In Vitro Activity of Some Medicinal Plants on Blood Coagulation

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ABSTRACT

Objectives: The concern for finding natural and curative agents without adverse side effects has prompted the interest in discovering hemostatic agents from plants. Therefore, in vitro activity of Aizoon hispanicum L. (Aizoaceae), Centaurea hyalolepis Boiss. (Asteraceae), Heliotropium maris-mortui Zohary. (Boraginaceae), Parietaria judaica L. (Urticaceae), Polygonum arenarium Waldst. & Kit. (Polygonaceae), and Verbascum sinuatum L. (Scrophulariaceae) on blood coagulation was estimated by two common tests, which are the prothrombin time test (PT) and the activated partial thromboplastin time test (aPTT).

Materials and Methods: The extracted powders from the plants under this study were adjusted to be 50 mg/mL. Then, in vitro effect of these extracts on the platelet poor plasma samples was measured by an automated coagulation analyzer using PT and aPTT tests.

Results: Based on the obtained results, all plant extracts affected the coagulation cascade by rising either PT or aPTT or both, except for V. sinuatum extract, which reduced only aPTT value. Moreover, the recorded PT values showed that A. hispanicum, H. maris-mortui, and P. arenarium significantly prolonged the PT (p<0.05). Additionally, the results clearly showed that V. sinuatum acted as a coagulant agent based on aPTT values, while all other plants, in contrast, acted as strong anticoagulants. Among the plant species under study, A. hispanicum, H. maris-mortui, and P. arenarium extracts prolonged both PT and aPTT significantly (p<0.05). This could be referred to their additional effect on the common pathway. However, C. hyalolepis, P. judaica, and V. sinuatum showed no significant effect on PT values (p>0.05).

Conclusion: The positive recorded data from this research could serve as identification of new hemostatic remedies that could be used for the commercial economic purposes and for managing several cardiovascular diseases.

Key words: Coagulation cascade, medicinal plants, prothrombin time, activated partial thromboplastin time

INTRODUCTION

Medicinal plants have been and still are used significantly in healthcare by different populations throughout the world.1 The natural compounds extracted from medicinal plants have been used as conventional or complementary remedies for both treatable and untreatable diseases.2 However, these natural products are considered as a valuable source of several compounds that exhibit various biological activities, which in turn be useful for developing alternative therapies.3 For example, herbal medicines prepared from garlic Allium sativum L. are supposed to inhibit platelet activation.2 Also, other herbs such as Salix alba L. are ethnomedicinally used as an anti-inflammatory agent. Later on, the extracted salicylic acid from the second plant species was transformed into a powerful anti-platelet drug acetylsalicylic acid that also called aspirin.4 For that reason, the current research worked on six different plants, which are Aizoon hispanicum L., Centaurea hyalolepis Boiss., Heliotropium maris-mortui Zohary., Parietaria judaica L., Polygonum arenarium Waldst. & Kit., and Verbascum sinuatum L. These plant species were selected for this research according to their medical histories in various fields. However, to date, there are no previous studies on the effect of these plant species on blood coagulation, which is closely related to thromboembolic diseases.5 The coagulation cascade is a complicated system of two pathways, which are the extrinsic (tissue factor) and intrinsic (contact factor) pathways. At the...
end of each of the two pathways, a solid hemostatic clot was formed from fluid blood. This clot must maintain hemostasis by repairing the damage vascular vessel and diminishing the blood flow through it. Thus, any malfunction in the coagulation cascade can be harmful to the human body. In this aspect, researchers have offered anticoagulant and antithrombotic drugs such as heparin, aspirin and warfarin that have been widely used nowadays as therapies for thromboembolic diseases. Also, researchers found that plants can be a useful source of new anticoagulant drugs due to their promising biological activities. Based on all previously mentioned information, the idea of this study emerged to assess in vitro activity of six plant species that are growing wild in Palestine in the coagulation cascade. The anticoagulant or coagulant properties of their leaf aqueous extracts were evaluated by measuring their effect on the prothrombin time (PT) and the activated partial thromboplastin time (aPTT).

MATERIALS AND METHODS

Plant collection and extract preparation

Six wild plant species [A. hispanicum (Jericho), C. hyalolepis (Jericho), H. maris-mortui (Tulkarm), P. judaica (Jericho), P. arenarium (Tulkarm), and V. sinuatum (Nablus)] were collected from the West Bank, Palestine, and identified by Ghadeer Omar, An-Najah National University, Palestine. Representative plant samples were deposited at An-Najah National University herbarium with specific voucher numbers (1940, 1950, 1960, 1954, 1965, and 1970 respectively). After collection, the plant leaves for coagulation study were washed, dried, crushed, and stored in a dry place at room temperature until the day of extract preparation. For aqueous extraction, 5 g from each crushed plant material was soaked in 100 mL sterile and warm distilled water with a continuous rotary shaking at 25°C. After 72 h, all plant mixtures were macerated using a probe sonicator (3 seconds sonication and 5 seconds rest) for 15 min at 25°C. Then, the macerated extracts were subjected to centrifugation at 4500 rpm for 10 min and the gained supernatants were dried by lyophilization. The lyophilized powder from each plant species was solubilized in sterile distilled water to obtain 50 mg/mL concentration.

Blood collection and platelet poor plasma (PPP) preparation

The study was approved by the Ethics Committee (IRB) of the Faculty of Health Sciences at An-Najah National University (ref: SC 12/1/20). Blood samples were collected from eight volunteers, who were healthy, not under any medication, and not smokers. Each collected blood sample was transferred to a citrated centrifugation tube. Then, the citrated tubes were centrifuged at 1500 rpm for 15 min to obtain PPP. All (PPP) samples were analyzed in duplicate by PT and aPTT tests within 2 h from the time of blood collection. The clotting time for two tests was automatically recorded by Coa DATA 4004 coagulation analyzer (LAberBioMedical Technologies, Germany), using standard reagent kits from Human technologies (Germany).

In vitro prothrombin time assay

In a single cuvette, 50 µL of (PPP) was incubated for 5 min with 50 µL of each plant aqueous extract at 37°C. Then, the clotting time was directly recorded after the addition of 100 µL PT reagent (Hemostat thromboplastin-SI, Human, Germany). Statistical analysis of the PT and aPTT results was performed using a statistical package SPSS by applying mean values using one-way ANOVA with post-hoc test. The purpose of this analysis was to determine whether there was any significant difference between the different plant aqueous extracts being studied and the controls. The obtained results were considered significant, when p value was less than 0.05.

RESULTS

This study aimed to evaluate the effect of different plant leaves aqueous extracts on the coagulation cascade by using PT and aPTT tests. PT measures the time required to produce fibrin after factor VII activation. However, aPTT measures the time required to create fibrin starting from intrinsic pathway initiation. According to the used kits and reagents in the running experiment, the normal range value for PT is between 10 and 15 sec and for aPTT is between 22 and 32 sec. Blood samples of the participants were considered to be a representative sample for the running study with no individual variations among them (p= 0.000). Based on the obtained results, all plant extracts influenced the coagulation cascade by increasing either PT or aPTT or both except for V. sinuatum extract, which reduced only the aPTT value (Table 1).

Moreover, the recorded PT values showed that A. hispanicum, H. maris-mortui and P. arenarium significantly prolonged PT with respect to the control (p<0.05) at the concentration under study (Figure 1). Additionally, the results clearly showed that V. sinuatum acted as a coagulant agent based on aPTT values. While all other plant species, in contrast, acted as strong anticoagulants. Thus, they could prevent blood coagulation (Figure 2). Among the plant species being studied, A. hispanicum, H. maris-mortui, and P. arenarium extracts prolonged both PT and aPTT significantly (p<0.05). This could be referred to their additional effect on the common pathway. However, C. hyalolepis, P. judaica, and V. sinuatum had no significant effect on PT values (p>0.05).

DISCUSSION

The blood coagulation cascade is a physiological phenomenon that comprises intrinsic, extrinsic, and common pathways. Briefly, the activation of the intrinsic pathway occurs because
of trauma and contact between kininogen, prekallikrein, and factor XII with underlining collagen on endothelium. Otherwise, activation of the extrinsic pathway occurs upon tissue injury, which causes the release of tissue factor into the bloodstream. Activation of the extrinsic pathway occurs upon tissue injury, which causes the release of tissue factor into the bloodstream. After activation, both the intrinsic and extrinsic pathways end with a common pathway, whose activation starts after the generation of factor Xa from one of the previous two pathways. This factor cleaves prothrombin into thrombin. Later on, thrombin converts fibrinogen to fibrin that in turn causes clot formation. In fact, blood coagulation is a highly regulated pathway and the imbalance due to genetic and environmental factors could alter the normal coagulation system that leads to the formation of unusual clots in the blood vessels. This pathological phenomenon called thrombosis, which is considered one of a cardiovascular and cerebrovascular risk.

### Table 1. Prothrombin time and activated partial thromboplastin time values of the study plant extracts on eight participants’ platelet-poor plasma samples

<table>
<thead>
<tr>
<th>Plant species</th>
<th>PT (sec)</th>
<th>p value*</th>
<th>aPTT (sec)</th>
<th>p value*</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Aizoon hispanicum</em></td>
<td>420</td>
<td>0.000</td>
<td>420</td>
<td>0.000</td>
</tr>
<tr>
<td><em>Centaurea hyalolepis</em></td>
<td>31.35</td>
<td>0.428</td>
<td>48.27</td>
<td>0.000</td>
</tr>
<tr>
<td><em>Heliotropium maris-mortui</em></td>
<td>325.45</td>
<td>0.000</td>
<td>420</td>
<td>0.000</td>
</tr>
<tr>
<td><em>Parietaria judaica</em></td>
<td>43.02</td>
<td>0.128</td>
<td>414.07</td>
<td>0.000</td>
</tr>
<tr>
<td><em>Polygonum arenarium</em></td>
<td>70.65</td>
<td>0.001</td>
<td>420</td>
<td>0.000</td>
</tr>
<tr>
<td><em>Verbascum sinuatum</em></td>
<td>27.19</td>
<td>0.806</td>
<td>16.63</td>
<td>0.018</td>
</tr>
</tbody>
</table>

*p value <0.05 was significant among the different study plant species relative to the control (blood sample without plant extract). PT: Prothrombin time, aPTT: Activated partial thromboplastin time.

![Figure 1](image1.png)

**Figure 1.** The effect of six plant species aqueous extracts on the prothrombin time for eight participants platelet-poor plasma samples

PT: Prothrombin time

![Figure 2](image2.png)

**Figure 2.** The effect of six plant species aqueous extracts on the partial thromboplastin time for eight participants’s platelet-poor plasma samples

aPTT: Activated partial thromboplastin time
The present study evaluated the effect of six different types of the leaf aqueous extracts, which belong to *A. hispanicum*, *C. hyalolepis*, *H. maris-mortui*, *P. judaica*, *P. arenarium*, and *V. sinuatum*, on the coagulation cascade. Since, clotting can be initiated and proceed according to two different cascading pathways, which are the intrinsic (contact factor) or the extrinsic (tissue factor), the impact of all aqueous extracts under study on blood coagulation was estimated by PT and aPTT tests. Consequently, compared to the standard values of PT and aPTT, it is noticeable that some of the plant extracts examined in this study had a coagulant or anticoagulant effect on the tested blood samples. This investigation considering the effects of previous plant species on the blood coagulation was not previously studied, which in turn provides the novelty of this research.

As noted in the results, all examined plant extracts prolonged aPTT values except *V. sinuatum*, which in contrast, demonstrated a pronounced decreasing effect on the aPTT at the studied concentration. The noticed anticoagulation effect of these plant species may be related to inhibition of the contact factors of intrinsic pathway, whereas extrinsic pathway tissue clotting factors may be inhibited by *A. hispanicum*, *H. maris-mortui*, and *P. arenarium* aqueous extracts as they prolong PT values. Remarkable anticoagulation activity was seen in *A. hispanicum*, *H. maris-mortui* and *P. arenarium* as they prolonged both PT and aPTT. This bioactivity indicated that those plant species acted on the common pathway in addition to their impact on the extrinsic and intrinsic pathways. Thus, the common pathway factors; X, V, II, and I, could be the target for their inhibition.

Regarding the obtained results, coagulation activity reduction may be mediated through inhibition or diminishment of activity of several factors including tissue factors, thrombin, and other clotting factors. Accordingly, it is recommended to evaluate the mechanism of action of these plant extracts on the coagulation cascade.

Actually, plants have unlimited ability to synthesize aromatic substances, including phenolics and polyphenols like simple phenols, phenolic acids, quinine, flavones, flavonoids, flavonols, tannins, coumarins, terpenoids, essential oils, alkaloids, lectins, and polypeptides. In this respect, several experiments relying on the chemical analysis of secondary metabolite content of plants in general and the plant species being studied in particular were carried out. Particularly, in some wild Aizoaceae species, phytochemical analysis indicated that they are rich in secondary compounds such as phenolic compounds, flavonoids, tannins, and alkaloids. Also, chemical studies on the genus *Centaurea* revealed the presence of many compounds belonging mainly to the groups of sesquiterpene lactones, flavonoids, coumarins, indole alkaloids, and lignans. A previous experiment showed that *C. depressa* flower extract had anticoagulant activity by extending both PT and aPTT times. These results match the obtained one in the current research concerning different species of *C. hyalolepis*. Moreover, many bioactive components, especially pyrrolidine alkaloids, quinones, terpenoids, flavonoids, and other phenolic compounds were reported to be present in *Heliotropium* genus. The results from the former research regarding other species *H. indicum* explain why leaves of these plant species have been traditionally used as a remedy for thrombosis. Hence, the ethanol, petroleum ether, carbon tetrachloride, and chloroform extracts of *H. indicum* leaves showed clot lysis activity. The same anticoagulant activity was observed for *C. hyalolepis* aqueous extract. Furthermore, phytochemical evaluation of *P. judaica* indicated phenolic and flavonoid compounds in this species. Recent research illustrated that the ethanol extract from *P. judaica* exhibited a prolonged effect on aPTT and no significant effect on PT, which coincide with the obtained results in the current study regarding the aqueous extract. Similarly, the main chemical ingredients of the genus *Polygonum* are flavonoids, quinones, phenyl propanoids, and terpenoids. However, the results obtained in this study agreed with anticoagulant activity of other species from the same genus, that is *P. cuspidatum*. It was noticed that *P. cuspidatum* ethanol extract-prolonged coagulation time via aPTT aPTT. Moreover, *P. cuspidatum* ethanol extract also exhibited effective fibrinolytic and antiplatelet activity. Subsequently, presence of these phytochemicals in the plant species being studied may elucidate their influence on the coagulation cascade. For example, the effect of some phenolic compounds on various stages of blood coagulation and fibrinolytic mechanism was examined. Here, phenol has a complicated action on blood coagulation as it accelerated thrombin-fibrinogen interaction. This in turn retards clot retraction, enhancement of streptokinase activity on plasminogen, and inhibition of plasmin. Additionally, PT and aPPT times were reported to be affected by flavonoids and tannins. The results presented in a previous study elucidated that flavonoids might be potential structural bases for the design of new naturally, safely, and orally bioavailable direct FXa inhibitors. Tannins demonstrated an efficient anticoagulant activity due to their significant inhibitory effect on thrombin. Likewise, terpenoids manifested a tendency to extend both PT and aPPT times that explain their inhibitory effect on platelet aggregation. Moreover, quinone is a strong anticoagulant, whose action is mediated through its ability to inhibit vitamin K-dependent carboxylase that controls blood clotting. From the previous literature, alkaloids are plentifully available in medicinal plants and most of them possess antiplatelet activity. From a perspective, plant alkaloids are mechanically very versatile and interfere with various mediators of clot formation. Moreover, coumarin derivatives are an important class of phytochemicals that display both *in vitro* and *in vivo* anticoagulant activity via PT and aPTT assays with no significant toxicity. Furthermore, plant carbohydrates like pectins and hemicelluloses exhibit antithrombin and thrombolytic activity. Similar to that, plant lectins increased both PT and aPTT times. Additionally, some plant polypeptides, such as cysteine proteases reduced clotting time because of their fibrinolytic activity.

However, *Verbascum* species contain biologically active compounds, such as iridoid glycosides, saponins, flavonoids, phenylethanoids, and neolignanglycosides. Iridoids glycosides showed no effect on both PT and aPTT tests as provided by a previous experiment. This may explain the activity of this
plant in the conducted research, as it demonstrated no effect on PT and significant low inhibitory effect on aPTT. The same observation was also recorded by previous research considering V. fruticosum. But, contrary to the coagulant activity of V. sinuatum, other Verbascum species have been shown to exert anticoagulant effect. Likewise, the aqueous and alcoholic extracts from the vegetative and generative organs of V. thapsus and V. densiflorum increased the blood clotting time.

CONCLUSION
The positive recorded data from this research could serve as identification of new hemostatic remedies that could be used for the commercial economic purposes and for managing several cardiovascular diseases. Though, it is not conclusive as in vivo studies are yet to be investigated. However, from the recorded results, it is apparent that these plants should be cautiously consumed with anticoagulant drugs (e.g. heparin) and stops their consumption before surgery. Furthermore, phytochemical and pharmacological elaborated experiments are required to purify and to characterize possible active constituents of the examined plant species in parallel to their cytotoxicity evaluation.

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Ethics
Ethics Committee Approval: The study was approved by the Ethics Committee (IRB) of the Faculty of Health Sciences at An-Najah National University (ref: SC 12/1/20).

Informed Consent: Not applicable.

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Authorship Contributions

Conflict of Interest: No conflict of interest was declared by the authors.

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