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PHENOLIC CHARACTERIZATION OF *CORIANDRUM SATIVUM* SEED EXTRACT AND EVALUATION OF ITS ANTIOXIDANT AND ANTITHROMBOTIC ACTIVITIES

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This study aimed to evaluate the antioxidant, antihemolytic, anticoagulant, and thrombolytic activities of the aqueous-methanolic extract of *Coriandrum sativum* seeds using *in vitro* assays, and to characterize its phenolic profile based on high-performance liquid chromatography (HPLC). Phytochemical analysis revealed high levels of bioactive compounds, with a total phenolic content of 69.87 ± 5.62 µg gallic acid equivalents mg^{-1} and a total flavonoid content of 26.54 ± 1.45 µg rutin equivalents mg^{-1}. HPLC analysis identified several phenolic acids and flavonoids, including gallic acid, chlorogenic acid, caffeic acid, ferulic acid, rutin, quercetin, and kaempferol, indicating a rich polyphenolic composition. The extract exhibited notable antioxidant activity: the half-maximal inhibitory concentration for the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay was 80 ± 5.13 µg mL^{-1}, and it had a total antioxidant capacity ($A_{0.5}$) of 39.33 ± 4.62 µg mL^{-1}. In addition, it showed a protective effect against oxidative hemolysis, with an IC_{50} of 0.244 mg mL^{-1}. The anticoagulant assay demonstrated a significant prolongation of clotting times, with prothrombin time and activated partial thromboplastin time of 57.66 and 68.66 seconds, respectively, at 75 mg mL^{-1}, suggesting an effect on both intrinsic and extrinsic pathways. Furthermore, the extract exhibited concentration-dependent thrombolytic activity, with clot lysis increasing from 15.82 % to 35.96 %. These findings suggest that *C. sativum* seeds represent a promising natural source of bioactive compounds with potential applications in the management of oxidative stress and thrombotic disorders.

Keywords: *Coriandrum sativum* L.; HPLC profiling; phenolic compounds; antioxidant activity; anticoagulant activity; clot lysis effect

ФЕНОЛНА КАРАКТЕРИЗАЦИЈА НА ЕКСТРАКТ ОД СЕМКИ НА *CORIANDRUM SATIVUM* И ЕВАЛУАЦИЈА НА НЕГОВАТА АНТИОКСИДАЦИСКА И АНТИТРОМБОТИЧНА АКТИВНОСТ

Оваа студија имаше за цел да ги евалуира антиоксидацискатa, антихемолитичната, антикоагулацискатa и тромболитичната активност на водно-метанолниот екстракт од семки на *Coriandrum sativum* со употреба на *in vitro* тестови, како и да го карактеризира неговиот фенолен профил со помош на високоефикасна течна хроматографија (HPLC). Фитохемиската анализа покажа високи нивоа на биоактивни соединенија, со вкупна содржина на фенолни соединенија од 69,87 ± 5,62 µg еквиваленти на гална киселина mg^{-1} и вкупна содржина на флавоноиди од 26,54 ± 1,45 µg еквиваленти на рутин mg^{-1}. HPLC анализата идентификуваше неколку фенолни киселини и флавоноиди, вклучувајќи гална киселина, хлорогена киселина, кафена киселина, ферулна киселина, рутин, кверцетин и кемпферол што укажува на богат полифенолен состав. Екстрактот покажа значајна антиоксидациска активност: половичната максимална инхибиторна концентрација (IC_{50}) во тестот за отстранување на радикалот 2,2-дифенил-1-пикрилхидразил (DPPH) изнесуваше 80 ± 5,13 µg mL^{-1}, додека вкупниот антиоксидациски капацитет ($A_{0.5}$) изнесуваше 39,33 ± 4,62 µg mL^{-1}. Дополнително, екстрактот покажа заштитен ефект против оксидациска хемолиза, со IC_{50} од 0,244 mg mL^{-1}. Антикоагулацискиот тест покажа значително

продолжување на времето на коагулација, при што протромбинското време (PT) и активираното парцијално тромбопластинско време (aPTT) изнесуваа соодветно 57,66 и 68,66 секунди, при концентрација од 75 mg mL^{-1}. Ова укажува на влијание врз внатрешниот и надворешниот пат на коагулацијата. Покрај тоа, екстрактот покажа тромболитична активност зависна од концентрацијата, при што лизата на згрутчувањето се зголеми од 15,82 % на 35,96 %. Овие наоди укажуваат дека семките од *C. sativum* претставуваат ветувачки природен извор на биоактивни соединенија со потенцијална примена во справувањето со оксидацискиот стрес и тромботичните нарушувања.

Клучни зборови: *Coriandrum sativum* L.; HPLC-профилирање; фенолни соединенија; антиоксидациска активност; антикоагулациска активност; ефект на лиза на згрутчување

1. INTRODUCTION

Medicinal plants have long been recognized as an important source of bioactive compounds with significant therapeutic potential. In recent years, there has been growing scientific interest in plant-derived natural products due to their antioxidant, anti-inflammatory, and cardioprotective properties. Many of these biological effects are largely attributed to the presence of phenolic compounds, which are well known for their ability to scavenge free radicals and modulate various physiological processes.[1]

Oxidative stress plays a crucial role in the development of several pathological conditions, including cardiovascular diseases and hematological disorders. Natural antioxidants derived from plants are considered promising alternatives to synthetic compounds because of their safety and potential health benefits. In addition, certain plant-derived phenolic compounds have been reported to influence blood coagulation pathways and platelet aggregation, suggesting their potential role in preventing thrombotic disorders.[2]

Coriandrum sativum L. (Apiaceae), commonly known as coriander, is an aromatic plant widely cultivated and used in traditional medicine in many regions of the world. Its seeds are particularly valued for their culinary and medicinal applications. Previous phytochemical investigations have revealed that coriander seeds contain various bioactive constituents including phenolic acids, flavonoids, essential oils, and other secondary metabolites. These compounds have been associated with a wide range of biological activities such as antioxidant, antimicrobial, anti-inflammatory, and cardioprotective effects.[3]

Specifically, the high antioxidant potential of polyphenols and flavonoids identified in *C. sativum* seeds provides a strong physiological foundation for protecting cellular structures against oxidative injury. Because oxidative stress directly damages erythrocyte membranes, leading to hemolysis, evaluating the antihemolytic capacity of coriander seed extracts represents a direct extension of their radical-scavenging properties.[4,5] Furthermore, polyphenols are increasingly recognized for their ability to interact with plasma proteins, coagulation factors, and fibrin networks.[6] This mechanical interplay provides a clear rationale for investigating the anticoagulant and thrombolytic potential of *C. sativum* as protective agents against vascular thrombosis.

Despite the extensive use of *C. sativum* in traditional medicine, studies exploring the combined antioxidant, antihemolytic, anticoagulant, and thrombolytic properties of its seed extracts remain limited. Therefore, the present study aimed to evaluate the antioxidant, antihemolytic, anticoagulant, and clot lysis activities of the hydromethanolic extract of *C. sativum* seeds. In addition, the phenolic profile of the extract was characterized using high-performance liquid chromatography (HPLC) to identify the major compounds that may contribute to the observed biological effects.

2. EXPERIMENTAL

2.1. *Plant material*

Mature *C. sativum* seeds were purchased from a local herbal market in Sétif, Algeria, in April 2025. The plant material was authenticated by Prof. Laouer, Department of Ecology, University of Setif1, Algeria. The seeds were washed with distilled water to remove impurities, air-dried at room temperature in a shaded and well-ventilated area, ground into a fine powder using an electric grinder, and stored in airtight amber glass containers protected from light until extraction.

2.2. *Preparation of the hydromethanolic extract*

The hydromethanolic extract was prepared by macerating 20 g of powdered seeds in 200 mL of methanol–water (80:20, v/v) for 24 hours under continuous agitation. The mixture was filtered and

the filtrate was concentrated at 40 °C until complete solvent evaporation. The resulting dry extract was stored in airtight containers and used for subsequent analyses.

2.3. *Determination of the total phenolic content*

The total phenolic content was determined using the Folin–Ciocalteu method.[7] Briefly, 200 µL of diluted extract were mixed with 1 mL of Folin–Ciocalteu reagent (10 %). After 4 minutes, 800 µL of sodium carbonate solution (7.5 %) were added. The mixture was incubated for 90 minutes in the dark at room temperature and absorbance was measured at 760 nm. The results are expressed as micrograms gallic acid equivalents per milligram of dry extract (µg GAE mg DE^{-1}).

2.4. *Determination of the total flavonoid content*

The total flavonoid content was determined using the aluminum chloride colorimetric method.[8] One milliliter of extract was mixed with 1 mL of $AlCl_3$ solution (2 %). After 15 incubation for 15 minutes at room temperature, absorbance was measured at 430 nm. The results are expressed as micrograms of rutin equivalents per milligram of dry extract (µg RE mg DE^{-1}).

2.5. *HPLC analysis*

Phenolic compounds in the hydromethanolic extract were analyzed by HPLC, using a device equipped with a diode-array detector. Separation was performed on a C_{18} column and detection was carried out at 280 nm. A mixed solution containing authentic phenolic standards was analyzed under the same chromatographic conditions as the extract, and peak identification was performed by comparing retention times with those of the reference standards.

2.6. *Antioxidant activity*

2.6.1. *2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay*

The antioxidant activity was evaluated using the DPPH radical scavenging method.[9] Briefly, 50 µL of extract at different concentrations were mixed with 1.95 mL of a methanolic DPPH solution (0.039 mg/mL). After incubation for 30 minutes in the dark at room temperature, absorbance was measured at 515 nm against methanol as blank. The control reaction consisted of 1.95 mL of DPPH solution mixed with 50 µL of methanol, representing 100 % of the initial DPPH radical concentration. Ascorbic acid was used as a reference standard and the radical scavenging activity was calculated according to the following equation:

$$\text{Inhibition (\%)} = \frac{(A_{control} - A_{sample})}{A_{control}} \times 100$$

The half-maximal inhibitory concentration (IC_{50}) is defined as the concentration of extract required to scavenge 50 % of DPPH radicals. It was calculated by linear regression analysis of the concentration–response curve using Microsoft Excel.

2.6.2. *Total antioxidant capacity*

The total antioxidant capacity was evaluated using the phosphomolybdenum method.[10] One-hundred microliters of extract was mixed with 1 mL of reagent solution containing sulfuric acid (0.6 M), sodium phosphate (28 mM), and ammonium molybdate (4 mM). The mixture was incubated at 95 °C for 90 minutes. After cooling, absorbance was measured at 695 nm. A standard calibration curve was generated using Trolox at different concentrations, and the total antioxidant capacity of the extract was calculated from the calibration equation and expressed as milligrams of Trolox equivalents per gram of dry extract (mg TE g DE^{-1}).

2.7. *Antihemolytic activity*

The antihemolytic activity of the extract was determined using human erythrocytes according to a previous study,[11] with slight modifications. Blood samples collected from healthy volunteers into ethylenediaminetetraacetic acid (EDTA)-containing tubes were centrifuged, and erythrocytes were washed three times with saline solution. A 4 % erythrocyte suspension was prepared in phosphate-buffered saline (PBS). One milliliter of the extract at different concentrations was mixed with 2 mL of erythrocyte suspension, followed by the addition of 1.5 mL of PBS. After incubation for 5 minutes at room temperature, oxidative stress was induced by adding 0.5 mL of hydrogen peroxide (H_2O_2, 0.82 M). The reaction mixture was incubated at 37 °C for 3 hours with shaking at 120 rpm, followed by centrifugation and measurement of the absorbance at 540 nm. Quercetin was used as a reference standard. Hemolysis is expressed relative to a negative control (H_2O_2-treated cells, 100 %

hemolysis), while PBS without H_2O_2 served as the control for 0 % hemolysis.

2.8. *Anticoagulant activity*

2.8.1. *Prothrombin time (PT)*

The PT assay was used to evaluate the extrinsic coagulation pathway. PT reflects the activity of key coagulation factors including factor VII, factor X, factor V, prothrombin (factor II), and fibrinogen, compared to a reference plasma (100 %). Under normal conditions, clotting occurs within 12–13 seconds (Quick time). A prolongation of clotting time compared with the negative control indicates that the tested sample has an anticoagulant effect. The effect of the extract was evaluated at concentrations of 20, 40, 50, and 75 mg mL^{-1}. Briefly, 20 µL of extract was mixed with 80 µL of citrated human plasma and incubated at 37 °C for 3 minutes. Coagulation was initiated by adding 200 µL of thromboplastin reagent from a commercial kit (Biolabo, maizy, France), and clotting time was recorded in seconds. The negative control consisted of plasma treated with physiological saline (0.9 % NaCl) instead of the extract.

2.8.2. *Activated partial thromboplastin time (aPTT)*

The aPTT assay was used to evaluate the intrinsic coagulation pathway. Under normal conditions, clotting time ranges from 30 to 34 seconds in adults. For the aPTT assay, 20 µL of plant extract (20, 40, 50, or 75 mg mL^{-1}) was mixed with 80 µL of plasma, followed by the addition of 100 µL of cephaline reagent (Biolabo). The mixture was incubated at 37 °C for 3 minutes, after which 100 µL of calcium chloride ($CaCl_2$) was added to initiate coagulation. lotting time was recorded manually in seconds. The negative control consisted of plasma treated with physiological saline (0.9 % NaCl) instead of the extract. An increase in aPTT relative to the negative control was considered indicative of anticoagulant activity.

2.8.3. *Clot lysis activity*

In vitro clot lysis activity was evaluated following Muduli et al.[12] Venous blood from healthy volunteers was transferred into pre-weighed sterile microcentrifuge tubes (1 mL per tube) and incubated at 37 °C for 45 minutes to allow clot formation. After complete removal of serum, the tubes were weighed to determine clot weight (clot weight = weight of clot-containing tube − weight of empty tube). Subsequently, 100 µL of plant extract at different concentrations (50, 25, 12.5, 6.25, and 3.125 mg mL^{-1}) was added to each tube. Distilled water was used as the negative control. The tubes were incubated at 37 °C for 90 minutes, then centrifuged, and the released fluid was removed. Tubes were reweighed, and clot lysis was calculated as the difference in weight before and after treatment. The percentage of clot lysis was determined using the formula:

$$\text{Clot lysis (\%)} = \frac{\text{weight of released clot}}{\text{initial clot weight}} \times 100$$

2.9. *Statistical analysis*

All experiments were performed in triplicate and the results are expressed as the mean ± standard deviation. Statistical analysis was performed using GraphPad Prism 8 (GraphPad Software, San Diego, CA, USA). Differences between groups were analyzed using one-way analysis of variance (ANOVA) followed by Dunnett's test, with $p < 0.05$ considered statistically significant.

3. RESULTS AND DISCUSSION

3.1. *Total phenolic and flavonoid contents*

Hydromethanolic extraction of *C. sativum* seeds resulted in a yield of 10.2 %, which was sufficient for the subsequent phytochemical and biological analyses. The hydromethanolic extract presented a high concentration of bioactive compounds, with a total phenolic content of 69.87 µg GAE mg DE^{-1} and a total flavonoid content of 26.54 ± 1.45 µg RE mg DE^{-1}. These values are notably higher than those reported in a previous study.[13] Such variations can be attributed to the efficiency of the hydromethanolic solvent system and the maceration process, which ensures prolonged solvent–sample contact, enhancing the diffusion and solubilization of phenolic and flavonoid compounds.[14]

3.2. *HPLC analysis*

HPLC analysis of the hydromethanolic extract revealed the presence of several phenolic acids and flavonoids. In total, 16 compounds were identified based on their retention times (Figure 1).

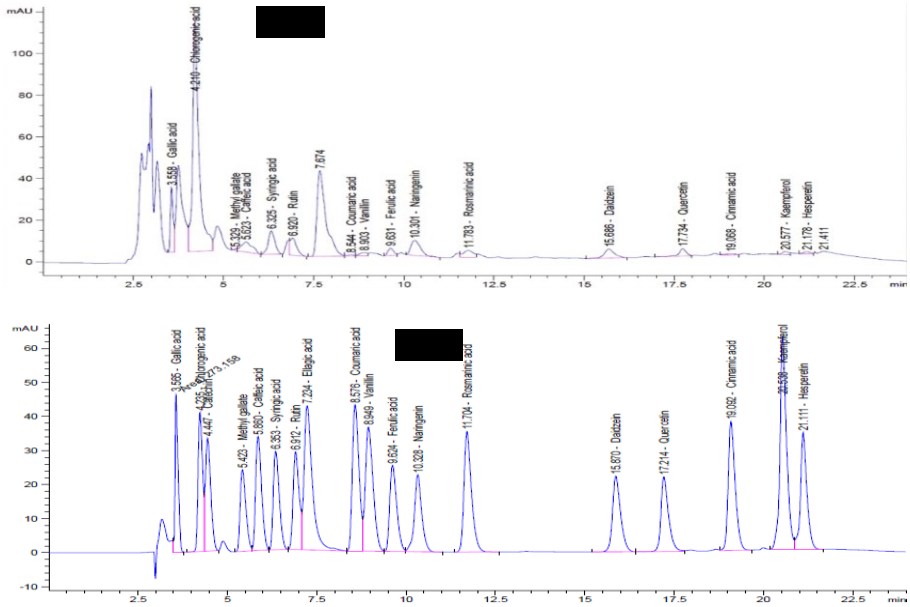


Fig. 1. High-performance liquid chromatogram of the methanolic extract of *Coriandrum sativum* seeds (A) and the mixed phenolic standards (B)

The hydromethanolic extract of *C. sativum* seeds possesses a diverse range of phenolic compounds, with chlorogenic acid (9347.5 μg g dry weight [DW]$^{-1}$) as the predominant compound, followed by rutin, naringenin, gallic acid, syringic acid, quercetin, rosmarinic acid, caffeic acid, and daidzein (Table 1). These findings confirm that *C. sativum* seeds constitute an important source of both phenolic acids and flavonoids. The predominance of chlorogenic acid observed in the present study is consistent with several reports describing this hydroxycinnamic acid as one of the principal phenolic constituents of *C. sativum* seeds.[15,16] Nevertheless, different phenolic profiles in coriander has been reported in other studies, with gallic acid, caffeic acid, rutin, or quercetin being identified as major constituents depending on the plant origin and extraction conditions.[13,15] Among the identified compounds, daidzein was also detected in the present extract, in agreement with the findings reported by Zeković et al.,[17] who previously reported this isoflavone in a *C. sativum* seed extract. Such discrepancies are not unexpected, as the biosynthesis of phenolic compounds is strongly influenced by genetic variability, climatic factors, soil characteristics, agricultural practices, seed maturity, solvent polarity, and chromatographic detection conditions.[18–20]

Table 1. Phenolic compounds identified in the *Coriandrum sativum* seed extract

Compound	Concentration (μg g^{-1})	Compound	Concentration (μg g^{-1})
Gallic acid	608.2	Ferulic acid	114.8
Chlorogenic acid	9347.5	Naringenin	613.5
Methyl gallate	12.6	Rosmarinic acid	304.1
Caffeic acid	268.4	Daidzein	242.9
Syringic acid	426.9	Quercetin	318.4
Rutin	805.9	Cinnamic acid	11.8
Coumaric acid	15.46	Kaempferol	23.14
Vanillin	45.76	Hesperetin	36.59

3.3. *Antioxidant Activity*

The DPPH radical scavenging assay measures the hydrogen atom donating capacity of plant extracts, indicated by the discoloration of a methanolic solution of DPPH. The *C. sativum* seed extract demonstrated notable DPPH radical scavenging activity, with an IC_{50} of 80 ± 5.1 μg mL^{-1}, whereas the reference antioxidant ascorbic acid showed stronger activity, with an IC_{50} of 12.47 ± 0.27 μg mL^{-1}.

The antioxidant activity of the *C. sativum* seed extract was also assessed using the total antioxidant capacity (TAC) method. The intensity of the absorbance of a plant extract reflects its efficiency in donating electrons, so this method provides an evaluation of the overall antioxidant capacity of an extract. In the present study, the total antioxidant capacity of the *C. sativum* seed extract expressed as Trolox equivalents, was 363.0 ± 35.8 mg TE g DE^{-1}, indicating a marked reducing ability,

although lower than that of the reference antioxidant Trolox.

The hydromethanolic extract of *C. sativum* seeds exhibited remarkable antioxidant activity, as demonstrated by its DPPH radical scavenging capacity and total antioxidant capacity determined by the phosphomolybdenum assay. These findings are consistent with previous studies reporting that coriander seeds are a rich source of phenolic antioxidants capable of neutralizing reactive oxygen species (ROS) and preventing oxidative damage.[13,21,22] The antioxidant potential observed in the present study is likely associated with the high abundance of chlorogenic acid together with appreciable concentrations of rutin, gallic acid, naringenin, quercetin, caffeic acid, rosmarinic acid, and syringic acid. These phenolic constituents have been extensively reported to possess strong free radical scavenging activity through hydrogen atom transfer, single-electron transfer, and metal-chelating mechanisms, thereby contributing significantly to the antioxidant capacity of plant extracts.[23,24]

Among these constituents, chlorogenic acid has been reported as an efficient hydrogen atom and electron donor, capable of scavenging DPPH radicals and inhibiting oxidative chain reactions through stabilization of phenoxyl radicals.[25] Likewise, gallic acid and caffeic acid possess multiple hydroxyl groups that facilitate electron transfer and radical quenching, thereby contributing substantially to the overall antioxidant capacity of plant extracts.[26,27] Flavonoids such as rutin, quercetin, naringenin, and kaempferol, further enhance antioxidant activity through complementary mechanisms including hydrogen atom transfer, single-electron transfer, transition metal chelation, and inhibition of lipid peroxidation.[28,29]

The antioxidant activity observed in the present study is therefore unlikely to result from the action of a single phenolic constituent. Instead, it probably reflects synergistic interactions among the various phenolic acids and flavonoids identified by HPLC. Similar synergistic effects have been described, where complex phytochemical mixtures exhibited greater antioxidant efficacy than isolated compounds.[30]

3.4. *Antihemolytic activity*

The hydromethanolic *C. sativum* seed extract exhibited a protective effect against H_2O_2-induced hemolysis of erythrocytes. Hemolysis decreased progressively as the extract concentration increased, reaching 13.63 % at 2 mg mL^{-1}. The calculated IC_{50} was 0.244 mg/mL^{-1}, whereas the reference compound quercetin showed a stronger activity with an IC_{50} of (0.106 mg mL^{-1}) (Figure 2).

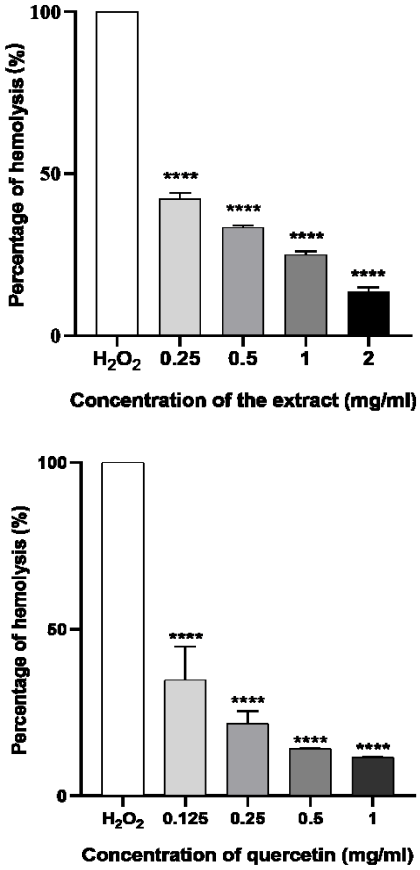


Fig. 2. The effect of the *Coriandrum sativum* extract and quercetin on H_2O_2 induced hemolysis

Due to their unique structural and biochemical characteristics, erythrocytes are highly sensitive to oxidative stress. This vulnerability is primarily attributed to the high content of polyunsaturated fatty acids in their plasma membranes, which are prone to peroxidation, as well as to the presence of hemoglobin, a redox-active molecule capable of undergoing auto-oxidation and generating reactive oxygen species[31] Consequently, erythrocyte membranes are particularly sensitive to oxidative damage, especially under exposure to oxidizing agents such as hydrogen peroxide. H_2O_2 can readily diffuse across erythrocyte membranes and, in the presence of ferrous ions (Fe^{2+}) derived from hemoglobin, participate in Fenton-type reactions, leading to the formation of highly reactive hydroxyl radicals (•OH). These radicals initiate lipid peroxidation and ultimately compromise membrane integrity, resulting in oxidative hemolysis.[32,33]

The protective capacity of the hydromethanolic *C. sativum* seed extract is likely associated with its abundant phenolic acids and flavonoids, which are known to stabilize erythrocyte membranes against oxidative stress.[13] This is further supported by the HPLC findings, which revealed the presence of several phenolic acids and flavonoids, including gallic acid, chlorogenic acid, rutin, quercetin, and kaempferol, all of which are widely reported for their antioxidant potential. These bioactive molecules act by neutralizing free radicals and chelating transition metals such as Fe^{2+}, thereby inhibiting the Fenton reaction and reducing the generation of highly reactive hydroxyl radicals.[34] In addition, they may limit the diffusion of H_2O_2 across the erythrocyte membrane and inhibit methemoglobin formation, thus preserving membrane integrity and preventing oxidative hemolysis.[35]

The antihemolytic activity observed in the present study is consistent with previous reports on *C. sativum*. A previous study reported that a methanolic *C. sativum* seed extract inhibited H_2O_2-induced hemolysis by 59.3 % at 500 μg mL^{-1}, with an IC_{50} of 347 μg mL^{-1}.[4] The hydromethanolic *C. sativum* seed extract evaluated in the present study exhibited a higher inhibition (66.53 %) at the same concentration and a lower IC_{50} (244 μg mL^{-1}), indicating a stronger protective effect against oxidative hemolysis. These differences may be attributed to variations in extraction solvent, phytochemical composition, and the geographical origin of the plant material, which are known to influence the accumulation of bioactive phenolic constituents. The observed protective effect is likely enhanced by the combined action of multiple phytochemicals identified in the extract, which may exert additive or synergistic effects, ultimately contributing to the overall antioxidant efficacy.

3.5. *Anticoagulant activity*

Blood coagulation is a tightly regulated physiological process essential for hemostasis, involving the intrinsic, extrinsic, and common pathways. Clinically, PT evaluates the extrinsic pathway, whereas aPTT reflects the intrinsic and common pathways. Therefore, prolongation of these clotting times is generally considered indicative of anticoagulant activity.[36,37]

As shown in Figure 3, the hydromethanolic *C. sativum* seed extract exhibited a concentration-dependent anticoagulant effect, evidenced by a significant prolongation of both PT and aPTT compared with the control (12.62 and 29.16 seconds, respectively). At 75 mg mL^{-1}, PT and aPTT increased markedly to 57.66 and 68.66 seconds, indicating a strong positive correlation between extract concentration and anticoagulant activity.

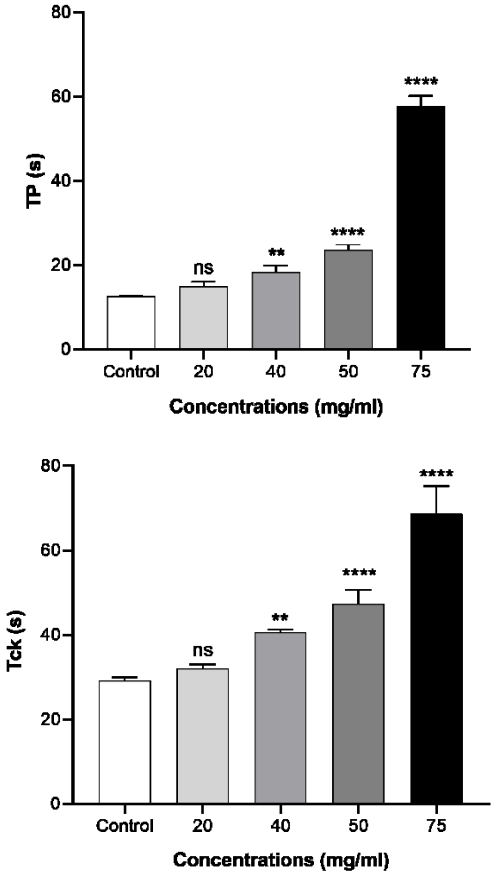


Fig. 3. The effect of the *Coriandrum sativum* extract on hemostatic parameters. The data are presented as the mean ± standard deviation. ** $p \leq 0.01$ and **** $p \leq 0.0001$ compared with the negative control

The prolongation of PT suggests interference with the extrinsic pathway (factor VII), while the increase in aPTT reflects inhibition of the intrinsic pathway (factors XII, XI, IX, and VIII). The concurrent elevation of both parameters indicates that the extract may also affect the common pathway, particularly factor Xa and thrombin (IIa), which are essential for fibrin formation.[38]

Since few studies have investigated the anticoagulant activity of *C. sativum* seed extracts using PT and aPTT assays, so direct comparison with the literature is limited. However, the observed activity may be related to the phenolic compounds identified by HPLC. Increasing evidence indicates that these compounds possess anticoagulant and antithrombotic properties through different mechanisms, including inhibition of platelet activation, attenuation of thrombin generation,

modulation of factor Xa activity, reduction of fibrin formation, and suppression of oxidative stress, which is closely associated with platelet hyperactivity and endothelial dysfunction.[39,40]

Among the identified compounds, chlorogenic acid has been reported to inhibit induced-platelet aggregation, thrombus formation, and the activity of thrombin, factor Xa, and factor XIIIa, thereby delaying coagulation and promoting an antithrombotic state.[41,42] Quercetin and rutin have also been shown to suppress platelet aggregation and interfere with thrombin- and factor Xa–mediated coagulation, whereas flavonoids such as naringenin, kaempferol, and hesperetin have been reported to regulate platelet signaling and oxidative pathways involved in hemostasis by inhibiting the enzymatic activities of thrombin and activated factor Xa, thereby reducing fibrin clot formation and platelet activation while prolonging aPTT.[43–46] Therefore, the anticoagulant activity observed in the present study is likely to result from the additive or synergistic interactions among the diverse phenolic acids and flavonoids identified in the extract.

3.6. *Clot lysis activity*

The hydromethanolic *C. sativum* seed extract exhibited concentration-dependent thrombolytic activity. Clot lysis increased from 15.82 % ± 1.45 % at a concertation of 3.125 mg mL^{-1} to 35.96 % ± 3.33 % at 50 mg mL^{-1}, compared with 9.1 % for the negative control, demonstrating significant thrombolytic potential (Figure 4). In the original *in vitro* clot lysis model described by Prasad et al.,[47] streptokinase (30,000 IU 100 μL^{-1}) achieved 70.80 % clot lysis under comparable experimental conditions.

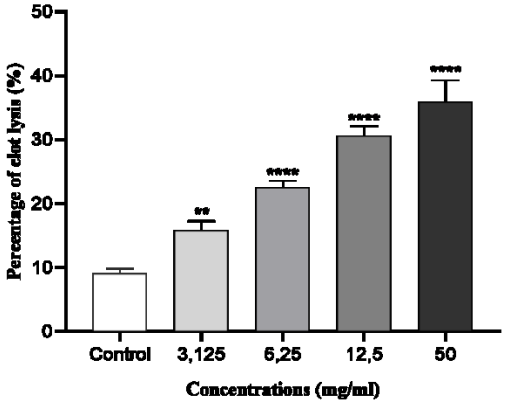


Fig. 4. *In vitro* clot lysis effect of the *Coriandrum sativum* seed extract. The data are presented as the mean ± standard deviation. ** $p \leq 0.01$ and **** $p \leq 0.0001$ compared with the negative control

The increase in clot lysis as the extract concentration increases suggests the presence of bioactive compounds capable of promoting clot degradation under the experimental conditions. HPLC analysis showed that the hydromethanolic *C. sativum* seed extract contains several phenolic compounds that have previously been associated with antithrombotic and fibrinolytic activities, including chlorogenic acid, rutin, quercetin, naringenin, gallic acid and kaempferol. Previous studies have shown that these compounds possess antithrombotic properties by reducing platelet activation, decreasing thrombin generation, limiting fibrin formation, and protecting the vascular endothelium against oxidative stress. [21,41,42,46,48] These mechanisms may contribute, directly or indirectly, to the clot lysis activity observed in the present study.

It should also be noted that the concentrations used in the present *in vitro* assay are relatively high. Therefore, the observed thrombolytic activity should be interpreted as a preliminary screening result. Additional studies using lower concentrations and *in vivo* models are required to confirm its physiological relevance.

Overall, the biological activities observed in the present study are closely associated with the phenolic composition of the hydromethanolic extract. The synergistic interactions between these phytochemicals likely contribute to its antioxidant, antihemolytic, anticoagulant, and thrombolytic effects, suggesting a potential role of *C. sativum* seed extracts in cardiovascular protection. Nevertheless, additional *in vivo* studies are required to validate these findings and assess the safety profile of the extract.

4. CONCLUSION

In conclusion, the hydromethanolic *C. sativum* seed extract exhibited notable antioxidant, antihemolytic, anticoagulant, and clot lysis activities *in vitro*. These effects are closely related to its phenolic composition, as confirmed by HPLC analysis, suggesting a multi-target mode of action involving free radical scavenging and modulation of the coagulation cascade. Overall, the findings support the potential of this plant as a natural source of bioactive compounds with possible cardiovascular benefits. However, further *in vivo* studies are required to confirm its efficacy and safety.

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