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In vitro Anticoagulant Activity of Leaf Essential Oil of *Artemisia absinthium* L. on Human Blood

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Abstract. The blood coagulation is a fast and efficient process that generates clot formation, which requires regulation. The blood coagulation derangement is a characteristic of many disease conditions. This study investigated the in vitro effects of leaf essential oil extract of *Artemisia absinthium* L. on blood coagulation. Essential oil was obtained by hydrodistillation. Then the phytochemical characterization of the essential oil was done by gas chromatography-mass spectrometry (GC-MS). Varying concentrations (1/10–1/40 v/v) of the essential oil were tested in vitro on blood coagulation profile; Quick time (QT) and Cephalin kaolin time (CKT). The leaf essential oil was characterized by a phytochemical profile dominated by β -thujene and chamazulene, among other monoterpenic and sesquiterpenic constituents. The essential oil significantly prolonged both Quick time (QT)

and Cephalin kaolin time (CKT). The leaf essential oil was characterized by a phytochemical profile dominated by β -thujene and chamazulene, among other monoterpenic and sesquiterpenic constituents. The essential oil significantly prolonged both Quick time (QT) and Cephalin-kaolin time (CKT), indicating a marked interference with both extrinsic and intrinsic coagulation pathways. These findings suggest a broad anticoagulant effect, likely mediated through the combined action of bioactive terpenoids affecting platelet function, coagulation cascade enzymes, and/or membrane-associated signaling processes. Overall, the essential oil contains bioactive compounds with potential anticoagulant properties, supporting its possible therapeutic relevance in the management of blood coagulation disorders.

Resumen. La coagulación sanguínea es un proceso rápido y eficiente de formación de coágulos, el cual requiere regulación. El desorden en la coagulación es una característica de muchas enfermedades. El presente estudio investigó los efectos in vitro del aceite esencial de las hojas de *Artemisia absinthium* L. El aceite esencial fue obtenido por hidrodestilación y la caracterización química fue realizada por cromatografía de gases – espectrometría de masas (CG-EM). Se probaron varias concentraciones (1/10–1/40 v/v) del aceite esencial in vitro en el perfil de la coagulación sanguínea por tiempo de respuesta rápida (QT) y por tiempo de cefalina con kaolina (CKT). El aceite esencial de las hojas se caracterizó por un perfil fitoquímico dominado por β -tujeno y chamazuleno, entre otros constituyentes monoterpénicos y sesquiterpénicos. El aceite esencial prolongó significativamente los tiempos QT y CKT, indicando una interferencia marcada con las rutas de coagulación intrínsecas y extrínsecas. Estos hallazgos indican un efecto anticoagulante amplio, mediado posiblemente por la acción combinada de terpenoides bioactivos que afectan a la función de las plaquetas, a las enzimas de la cascada de coagulación, y/o a procesos de señalización asociados con la membrana. En general, el aceite esencial contiene principios bioactivos con propiedades anticoagulantes, lo cual da cuenta de su posible relevancia terapéutica en el manejo de los desórdenes de coagulación sanguínea.

Introduction

Thrombosis, defined as the formation of blood clots within blood vessels, is a major pathological process underlying several life-threatening conditions, including pulmonary embolism, deep vein thrombosis, and cardiovascular diseases, leading to significant global mortality [1]. Recent clinical evidence has also highlighted that patients with coronavirus disease 2019 (COVID-19) frequently develop a hypercoagulable state associated with an increased risk of venous thrombotic events [2,3]. Although conventional antithrombotic agents such as heparin, aspirin, warfarin, and vitamin K antagonists are widely used in clinical practice, their therapeutic application is often limited by severe adverse effects, including bleeding complications, hematological disorders, gastrointestinal disturbances, and drug interactions [4-6]. Therefore, the development of safer and effective alternative antithrombotic agents remains an important research priority [7]. In this context, medicinal plants represent a valuable source of bioactive compounds with potential antithrombotic properties [8]. Several studies have reported that plant-derived secondary metabolites, including flavonoids, coumarins, alkaloids, phenylpropanoids, and terpenoids [9-11], exhibit antiplatelet, fibrinolytic, and anticoagulant activities through modulation of key pathways involved in hemostasis [10,12]. These findings provide a chemical–biological basis supporting the investigation of natural products as potential therapeutic agents.

Artemisia absinthium L. (*A. absinthium*) is a medicinal plant belonging to the family Asteraceae, widely used in traditional medicine for the treatment of gastrointestinal disorders, hepatic diseases, neurocognitive impairments (memory enhancement), osteoarthritis and joint pain, parasitic infections, inflammatory conditions, and hypertension [13]. In addition to these ethnomedicinal uses, the plant has been extensively investigated for its pharmacological properties, particularly its antioxidant activity [14,15], which is mainly attributed to its essential oil and various solvent extracts [16]. Phytochemical studies have identified a rich profile of secondary metabolites, including flavonoids, coumarins, and terpenoid compounds [13], which are known to contribute to its anti-inflammatory [17-19], hepatoprotective [14], and analgesic effects. Although antioxidant, anti-inflammatory, hepatoprotective, and analgesic properties may, in a theoretical manner,

contribute indirectly to hemostatic regulation [20-22], the direct anticoagulant activities of *A. absinthium* remain insufficiently studied.

The present study was therefore designed to evaluate the anticoagulant activity of its essential oil extract, providing preliminary data to guide further investigations into natural products for the treatment and prevention of thrombosis and cardiovascular diseases.

Experimental

Plant materials

The leaf of *A. absinthium* growth stage was collected before the flowering of the plant in March 2025, from Keddara in the Boudouaou region, Boumerdes, Algeria. The leaf was authenticated by a voucher specimen available in the herbarium of the Department of Botany, National Superior School of Agronomy (E.N.S.A.) El Harrach, Algeria.

Hydrodistillation of the essential oil

A hundred grams of powdered *A. absinthium* was distilled during 3 hours by hydro-distillation, using a steam distillation apparatus fitted to a 1000 mL flask, with 500 mL of water as distillation liquid. The distillate was collected in a Separatory funnel. The extraction yield of essential oil was calculated after determining the weight of the oil as % w/w [23]. The oil sample was kept at -20 °C until it was analyzed.

Density of *A. absinthium* essential oil

The density of *A. absinthium* essential oil is an important physicochemical metric for determining its identity and quality.

The density of each oil varies, but it is lower than the density of water, hence the oil floats above it.

The density of an essential oil is a physical attribute that corresponds to the ratio of its specific mass to that of water and is typically measured at a reference temperature (20 °C). It specifies whether the oil is lighter or heavier than water; a density less than one means the oil floats, whilst a density larger than one means it sinks. This parameter is crucial for the identification, quality control, and characterisation of essential oils since it depends on their chemical makeup [23].

The formula for an essential oil's density is as follows:

where:

d = density

ρ = mass density of the essential oil

ρ_w = mass density of water ($\approx 1 \text{ g/cm}^3$ at 20 °C)

It can also be expressed using mass and volume: $\rho = m/v$

$$\text{So: } d = \frac{(m/V)/(\text{oil})}{m/V(\text{water})}$$

In practice, since the density of water is close to 1 g/cm³, the density of an essential oil is numerically close to its mass density expressed in g/cm³.

Determination of the chemical composition of essential oil by GCMS

The chemical composition of the essential oil was determined by gas chromatography coupled with mass spectrometry (GC–MS) using an Agilent 7890B gas chromatograph equipped with electronic pressure control and coupled to an Agilent 5977 mass selective detector. Electron impact ionization was performed at 70 eV. Separation was achieved on an HP-5MS capillary column (30 m × 0.25 mm i.d., 0.25 µm film thickness). The injection was performed in splitless mode (split ratio 1:50), with the injector temperature set at 250 °C. The oven temperature program was set from 60 °C to 250 °C at a rate of 3 °C min⁻¹. Helium was used as the carrier gas at a constant flow rate of 1.0 mL min⁻¹ [24]. Compound identification was based on comparison of their mass spectra with those in the NIST98 library.

Determination of total polyphenol content

The total phenolic amount in the essential oils was quantified spectrophotometrically according to the Folin–Ciocalteu method described by Singleton and Rossi (1965) [25]. Briefly, 100 μ L extract (1000 mg/L) was added with diluted Folin–Ciocalteu reagent (1/10, 500 μ L). After a 1-minute reaction, sodium carbonate (Na_2CO_3) (20 %, 2 mL) was added to each tube, then the tubes were vortexed and incubated for 30 min at room temperature. The absorbance was measured at 760 nm using an ultraviolet-visible spectrophotometer. The analysis was performed in triplicate. The standard curve was prepared using 0.015–0.25 mg/mL solutions of gallic acid in methanol. Total phenol values were expressed as gallic acid equivalents (mg GA/g of essential oil), which is a common reference compound for phenolic compounds.

Anticoagulation assay

The anticoagulant activity was evaluated by the Cephalin kaolin time (CKT) and the Quick time (QT), commonly known as the Prothrombin time (PT). When CKT allows exploring the activity of the factors VIII, IX, XI, and XII of the intrinsic pathway. Then the PT allows exploring the activity of the factors V, VII, and X, II of the extrinsic path.

The CKT tests are carried out according to the method of Mourao et al. (1996) [26]. CKT is measured as follows 90 μ L of normal citrated human plasma sample to which a 10 μ L of the *A. absinthium* essential oil dilution is added, after incubation at 37 °C (for 1mn), 100 μ L of the preheated cephalin Kaolin reagent is added and then incubated at 37 °C (for 2 mn).100 μ L calcium chloride at 0.25mol / l is added and the clot formation time is recorded thanks to a coagulometer Bio Solea 2.

The QT tests are carried out according to the method of Qi et al. (2012) [27]. QT is measured as follows: 90 μ L of normal citrated human plasma is mixed with 10 μ L of *A. absinthium* essential oil dilution, then incubated at 37 °C (for one minute), and 200 μ L of an activator pre-incubated at 37 °C. for 10 minutes is added, and the coagulation time is recorded thanks to a coagulometer Bio Solea 2.

Statistical analysis

Data are expressed as mean $\pm$ standard deviation (S. D). Statistical analysis involved a one-way analysis of variance (ANOVA). A value of P less than 0.05 ($p < 0.05$) was considered statistically significant.

Results

Yield and chemical composition

The hydrodistillation extractions give a dark greenish-blue essential oil with a very strong and persistent aromatic odor and a greasy appearance. The yield of essential oil from 5 extractions is 0.3 % $\pm$ 0.07.

The essential oil's relative density at 20 °C is 0.922 $\pm$ 0003. The average score suggests a chemical composition compatible with an essential oil high in light volatile molecules. The predominant presence of monoterpenic ketones, particularly thujones, as well as hydrocarbon monoterpenes, contributes to a lower density than water.

This characteristic refers to the chemical composition of the oil, which is influenced by a variety of parameters such as phenotype, harvest time, soil type, storage, processing, and extraction conditions [28].

These findings are consistent with the literature, which suggests that the density of this essential oil ranges between 0.91 and 0.94 at 20 °C. This agreement implies that the investigated oil has physicochemical properties consistent with generally acknowledged criteria [29].

The constituents of the essential oils were identified based on their retention times and mass spectra in comparison with the literature data and the laboratory personnel's library. The result of the chemical analysis of essential oils is shown in Table 1 and Fig 1.

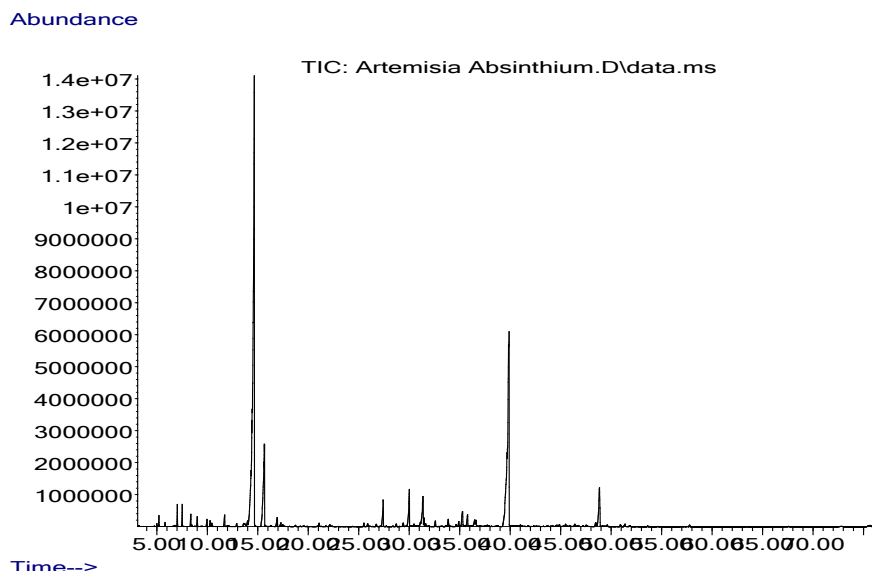


Fig 1. Chromatogram of *Artemisia absinthium*. L essential oil.

The identification of the volatile constituents was further supported by the calculation of Kovats retention indices (IK) using linear interpolation relative to a homologous series of n-alkanes analyzed under identical chromatographic conditions. The calculated IK values are presented in Table 1 and showed good agreement with those reported in the NIST database and relevant literature, confirming the reliability of compound identification.

Table 1. GC–MS profiling of *A. absinthium* essential oil.

Peak	Rt (min)	Compound	Area (%)	IK _{exp}	IK _{NIST}
1	4.754	1,4-Octadiene	0.011	858	810
2	4.993	Ethylbenzene	0.084	873	870
3	5.205	p-Xylene	0.332	888	880
4	7.030	α -Pinene	0.579	932	939
5	7.515	Camphene	0.601	949	954
6	8.366	Sabinene	0.438	971	969
7	9.007	Myrcene	0.377	988	991
8	9.969	α -Terpinene	0.311	1003	1014
9	10.277	p-Cymene	0.221	1024	1020
10	10.445	Limonene	0.149	1028	1024
11	11.714	γ -Terpinene	0.519	1052	1059
12	14.654	β -Thujone	46.568	1112	1115

Peak	Rt (min)	Compound	Area (%)	IK _{exp}	IK _{NIST}
13	15.663	Terpinen-4-ol	8.331	1126	1174
14	16.930	Terpineol	0.558	1145	1186
15	20.915	Perillal	0.016	1195	1265
16	22.128	Perillol	0.102	1210	1370
17	25.509	Germacrene D	0.164	1385	1495
18	25.878	Bourbonene	0.152	1392	1395
19	26.739	Methyl eugenol	0.128	1408	1410
20	27.416	Caryophyllene	0.481	1416	1417
21	30.008	Germacrene	2.355	1478	1480
22	31.366	δ -Cadinene	2.396	1515	1522
23	32.587	Elemol	0.283	1540	1548
24	33.866	Trifluoroacetyl lavandulol	0.428	1555	1400
25	34.625	Geranyl acetate	0.112	1575	1385
26	35.779	3,6-Dihydrochamazulene	0.692	1590	1520
27	36.483	β -Eudesmol	0.794	1651	1660
28	39.889	Chamazulene	26.205	1718	1740
29	48.846	p-Cymene (late trace)	3.466	1829	1025

The essential oil of Absinthe is a monoterpene type (89.68 %), with a proportion of substantially identical hydrocarbon monoterpenes and oxygenated monoterpenes. The most remarkable of which are β -thujone (46.56 %) and Chamazulene (26.2 %). The main constituent of the oxygenated fraction is thujone. Among the monoterpenes hydrocarbons, there may be mentioned 4 Terpinol (8.33 %).

Anticoagulant activity

The in vitro anticoagulant power of *A. absinthium* studied was evaluated for the endogenous pathway and the exogenous way of coagulation using two chronometric tests, CKT and QT, respectively. The results of the essential oil dilutions' anticoagulant capacity studied by their against coagulation test of the exogenous pathway, performed using the QT, are shown in the following graph in Fig 2.

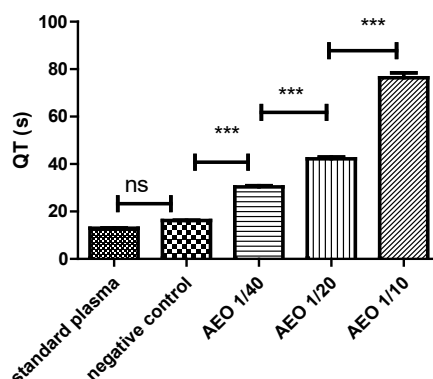


Fig 2. Effect of AEO on Quick Time (extrinsic pathway test).

AEO: *Artemisia absinthium* essential oil

The results showed significant prolongation of the QT by the essential oil extract in a concentration-dependent manner ($p < 0.0001$). (Fig. 2), noting that the standard plasma also gave an estimated coagulation time of 13 s. The best QT is found with the dilution 1/10, with a value of 76.36 s. The anticoagulant activity exists through the extension of QT more than the negative control (16.2 ± 0.08 s). ANOVA yielded a significant result, and Tukey's Multiple Comparison Test (Table 3) confirms that the results are significant ($p < 0.0001$).

The evaluation of the anticoagulant capacity of the essential oil dilutions against the endogenous pathway was performed using the CKT test. In this test, this coagulation pathway is activated by the contact between the factor XII and the electronegative surface of the activator, which is kaolin (a substitute for collagen and connective tissue in vivo). This interaction induces the activation of factor XII and, consequently, the sequential activation of factors XI, IX, X, and thrombin (factor II).

An extended coagulation time compared to the positive control, where the sample is replaced by DMSO, reflects an anticoagulant activity of the tested material (Table 2-3).

CKT results in the presence of the essential oil dilutions were evaluated at optimal incubation time, 15 minutes (Table 2). The values obtained revealed that these extracts exert a strong anticoagulant activity.

Table 2. Effect of AEO on *Cephalin kaolin* time (intrinsic coagulation test).

Dilution	CKT (s)	Comment
AEO 1/ 10	>300	Incoagulable
AEO 1/20	>300	Incoagulable
AEO 1/40	>300	Incoagulable
Heparin (0.25µg/mL)	30.6 ± 1.5	
Heparin (0.5µg/mL)	65.6 ± 0.9	
Heparin (0.8µg/mL)	147.1 ± 1.46	
Standard plasma	30.4 ± 0.43	
Negative Control	42.2 ± 0.4	

AEO/ *Artemisia absinthium* essential oil CKT/ Cephalin kaolin time

The analyses show that in the presence of extracts, the lengthening of the CKT compared to standard plasma (30.4 ± 0.43) is significantly well-marked by a value exceeding 300s ($p<0.0001$).

Table 3. Tukey's Multiple Comparison Test.

Tukey's Multiple Comparison Test	MeanDiff	q	Significant? $P < 0.05?$	Summary	95 % CI of diff
QT					
standard plasma vs negative control	-3,188	2,984	No	ns	-7.817 to 1.441
standard plasma vs AEO 1/40	-17,34	16,23	Yes	$p<0.0001$	-21.97 to -12.71
standard plasma vs AEO 1/20	-29,21	27,34	Yes	$p<0.0001$	-33.84 to -24.58
standard plasma vs AEO 1/10	-63,36	59,31	Yes	$p<0.0001$	-67.99 to -58.73
negative control vs AEO 1/40	-14,16	13,25	Yes	$p<0.0001$	-18.79 to -9.527
negative control vs AEO 1/20	-26,02	24,35	Yes	$p<0.0001$	-30.65 to -21.39
negative control vs AEO 1/10	-60,18	56,33	Yes	$p<0.0001$	-64.81 to -55.55
AEO 1/40 vs 1/20	-11,86	11,10	Yes	$p<0.0001$	-16.49 to -7.233
AEO 1/40 vs 1/10	-46,02	43,08	Yes	$p<0.0001$	-50.65 to -41.39
AEO 1/20 vs 1/10	-34,16	31,97	Yes	$p<0.0001$	-38.79 to -29.53
CTK					
standard plasma vs Negative Control	-11,74	37,36	Yes	$p<0.0001$	-13.07 to -10.41
standard plasma vs Heparin (0.25g/mL)	-0,2000	0,636 4	No	ns	-1.530 to 1.130
standard plasma vs Heparin (0.5g/mL)	-35,20	112,0	Yes	$p<0.0001$	-36.53 to -33.87
standard plasma vs Heparin (0.8g/mL)	-116,8	371,7	Yes	$p<0.0001$	-118.1 to -115.5
Negative Control vs Heparin (0.25g/mL)	11,54	36,72	Yes	$p<0.0001$	10.21 to 12.87
Negative Control vs Heparin (0.5g/mL)	-23,46	74,65	Yes	$p<0.0001$	-24.79 to -22.13
Negative Control vs Heparin(0.8g/mL)	-105,1	334,4	Yes	$p<0.0001$	-106.4 to -103.8
Heparin (0.25g/mL) vs Heparin (0.5g/mL)	-35,00	111,4	Yes	$p<0.0001$	-36.33 to -33.67
Heparin (0.25g/mL) vs Heparin (0.8g/mL)	-116,6	371,1	Yes	$p<0.0001$	-118.0 to -115.3
Heparin (0.5g/mL) vs Heparin(0.8g/mL)	-81,62	259,7	Yes	$p<0.0001$	-82.95 to -80.29

AEO/ *Artemisia absinthium* essential oil CKT/ Cephalin kaolin time, QT Quick time

Discussion

The Essential oil yield is relatively lower than that obtained by Bouchenak et al. 2018 in Blida [24], Lopes et al., 2008 in Canada [30] and by Derwich et al., 2009 in Morocco [31], it was evaluated at 0.5 %, respectively. 0.5 % and 0.57 %. However, Riahi et al. 2013 reported a higher return of 1.87 % in Tunisia [32]. These differences are due to several factors: geographical origin, ecological factors including climatic (temperature and humidity), and the plant species itself. We recall here that we harvested our plant after its flowering; the content of essential oils also depends on the harvesting time. The flowering tops and leaves should be harvested before flowering because, according to Salle et Pelletier 1991 [33], after flowering, 70 % of the essential oils evaporate in the air. On the other hand, the whole plant is usually harvested during flowering [34].

These results are in agreement with those obtained by Bouchenak et al (2018) [24]. Other than the main components of *A. absinthium* essential oils in Turkey were camphor and 1,8-cineole [35]. In *A. absinthium* leaf oil taken from Ardabil, 1,8-cineole, borneol, and camphor were the main oils [36]. In another research on *A. absinthium* essential oils, the main constituents were thujone and trans-sabinyl acetate [37]. In another investigation on the chemical composition of the essential oil extracted from the leaves of *A. absinthium* was conducted. In the region of Guigou and Errachidia, α -thujone, sabinyl acetate, and β -thujone were the main components of this oil [31]. This is consistent with our results; thujone is probably the most important biologically active compound of wormwood [38].

The total phenolic content of essential oils in terms of gallic acid equivalents is 18.18 mg GAE/g dry weight. This rate represents 49.7 % of the phenolic compound content in the crude hydroalcoholic extract (methanol-water: 80 w /20 w). This result is different from the result obtained by Mohammadi et al. (2015) in the pre-flowering stage, 48.49 mg GAE/g dry weight; this difference is justified by the growing times and changes of seasons [39].

The GCMS analysis of the essential oil revealed a complex phytochemical composition dominated by monoterpenes and sesquiterpenes. Monoterpenes such as (α -pinene, camphene, sabinene, myrcene, α -terpinene, p-cymene, limonene, γ -terpinene, and terpinen-4-ol), along with related terpenoid derivatives including (perillyl alcohol and β -thujone), and several sesquiterpenes such as (caryophyllene, germacrene, cadinene, elemol, and eudesmol), as well as azulene derivatives like (chamazulene and 3,6-dihydrochamazulene), have been widely reported to exhibit anti-inflammatory, antioxidant, and indirect cardiovascular-related activities, mainly through modulation of oxidative stress and inflammatory signaling pathways. This chemical diversity suggests that the observed antithrombotic activity likely arises from a multi-component synergistic network rather than a single dominant bioactive molecule.

β -thujone, as a major constituent, has demonstrated antioxidant activity, including nitric oxide (NO•) radical scavenging capacity (74.12 ± 0.66 % inhibition at 100 $\mu\text{g/mL}$) [40], which may contribute to the modulation of reactive nitrogen species such as peroxynitrite (ONOO⁻). Given that NO is a key endogenous inhibitor of platelet activation via stimulation of soluble guanylate cyclase (sGC) and elevation of cyclic guanosine monophosphate (cGMP), leading to reduced intracellular Ca^{2+} levels and inhibition of platelet aggregation [41,42]. Oxidative stress-mediated NO depletion and peroxynitrite formation can compromise this protective pathway [43].

In parallel, Chamazulene acts as a specific 5-lipoxygenase (5-LOX) inhibitor with an IC_{50} of 10-15 μM in human neutrophils, preventing the conversion of arachidonic acid to LTB₄ through the leukotriene pathway without affecting COX [44,45]. Reduced LTB₄ formation decreases leukocyte-platelet interactions via BLT1 receptors, limiting cellular aggregates involved in thrombosis [46,48]. Additionally, its potent antioxidant activity ($\text{IC}_{50}=2$ μM for arachidonic acid peroxidation) reduces ROS-mediated platelet priming [44], while indirectly competing with TxA₂ by limiting arachidonic acid availability for the COX pathway [49].

Monoterpenes have been reported to modulate platelet-relevant signaling pathways [11]. In particular, α -pinene has shown inhibitory effects on ADP-induced platelet aggregation ($\text{IC}_{50} = 8.23$ $\mu\text{g/mL}$), suggesting interference with purinergic signaling [50]. Consistently, in the endothelial cells pre-incubated with γ -terpinene (50-200 μM for 24h), platelet aggregation was reduced by 51.57 % at 50 μM and 44.20 % at 100 μM using platelet-rich plasma. The compound showed strong *in silico* affinity for P2Y₁₂ purinergic receptors (-6.450 ± 0.232 kcal/mol), suggesting antagonism of ADP-induced platelet activation via calcium release inhibition from dense granules [51]. More broadly, monoterpenes modulate intracellular Ca^{2+} dynamics and inflammatory pathways, including NF- κB , in a model-dependent manner [52,53]. Monoterpenes such as limonene, p-cymene, terpinen-4-ol, and α -Pinene has also been shown to enhance antioxidant defenses and reduce oxidative stress,

thereby indirectly preserving NO bioavailability through ROS scavenging and modulation of redox-sensitive pathways [54].

β -Caryophyllene selectively activates CB2 receptors, reducing platelet activation and platelet-leukocyte interactions through downregulated P-selectin expression [55,56], and NF- κ B inhibition in blood components [57]. This CB2-dependent mechanism decreases platelet reactivity, critical for intrinsic pathway stability (contact phase XII $\rightarrow$ amplification VIII/IX) rather than extrinsic tissue factor-driven coagulation [58,59].

β -Eudesmol has been reported to suppress caspase-1 activation and downregulate RIP2 expression in activated mast cells, indicating inhibition of upstream inflammatory signaling pathways [60]. Such modulation of mast cell-driven inflammatory cascades may indirectly influence platelet reactivity by reducing pro-inflammatory mediators involved in vascular and hemostatic regulation [61]. Due to its reported activity on platelet aggregation and potential impact on hemostatic processes, caution has been suggested regarding its use in physiologically sensitive conditions such as childbirth [62].

Phenylpropanoid derivatives, including methyl-eugenol, have been associated with modulation of platelet function via calcium-dependent and thromboxane-related pathways [7,63]. Recent evidence indicates that methyl-eugenol can act as a dose-dependent inhibitor of platelet function by suppressing collagen-induced activation through GPVI signaling, leading to inhibition of PLC γ 2 activation, reduced intracellular Ca²⁺ mobilization, and decreased granule secretion, ultimately impairing platelet aggregation [64-66].

Synergistic effects of essential oils have been widely documented in the literature, where multi-component interactions among bioactive constituents enhance biological activity compared with isolated compounds [67-69]. In this context, β -thujone-associated redox modulation may act in concert with monoterpene-mediated ROS scavenging, leading to improved preservation of nitric oxide (NO) bioavailability. Nitric oxide (NO) exerts its biological effects by activating soluble guanylate cyclase (sGC), which increases the production of cyclic guanosine monophosphate (cGMP); this signaling cascade subsequently reduces intracellular Ca²⁺ levels and inhibits platelet activation [69]. These integrated antioxidant effects may contribute to the reduction of platelet hyperreactivity [43,70].

Functionally, the absence of clot formation in the CKT assay, together with a comparatively weaker effect on QT, suggests a preferential impairment of intrinsic coagulation processes. This indicates that the essential oil primarily affects early hemostatic events, including platelet activation and intrinsic pathway amplification, rather than directly targeting the extrinsic coagulation cascade.

Overall, these findings support a multi-target, system-level mechanism involving coordinated modulation of platelet activation, suppression of thromboxane-mediated amplification, and reinforcement of redox-sensitive (NO) signaling. This integrated network of interactions likely underlies the pronounced disruption of intrinsic coagulation observed in the CKT assay.

Conclusions

This study demonstrates that the plant's essential oil significantly affects both extrinsic and intrinsic coagulation pathways, with greater prolongation of Cephalin-kaolin time compared to Quick time.

The in vitro results using extrinsic and intrinsic coagulation tests show a more marked effect on the intrinsic pathway, as indicated by the stronger prolongation of Cephalin-kaolin time compared to Quick time. This suggests that the anticoagulant activity of the essential oil is mainly related to interference with early hemostatic events, including platelet function and redox-sensitive signaling pathways. However, further in vivo studies are required to clarify the precise molecular mechanisms and to confirm the pharmacodynamic relevance of these effects under physiological conditions.

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