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Research Article

The Effect of Oregano (*Origanum vulgare*) Leaf Extract on Selected Clot-Based Assays

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ABSTRACT

Aim: This study investigates the anticoagulant capabilities of Oregano (*Origanum vulgare*) leaf extract using clot-based tests. **Materials and Methods:** Fifteen (15) healthy male volunteers completed medical assessments and had blood samples collected through venipuncture. The components of oregano leaves were identified by subjecting them to methanol extraction. Prothrombin Time (PT) and activated Partial Thromboplastin Time (aPTT) assays were performed on the human plasma samples, each repeated three times. **Results:** The results demonstrated a substantial increase in the PT and aPTT at extract doses of 25 mg/mL, 50 mg/mL, and 100 mg/mL. There were notable differences in the results between the ideal concentration (50 mg/mL) and the baseline control (0 mg/mL) for PT, as well as between the ideal concentration (25 mg/mL) and the baseline control for aPTT. There also were significant differences between the concentrations of the extract and the baseline control for both PT and aPTT. **Conclusion:** The results indicate that the leaf extract of *O. vulgare* has an anticoagulant effect and can potentially prevent blood clotting. Future research should focus on analyzing outcomes in distinct demographic groups, investigating alternate clot-based examinations, and exploring the chemical makeup of *O. vulgare* for pharmaceutical and medical laboratory applications.

Keywords: Anticoagulation, Clot-based assays, Flavonoids, *Origanum vulgare*.

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INTRODUCTION

The biological process of hemostasis is crucial in the control of bleeding caused by trauma to the blood vessel lining.[1] Influenced by procoagulant and anticoagulant factors, disruption of this process leads to bleeding conditions, which are usually assessed by clot-based assays.

Prothrombin Time (PT) and activated Partial Thromboplastin Time (aPTT) are two of the most common coagulation tests. PT represents the extrinsic and common pathways of the coagulation cascade, while aPTT assesses the intrinsic and the common coagulation cascade function. These two assays are also used to monitor antiplatelet and anticoagulant therapy, which often pose dangerous side effects and associated complications. Vitamin K Antagonists (VKAs) being one of the predominant treatments for venous thromboembolism, for instance, are related to greater bleeding risks. Hence, there exists an imperative to investigate safer coagulation remedies, prompting research on the anticoagulant properties of medicinal plants.

Plants are a great source of anticoagulants as they have been historically used for therapeutic purposes due to their bioactivity.[2] Moreover, it was discovered that they contain polyphenols and flavonoids, which are capable of improving blood flow and regulating platelet aggregation.[3] In this study, *Origanum vulgare* is examined for anticoagulant properties due to the phenolic compounds found in the plant. The study aimed to investigate the effect of oregano leaf extracts on selected clot-based assays, the PT and aPTT.

This study hypothesizes that employing varying concentrations of oregano leaf extract has a significant effect on prolonging the clotting process. Furthermore, it sought a significant difference between the optimal concentration and the baseline control and a statistically significant variance between the concentration used and the baseline control in PT and aPTT assays.

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MATERIALS AND METHODS

Plant Collection and Species Identification

The *O. vulgare* leaves used in the study were collected at 2 Sto. Niño St., Brgy. San Jose, Antipolo City, and authenticated by the Far Eastern University Herbarium.

Plant Preparation

The collected *O. vulgare* leaves were sealed in ziplock bags for transport to the laboratory. They were cleared of debris with tap and distilled water. Next, the researchers oven-dried the leaves at 40°C for six (6) days and ground them using a mechanical blender afterward. The yield was 52.5 g of powdered dried oregano leaves.[4]

Oregano Leaf Extraction

Methanol extraction has been the predominant method of choice for workups focused on flavonoids and phenolic acids for *Origanum* spp.[5] Given this, the researchers extracted the sample with 500 mL of 70.0% methanol. The plant powder was mixed and macerated in methanol for three (3) days and filtered afterward. The filtrate was subjected to a rotary evaporator at 40°C. The resulting extract was contained in an amber flask kept at -16°C.

Before the experiment, the leaf extract was diluted to three (3) concentrations: 25, 50, and 100 mg/mL in normal saline.[6]

Qualitative Test for Flavonoids

To confirm the presence of flavonoids in the methanolic extract, the researchers performed the alkaline reagent test at FEU-Manila. The leaf extract was mixed with 2 mL of 2.0% sodium hydroxide solution. Next, a few drops of diluted acid were added. The formation of a yellow solution qualitatively determined the presence of flavonoids.[7] The results were verified by a registered chemist.

Quantitative Phytochemical Analysis

The quantitative analysis of oregano leaf extracts for flavonoids conducted at Adamson University utilized ultra-performance liquid chromatography. This technique has proven effective in quantifying phytochemicals, offering insights into the complex composition of plant extracts.[8]

Participant Selection

In the selection of the participants, purposive sampling was applied. The blood samples were extracted from 15 volunteered male adults aged 18 to 59 years old. The participants must be healthy and disease-free. There should be no medication history for at least one week before blood sample collection. Recent alcohol consumption and cigarette smoking are not allowed. All participants underwent a health screening survey before the blood collection to determine if they met the criteria. The participants signed an informed consent form provided by the researchers to indicate their willingness to participate in the study.[9]

Determination of Blood Coagulation Parameters

Blood samples were collected in three citrated tubes and centrifuged at 3000 rpm for 15 min to obtain the Platelet-Poor Plasma (PPP). The normal control plasma was tested for five repetitions to establish quality control. The participant samples were then subjected to PT and aPTT using a digital coagulation analyzer, each assay carried out thrice. The PPP was stored in a freezer until further testing to prevent the deterioration of the coagulation factors. They were thawed rapidly at 37°C and tested within 1 hr.[10] Finally, the results were compared with the baseline control to the PPP with various concentrations of oregano leaf extract.[11]

The PT and aPTT assays were conducted utilizing the CoaDATA 504 machine and the manufacturer's manual was followed. This machine employs a turbo-densitometric measuring principle, combining a high-resolution LED photometer unit with mechanical stirring for precise analysis.

Statistical Analysis

Independent samples t-test and Mann-Whitney U test assessed whether there was a significant difference between the optimum concentration of oregano leaf extract and the baseline control. The Shapiro-Wilk Normality Test was utilized to determine whether the data followed a Gaussian distribution. The alpha level is 0.05 and a p-value ≤ 0.05 indicates that the result is significant.

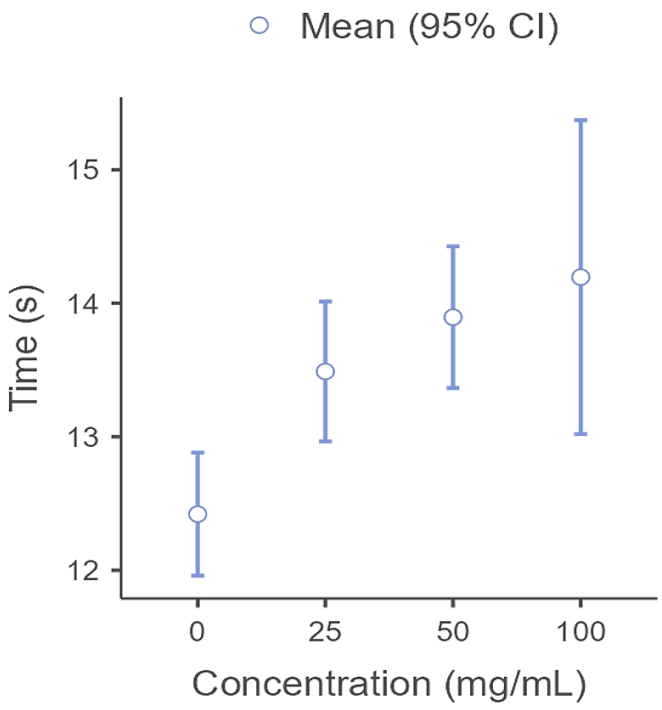

Figure 1: Effect of *O. vulgare* methanolic extract on Prothrombin Time.

To determine whether there is a significant variation between the extract concentrations and the baseline control, the researchers used the Kruskal-Wallis test. The setup is as follows: baseline control (0 mg/mL), 25 mg/mL, 50 mg/mL, and 100 mg/mL of the extract. The significance level was also 0.05. Dwass-Steel-Critchlow-Fligner Pairwise Comparisons show the concentration/s that has/have a significant difference compared to the baseline control and each other.

SAFETY CONSIDERATIONS/ETHICS CONSIDERATIONS

The researchers adhered to the laboratory protocols for oregano extraction and blood collection, overseen by a research adviser and two medical technologists. Personal protective equipment and proper disposal of biohazardous waste were observed. Confidentiality measures were in strict accordance with the Data Privacy Act of 2012. Furthermore, participants were assured the right to refuse or withdraw from the study without consequence. The study underwent ethical approval from the FEU Ethics Review Committee, underscoring the commitment to ethical research standards. The researchers accomplished the Good Clinical Practice course ensuring adherence to international clinical trial norms.

RESULTS

Figure 1 represents the effect of different concentrations (in mg/mL) of *O. vulgare* methanolic extract on PT, measured in seconds. The graph shows the mean value for each concentration and the range of values closely scattered near the average. At a concentration of 0 mg/mL (baseline control), the mean PT is 12.4 sec. For concentrations of 25 mg/mL, 50 mg/mL, and 100 mg/mL, the average PT values are 13.5 sec, 13.9 sec, and 14.2 sec respectively. Notably, there is a trend of increasing PT with higher extract concentrations.

Figure 2 illustrates the effect of different concentrations (in mg/mL) of *O. vulgare* methanolic extract on aPTT, measured in seconds. At a concentration of 0 mg/mL (baseline control), the mean aPTT is 34.2 sec. For concentrations of 25 mg/mL, 50 mg/mL, and 100 mg/mL, the aPTT averages are 43.2 sec, 41.6 sec, and 40.8 sec, respectively. It is a straightforward representation of the decreasing trend of aPTT with increasing concentrations of *O. vulgare* methanolic extract (25 mg/mL, 50 mg/mL, and 100 mg/mL) and the effect of the extract addition on the baseline control.

Additional examination through post-hoc pairwise comparisons utilizing the Dwass-Steel-Critchlow-Fligner approach revealed the precise differences among the concentrations. These findings indicate that the 50 mg/mL oregano concentration significantly affected the PT more than other concentrations.[14]

Additional examination utilizing a post-hoc test employing Dwass-Steel-Critchlow-Fligner pairwise comparisons reveals that the 25 mg/mL oregano concentration is the most noticeable distinction, suggesting a substantial divergence in aPTT compared to the baseline control.[14] Based on the results, it has been determined that the oregano concentration of 25 mg/mL is the most effective extract concentration for modulating aPTT.

Table 1: Significant Difference between the Optimum Concentration of Oregano Leaf Extract and the Baseline Control in Terms of Prothrombin Time.

Group Descriptives

	Concentration (mg/mL)	N	Mean	SD	SE
Time (s)	0	45	12.4	1.53	0.229
	50	45	13.9	1.77	0.263

Normality Test (Shapiro-Wilk)

	W	*p*
Time (s)	0.962	0.077

Independent Samples T-test

		Statistic	d_f	*p*
Time (s)	Student's t	-4.23	88.0	<0.001

Table 1 compares the PT of the optimal concentration of oregano leaf extract (50 mg/mL) to that of the baseline control at a significance level of 0.05. The Shapiro-Wilk normality test produced a *p*-value of 0.077, signifying sufficient evidence that the data follows a normal distribution. The independent samples t-test yielded a p-value of <0.001, which led to the rejection of the null hypothesis. Therefore, there is enough evidence to establish a significant difference between the optimum concentration and the baseline.

Table 2: Significant Difference between the Optimum Concentration of Oregano Leaf Extract and the Baseline Control in Terms of Activated Partial Thromboplastin Time.

Group Descriptives

	Concentration (mg/mL)	N	Mean	SD	SE
Time (s)	0	45	34.4	3.15	0.470
	25	45	43.2	5.84	0.871

Normality Test (Shapiro-Wilk)

	W	*p*
Time (s)	0.886	<0.001

Mann-Whitney U Test

		Statistic	d_f	*p*
Time (s)	Student's t	-9.11	88.0	<0.001
	Mann-Whitney U	66.5		<0.001

Table 2 compares the aPTT of the optimal concentration of oregano leaf extract (25 mg/mL) to that of the baseline control at a significance level of 0.05. The Shapiro-Wilk test yielded a *p*-value of <0.001, suggesting that the data is not normally distributed.[12] As a result, a non-parametric analysis method was chosen. The Mann-Whitney U test showed a significantly low *p*-value of <0.001. Therefore, there is a significant difference in aPTT between the two groups.

Table 3: Significant Difference among the Extract Concentrations and the Baseline Control in Terms of Prothrombin Time.

Group Descriptives

	Concentration (mg/mL)	*N*	*Mean*	*SD*	SE
Time (s)	0	45	12.4	1.53	0.229
	25	45	13.5	1.74	0.260
	50	45	13.9	1.77	0.263
	100	45	14.2	3.91	0.583

Normality Test (Shapiro-Wilk)

	W	p
Time (s)	0.906	<0.001

Kruskal-Wallis Test

	x^2	d_f	p
Time (s)	80.8	3	<0.001

Dwass-Steel-Critchlow-Fligner Pairwise Comparisons

Concentration (mg/mL)		*W*	*p*
0	25	10.80	<0.001
0	50	9.93	<0.001
0	100	9.77	<0.001
25	50	-1.55	0.694

Concentration (mg/mL)		*W*	*p*
25	100	-2.93	0.162
50	100	-1.09	0.868

Table 3 shows the evaluation of the influence of different concentrations of extracts on PT, employing statistical tests to identify significant disparities between the concentrations and the baseline control. The Shapiro-Wilk test indicated a substantial deviation from normality ($p<0.001$). Therefore, the Kruskal-Wallis test was used to analyze the distributions of PT among various doses. The test yielded a *p*-value of 0.002, showing statistically significant differences between the concentrations and the baseline control.[13] Thus, the effect of at least one concentration was different from the others.

Table 4: Significant Difference among the Extract Concentrations and the Baseline Control in Terms of Activated Partial Thromboplastin Time.

Group Descriptives

	Concentration (mg/mL)	N	Mean	SD	SE
Time (s)	0	45	34.2	3.15	0.470
	25	45	43.2	5.84	0.871
	50	45	41.6	4.39	0.654
	100	45	40.8	4.24	0.632

Normality Test (Shapiro-Wilk)

	W	*p*
Time (s)	0.848	<0.001

Kruskal-Wallis Test

	x^2	d_f	*P*
Time (s)	15.1	3	0.002

Dwass-Steel-Critchlow-Fligner Pairwise Comparisons

Concentration (mg/mL)		*W*	*p*
0	25	3.968	0.026
0	50	5.440	<0.001
0	100	3.431	0.072
25	50	1.735	0.610
25	100	0.194	0.999
50	100	-0.748	0.952

The analysis of the significant difference between the extract concentrations and the baseline control regarding aPTT starts by testing the normality assumption using the Shapiro-Wilk test. As shown in Table 4, the *p*-value obtained, <0.001, indicates that the data does not conform to a normal distribution.[12] Therefore, the Kruskal-Wallis test, which is a non-parametric method, is used to compare the distributions of the four concentrations. The obtained *p*-value, <0.001, provides evidence of a significant difference in the distribution of at least one concentration concerning aPTT.[13]

DISCUSSION

Hemostasis has four phases-vasoconstriction, "platelet plug," coagulation cascade, and fibrin clot.[1] Anticoagulants interfere with this process and are used to treat atrial fibrillation and venous thromboembolism.[15] New plant-based anticoagulants may be cheaper and safer than existing ones. Methanol leaf extract containing phenolic and flavonoid components has shown potential as an anticoagulant in clot-based tests like PT and aPTT. PT assesses tissue factors and the extrinsic hemostatic pathway. Thus, coagulation factors II, V, VII, X, and fibrinogen affect the testing.[16] It is the primary test for monitoring warfarin therapy response.[17] The *O. vulgare* methanolic leaf extract produced increasingly prolonged PT results compared to the baseline control with increasing concentration. Other studies examined the flavonoid content of *Calotropis procera* leaf extracts and found that 50 and 100 mg/mL extended PT.[6] Research on *Thymus spp*. reveals extended PT outcomes with leaf extract concentration.[18]

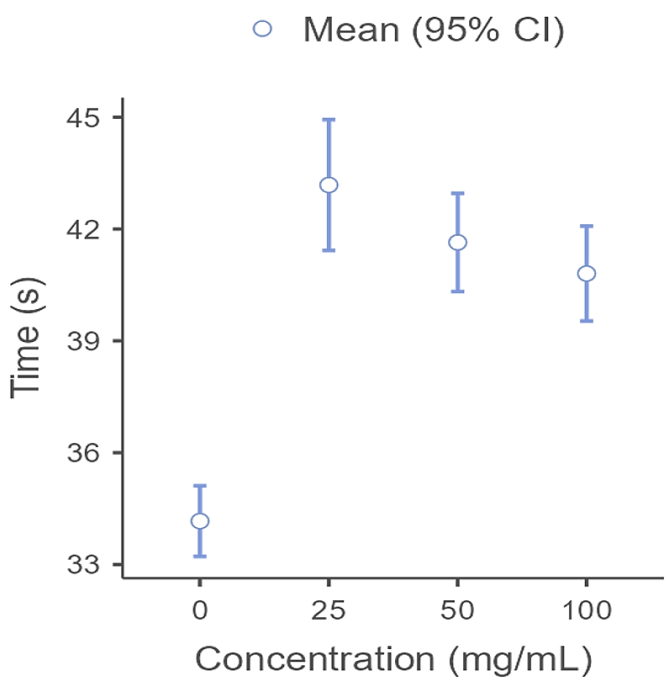

Figure 2: Effect of *O. vulgare* methanolic extract on Activated Partial Thromboplastin Time.

The oregano leaf extract concentrations and PT appear to be dose-dependent.

Additionally, this study also showed that the PT result differs significantly between the optimal concentration and baseline control. Another study found that tarragon leaf extract exceeded methanol extract (at the highest concentration) and baseline control in PT.[19] This study also found that the 50 mg/mL *O. vulgare* concentration significantly affected PT, making it the optimum extract concentration. Another study found that the methanol extract of *C. crepidioides* at 10 mg/mL prolonged PT but shortened it to 20 mg/mL, making it the optimum concentration.[9]

aPTT tests the intrinsic and common pathways of the coagulation cascade.[20] The intrinsic pathway factors are I, II, IX, X, XI, and XII.[21] This study shows that *O. vulgare* leaf extract concentrations prolonged aPTT results compared to the baseline control, showing a decreasing trend as the concentration increases. A similar study indicates a longer aPTT from 32.03±0.20 sec to 37.43±1.60 sec at 500 µM quercetin concentration.[22] Studies on flavonoid-rich plants, including *Thymus atlanticus, Thymus zygis, and Licania rigida*, showed prolonged aPTT findings with consistent concentrations.[2] This suggests a dose-dependent link between *O. vulgare* methanolic extract content and aPTT.

Moreover, this study shows that the optimal concentration and baseline control aPPT results varied significantly. A study on *Berberis vulgaris, Teucrium polium*, and *Orthosiphon aristatus* extracts showed prolonged aPTT results compared to the baseline. This investigation discovered that 25 mg/mL *O. vulgare* extract modulates aPTT best, which is also the concentration that inhibited coagulation the best for the study mentioned prior.[23]

Multiple research trends demonstrate the anticoagulant activities of flavonoid-rich plants. PT expands with plant extract concentration, similar to *Calotropis procera* leaf extract and *C. crepidioides* extracts.[6,9] This suggests that certain plant extracts can significantly affect the intrinsic blood coagulation pathway when quantities are reduced. Further research has shown that aPTT durations vary with plant extract content and dose.[2,22,23] After investigations indicated that flavonoid chemical levels were positively connected, aPTT was prolonged. The association suggests that plant active metabolites regulate coagulation processes, stressing the relevance of natural compounds in clotting problem medicine formulation.

The systematic integration of several studies examining the anticoagulant capabilities of diverse plant extracts provides a detailed comprehension of their impact on blood coagulation parameters. There is a predictable pattern showing that plant extract content directly affects coagulation parameters. Plant extracts significantly impact coagulation processes because they affect extrinsic and intrinsic pathways. Despite using different plant species and methodologies in multiple studies, the reported benefits remain solid and reliable. These findings have profound consequences for treating clotting issues with plant extracts.

CONCLUSION

The study provides convincing evidence of the anticoagulant effects of *O. vulgare* leaf extract, as indicated by a substantial increase in PT and aPTT in clot-based tests. The discovered dose-response relationship highlights the potential effectiveness of the extract in regulating blood coagulation mechanisms. The presence of flavonoids in the extract offers a reasonable explanation for its observed effects, consistent with the established understanding of this chemical's capacity to modulate platelet activity and enhance blood circulation. These findings show possibilities for creating safer options to traditional anticoagulant treatments, with Oregano leaf extract potentially providing a natural, bioactive remedy. Nevertheless, additional investigation into the chemical composition of the extract and its impact on various demographic groups is necessary to thoroughly understand its therapeutic capabilities and facilitate its use in pharmaceutical and medical settings.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

APTT: Activated Partial Thromboplastin Time; **PT:** Prothrombin Time; **PPP:** Platelet-Poor Plasma.

SUMMARY

This study offers insight into the anticoagulant potential of Origanum vulgare. The findings could support the development of new drugs to meet therapeutic needs.

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