://doi.org/10.3390/ijms24076280

Jeon JS, Kim J, Park S, Ryou C, Kim CY. 2016. Preparative purification of plasmin activity stimulating phenolic derivatives from *Gastrodia elata* using centrifugal partition chromatography. *Biomedical Chromatography* **30**: 976-982. DOI: https://doi.org/10.1002/bmc.3640

Kakkar S, Bais S. 2014. A review on protocatechuic Acid and its pharmacological potential. *ISRN Pharmacology* **2014***:* 952943. DOI: https://doi.org/10.1155/2014/952943

Kim K, Bae ON, Lim KM, Noh JY, Kang S, Chung KY, Chung JH. 2012. Novel antiplatelet activity of protocatechuic acid through the inhibition of high shear stress-induced platelet aggregation. *The Journal of Pharmacology and Experimental Therapeutics* **343**: 704-711. DOI: https://doi.org/10.1124/jpet.112.198242

Lamponi S, Baratto MC, Miraldi E, Baini G, Biagi M. 2021. Chemical Profile, Antioxidant, Anti-Proliferative, Anticoagulant and Mutagenic Effects of a Hydroalcoholic Extract of Tuscan *Rosmarinus officinalis*. *Plants (Basel)* **10:** 97. DOI: https://doi.org/10.3390/plants10010097

Lee CL, Yen MH, Chang FR, Wu CC, Wu YC. 2014. Antiplatelet aggregation effects of phenanthrenes from *Calanthe arisanensis*. *Natural Product Communications* **9**: 83-84.

Li C, Hu M, Jiang S, Liang Z, Wang J, Liu Z, Wang HMD, Kang W. 2020. Evaluation Procoagulant Activity and Mechanism of Astragalin. *Molecules* **25:** 177. DOI: https://doi.org/10.3390/molecules25010177

Lin Q, Li XF, Li WJ, Dai R, He XS. 2006. Antiaggregation effects of fractions from Gastrodia rhizome. *Journal of Chinese Microcirculation* **10**: 33-35.

Li M, Li J, Wang X, Hui X, Wang Q, Xie S, Yan P, Tian J, Li J, Xie P, Yang K, Yao L. 2023. Oral direct thrombin inhibitors or oral factor Xa inhibitors versus conventional anticoagulants for the treatment of pulmonary embolism. *The Cochrane Database of Systematic Reviews* **4**: CD010957. DOI: https://doi.org/10.1002/14651858.CD010957.pub3

López-Pérez A, Hernández-Juárez J, Solano R, Majluf-Cruz A, Hernández-Cruz PA, Lagunez-Rivera L. 2022. *Laelia furfuracea* Lindl.: an Endemic Mexican Orchid with Anticoagulant Activity. *Journal of Mexican Chemical Society* **66**: 1-16. DOI: https://doi.org/10.29356/jmcs.v66i1.1588

Mann KG. 2011. Thrombin generation in hemorrhage control and vascular occlusion. *Circulation* **124**: 225-235. DOI: https://doi.org/10.1161/CIRCULATIONAHA.110.952648

Margetić S, Goreta SŠ, Ćelap I, Razum M. 2022. Direct oral anticoagulants (DOACs): From the laboratory point of view. *Acta Pharmaceutica* **72**: 459-482. DOI: https://doi.org/10.2478/acph-2022-0034

May JE, Moll S. 2024. How I treat the co-occurrence of venous and arterial thromboembolism: anticoagulation, antiplatelet therapy, or both? *Blood*. **6**: 2351-2362. DOI: https://doi.org/10.1182/blood.2023021638

Mu K, Liu Y, Liu G, Ran F, Zhou L, Wu Y, Peng L, Shao M, Li C, Zhang Y. 2023. A review of hemostatic chemical components and their mechanisms in traditional Chinese medicine and ethnic medicine. *Journal of Ethnopharmacology* **307:** 116200. DOI: https://doi.org/10.1016/j.jep.2023.116200

Olas B, Żuchowski J, Lis B, Skalski B, Kontek B, Grabarczyk Ł, Stochmal A. 2018. Comparative chemical composition, antioxidant and anticoagulant properties of phenolic fraction (a rich in non-acylated and acylated flavonoids and non-polar compounds) and non-polar fraction from *Elaeagnus rhamnoides* (L.) A. Nelson fruits. *Food Chemistry* **247**: 39-45. DOI: https://doi.org/10.1016/j.foodchem.2017.12.010

Paik YS, Song JK, Yoon CH, Chung KS, Yun-Choi HS. 1995. Anti-platelet and anti-thrombotic effects of *Gastrodia elata*. *Korean Journal of Pharmacognosy* **26**: 385-389.

Pasqualotto E, Gewehr DM, Ferreira ROM, Chavez MP, Silva CH, Cruz SA, Limachi-Choque J, Park A, Coutinho

MSSA, Kubrusly LF. 2024. Direct oral anticoagulants versus Vitamin K antagonists for left ventricular thrombus: A Meta-Analysis with trial sequential analysis. *Arquivos Brasileiros de Cardiologia* **121**: e20230738. DOI: https://doi.org/10.36660/abc.20230738

Pawlaczyk I, Czerchawski L, Pilecki W, Lamer-Zarawska L, Gancarz R. 2009. Polyphenolic-polysaccharide compounds from selected medicinal plants of Asteraceae and Rosaceae families: Chemical characterization and blood anticoagulant activity. *Carbohydrate Polymers* **77**: 568-575. DOI: https://doi.org/10.1016/j.carbpol.2009.01.030

Pawlaczyk I, Czerchawski L, Kuliczkowski W, Karolko B, Pilecki W, Witkiewicz W, Gancarz R. 2011. Anticoagulant and anti-platelet activity of polyphenolic-polysaccharide preparation isolated from the medicinal plant *Erigeron canadensis* L. *Thrombosis Research* **127**: 328-340. DOI: https://doi.org/10.1016/j.thromres.2010.11.031

Pawlaczyk-Graja I, Balicki S, Wilk KA. 2019. Effect of various extraction methods on the structure of polyphenolic-polysaccharide conjugates from *Fragaria vesca* L. leaf. *International Journal of Biological Macromolecules* **130**: 664-674. DOI: https://doi.org/10.1016/j.ijbiomac.2019.03.013

Pawlaczyk-Graja I, Balicki S, Ziewiecki R, Matulová M, Capek P, Gancarz R. 2016. Polyphenolic-polysaccharide conjugates of *Sanguisorba officinalis* L. with anticoagulant activity mediated mainly by heparin cofactor II. *International Journal of Biological Macromolecules* **93**: 1019-1029. DOI: https://doi.org/10.1016/j.ijbiomac.2016.09.082

Pollack CV Jr, Peacock WF, Bernstein RA, Clark CL, Douketis J, Fermann GJ, Fiore GJ, Frost A, Jahromi B, Johnson C, Merli G, Silber S, Villines TC, Fanikos J; Hospital Quality Foundation. 2020. The safety of oral anticoagulants registry (SOAR): A national, ED-based study of the evaluation and management of bleeding and bleeding concerns due to the use of oral anticoagulants. *The American Journal of Emergency Medicine* **38:** 1163-1170. DOI: https://doi.org/10.1016/j.ajem.2019.12.023

Pyo MK, Jin JL, Koo YK, Yun-Choi HS. 2004. Phenolic and furan type compounds isolated from *Gastrodia elata* and their anti-platelet effects. *Archives of Pharmacal Research* **27**: 381-385. DOI: https://doi.org/10.1007/BF02980077

Rahman M, Surag AT, Begum R, Hossain MS, Huda MK. 2022. Phytochemical, antioxidant, anti-inflammatory, and thrombolytic properties of *Cleisomeria lanatum* (Lindl.) Lindl. ex G. Don. *Scientifica* **26**: 5660527. DOI: https://doi.org/10.1155/2022/5660527

Raskob GE, Angchaisuksiri P, Blanco AN, Buller H, Gallus A, Hunt BJ, Hylek EM, Kakkar A, Konstantinides SV, McCumber M, Ozaki Y, Wendelboe A, Weitz JI. 2014. Thrombosis: a major contributor to global disease burden. *Arteriosclerosis, Thrombosis, and Vascular Biology* **34**: 2363-2371. DOI: https://doi.org/10.1161/ATVBAHA.114.304488

Rivera-Caravaca JM, Camelo-Castillo A, Ramírez-Macías I, Gil-Pérez P, López-García C, Esteve-Pastor MA, Orenes-Piñero E, Tello-Montoliu A, Marín F. 2021. Antithrombotic therapy in patients with peripheral artery disease: A focused review on oral anticoagulation. *International Journal of Molecular Sciences* **22**: 7113. DOI: https://doi.org/10.3390/ijms22137113

Sadeghi M, Safaeian L, Ghazvini MA, Ramezani M. 2017. Evaluation of fibrinolytic and antioxidant effects of *Allium affine* hydroalcoholic extract. *Research in Pharmaceutical Sciences* **12**: 299-306. DOI: https://doi.org/10.4103/1735-5362.212047

Sharma V, Jaiswal PK, Kumar K, Saran M, Mathur M, Swami AK, Chaudhary S. 2018. An efficient synthesis and biological evaluation of novel analogues of natural product Cephalandole A: A new class of antimicrobial and antiplatelet agents. *Fitoterapia* **129**: 13-19. DOI: https://doi.org/10.1016/j.fitote.2018.06.003

Shi ZH, Li NG, Tang YP, Wei-Li, Lian-Yin, Yang JP, Hao-Tang, Duan JA. 2012. Metabolism-based synthesis, biologic evaluation and SARs analysis of O-methylated analogs of quercetin as thrombin inhibitors. *European Journal of Medicinal Chemistry* **54**: 210-222. DOI: https://doi.org/10.1016/j.ejmech.2012.04.044

Silva PEDCE, Barros RC, Albuquerque WWC, Brandão RMP, Bezerra RP, Porto ALF. 2018. In vitro thrombolytic activity of a purified fibrinolytic enzyme from *Chlorella vulgaris*. *Journal of Chromatography*. **1092**: 524-529. DOI: https://doi.org/10.1016/j.jchromb.2018.04.040

Singh MK, Rajagopalan A, Tanimu H, Sukumaran BO. 2021. Purification, characterization and fibrino(geno)lytic ac-

tivity of cysteine protease from *Tabernaemontana divaricata* latex. *Biotech* **11**: 106. DOI: https://doi.org/10.1007/s13205-021-02643-9

Swe HN, Sritularak B, Rojnuckarin P, Luechapudiporn R. 2021. Inhibitory mechanisms of lusianthridin on human platelet aggregation. *International Journal of Molecular Sciences* **22**: 6846. DOI: https://doi.org/10.3390/ijms22136846

Tao M, Liu Y, Zhou X, Yu X, Zhang Y, Guo L, Meng D. 2023. Polycephalums A and B, new anticoagulant constituents isolated from *Clinopodium polycephalum*. *Chemistry Biodiversity* **20:** e202300448. DOI: https://doi.org/10.1002/cbdv.202300448

van der Hulle T, Kooiman J, den Exter PL, Dekkers OM, Klok FA, Huisman MV. 2014. Effectiveness and safety of novel oral anticoagulants as compared with vitamin K antagonists in the treatment of acute symptomatic venous thromboembolism: a systematic review and meta-analysis. *Journal of Thrombosis and Haemostasia* **12**: 320-328. DOI: https://doi.org/10.1111/jth.12485

Wendelboe AM, Raskob GE. 2016. Global burden of thrombosis: Epidemiologic aspects. *Circulation Research* **118**: 1340-1347. DOI: https://doi.org/10.1161/CIRCRESAHA.115.306841

Yang X, Wang Y, Gong S, Xiong T, Xie L. 2024. Integrated metabolomics and network pharmacology reveal the procoagulant mechanisms of *Cirsium setosum* extracts. *Journal of Chromatography. B, Analytical Technologies in the Biomedical and Life Sciences* **1247:** 124335. DOI: https://doi.org/10.1016/j.jchromb.2024.124335

Yong J, Toh CH. 2023. Rethinking coagulation: from enzymatic cascade and cell-based reactions to a convergent model involving innate immune activation. *Blood* **142**: 2133-2145. DOI: https://doi.org/10.1182/blood.2023021166

Yoon SJ, Pereira MS, Pavão MS, Hwang JK, Pyun YR, Mourão PA. 2002. The medicinal plant *Porana volubilis* contains polysaccharides with anticoagulant activity mediated by heparin cofactor II. *Thrombosis Research* **106**: 51-58. DOI: https://doi.org/10.1016/s0049-3848(02)00071-3

Associate editor: Elihú Bautista

Author's contribution: LLR and RS designed the experiments, analyzed the results, and reviewed the article writing. BFR, EGS, and GSBZ analyzed the results and reviewed the article writing. AMC selected the patients, designed the experiments, analyzed the results, and reviewed the article writing. ALP and JHJ designed and carried out the experiments, analyzed the results, wrote, and reviewed the article writing.

Supporting agencies: The financial support provided by Consejo Nacional de Ciencia y Tecnología (CONACYT, project 270428) and Instituto Politécnico Nacional (IPN, projects SIP 2016-RE/50, SIP-20161270, SIP-20170263, SIP-20180382, SIP-20195897, SIP-20240944). The postdoctoral fellowship from SECIHTI to the first author.

Conflict of interests: The authors declare that there is no conflict of interest, financial or personal, in the information, presentation of data, and results of this article.