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Potential Anticoagulant Activity of *Thymus atlanticus* Leaves Part *in vitro* on Normal Plasma Samples

Mothana Abdullah Elhussein[1]; Albara Ahmad[2]; Yousif Mohammed Elmosaad[3]; Hisham Ali Waggiallah[4*]

[1]*Department of Hematology and Immunohematology, Faculty of Medical Laboratory, National University, Sudan*
[2]*Department of Hematology, Medical Laboratory Program, Alfajr College for Sciences and Technology, Sudan*
[3]*Department of Public Health, College of Applied Medical Sciences, King Faisal University, Saudi Arabia*
[4]*Department of Medical Laboratory Sciences, College of Applied Medical Sciences, Prince Sattam Bin Abdulaziz Universiy, Alkharj, Saudi Arabia*

Abstract

The purpose of this study was to evaluate the anticoagulant activity of *Thymus atlanticus* leaves.

Methods and Results: This cross-sectional study was carried out in Khartoum state from March 2021 to June 2021. This study comprised 60 healthy volunteers (40% men and 60% women) aged 19-25 years. Activated partial thromboplastin time (APTT) and prothrombin time (PT) were evaluated in the blood plasma with different amounts of *Thymus atlanticus* leaves using Coatron M1 (TECO Medical Devices). *Thymus atlanticus* leaves considerably lengthened APTT and PT in all tested concentrations. Furthermore, only the APTT test at a concentration of 5.17 µl revealed a significant difference in the anticoagulant activity of *Thymus atlanticus* leaves between males and females. No correlation between age and anticoagulant activity of *Thymus atlanticus* leaves on PT and APTT in the study was found. (**International Journal of Biomedicine. 2022;12(2):299-302.**)

Key Words: activated partial thromboplastin time • prothrombin time • *Thymus atlanticus*

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Introduction

In underdeveloped countries, thromboembolic diseases, such as heart attacks, deep vein thrombosis, and pulmonary emboli, are among the leading causes of death. Furthermore, anticoagulant medications, which inhibit blood coagulation, are a powerful means of preventing thrombosis illnesses.[1] In this regard, numerous studies have found that medicinal herbs are a good source of natural anticoagulant and antioxidant chemicals.[2] The plant kingdom is an important source of antioxidant chemicals, particularly phenolic content.[3] As a result, plant-based diets can help to minimize destruction caused by oxidative stress. Atherosclerosis, on the other hand, is a vascular disease characterized by the formation of atherosclerotic plaques, which involves numerous mechanisms such as endothelial dysfunction, degradation, thrombosis, inflammation, and oxidative stress.[4] As a result, medicinal plants can be used to prevent and treat various illnesses. Thyme, a member of the Lamiaceae family, is a medicinal and aromatic plant of the Mediterranean flora that has been used in traditional medicine to treat a variety of chronic conditions,

***Corresponding author**: Dr. Hisham Ali Waggiallah, Department of Medical Laboratory Sciences, College of Applied Medical Sciences, Prince Sattam Bin Abdulaziz University. Alkharj, Saudi Arabia. E-mail: hishamwagg30@hotmail.com*

including hypertension, heart disease, and diabetes mellitus. Moreover, various investigations have found that *Thymus* species have anti-inflammatory, antioxidant, and antibacterial properties.[5]

The purpose of this study was to evaluate the anticoagulant activity of *Thymus atlanticus* leaves.

Materials and Methods

Study design

This cross-sectional study was carried out in Khartoum state from March 2021 to June 2021. This study comprised 60 healthy volunteers (40% men and 60% women) aged 19-25 years.

Inclusion criteria: Physically fit individuals who do not have bleeding disorders or thrombosis problems and do not use anticoagulant medications.

Exclusion criteria: Participants with a history of bleeding disorders or thrombosis illness, as well as those on anticoagulant medications.

Sampling

Each subject had 2.5 ml of fresh venous blood collected in a trisodium citrate bottle, and activated partial thromboplastin time (APTT) and prothrombin time (PT) were evaluated with different amounts of *Thymus atlanticus* leaves. The anticoagulant activity was assessed using Coatron M1 (TECO Medical Devices). Participants' demographic information was gathered using a structured questionnaire.

Plant Material Collection and Preparation

The leaves of *Thymus atlanticus* were obtained from a local market. They were ground, shade-dried, and ground again into a fine powder for extraction. At the Medicinal and Aromatic Plants Research Institute (MAPRI), the plant components were authenticated, and a voucher specimen was placed in the herbarium.

Preparation of Aqueous Extracts

Each extraction process used the air-dried leaf portion of *Thymus atlanticus* (20g). Plant pieces were ground to fine particles in a blender and mixed with 500 ml of double-distilled water. The sample was filtered after extraction using the techniques indicated; subsequently, the extracts were concentrated to dryness, and the residues were kept at 4°C. Each extraction experiment was repeated three times.[6] In the decoction extracting technique, aqueous preparations of plant components were cooked in water for 30 minutes before being filtered and cooled.

Soxhlet Extraction Method

In the Soxhlet extraction procedure, aqueous preparations of plant components were extracted for 5 hours using Soxhlet extractors, and the resulting boiled preparation was filtered and chilled. The powered plant was placed in a stoppered container holding 500 ml of double-distilled water in the dark for 12 hours at room temperature with constant stirring for this approach.[6]

Phenolic Compounds Determination

The total phenolic composition of the plant was measured using the method outlined by Alem et al.[7] Gallic acid was used to create the calibration curve. The total phenolic compounds were expressed in mg/g dry matter plant as gallic acid equivalent. The determination of the total flavonoid content of a plant was studied using a method proposed by Bammou et al.[8]

In Vitro Anticoagulant Activity

With only minor modifications, in vitro anticoagulant activity was assessed using the methods mentioned by Athukorala et al.[9]) For the APTT experiment, 50 µl of citrated normal human plasma was combined with 25 µl of aqueous extract and incubated for 10 minutes at 37 °C before adding 50 l of APTT reagent and incubating for 3 minutes at 37 °C.

Thus, clotting was induced and time was monitored by adding 50 µl of $CaCl_2$ (0.025 mol/L). 50 µl of citrated normal human plasma was combined with 25 µl of aqueous extract and incubated for 10 minutes in the PT assay. Then, 100 ml PT reagents were added after being pre-incubated for 10 minutes at 37°C, and the clotting time was recorded.

Statistical analysis was performed using the standard Statistical Package for the Social Sciences (IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY: IBM Corp). Continuous variables were presented as mean±standard deviation (SD). For data with normal distribution, inter-group comparisons were performed using Student's t-test. A probability value of $P \leq 0.05$ was considered statistically significant.

The study was approved by the Ethics Committee of the College of Medical Laboratory Sciences, Prince Sattam Bin Abdulaziz University. Written informed consent was obtained from all participants.

Results

Thymus atlanticus leaves were examined for blood coagulation effects in normal human plasma and were shown to be extremely significant prolonged APTT and PT of the human plasma ($P \leq 0.001$, Eta squared ($^2\eta$) value between 0.50 and 1.0) (Table 1). Also, there was no significant difference in *Thymus atlanticus* activity on PT according to gender; however, APTT indicated a significant difference ($P \leq 0.05$) between males and females at concentration 5.17 µl (Table 2).

Table 1.

Effects of different concentrations of Thymus atlanticus in the PT and APTT tests

Test	Concentration, µl	Mean±SD	T	*P*	Eta Squared
PT, sec	Control	14.6±1.2			
	0.18	13.4±2.1	-4.01	0.000	0.12
	1.34	25.3±1.8	38.4	0.000	0.93
	5.17	97.4±1.4	138.2	0.000	0.99
APTT, sec	Control	23.2±2.9			
	0.18	35.8±6.8	13.2	0.000	0.56
	1.34	63.3±9.5	31.2	0.000	0.89
	5.17	108.5±6.2	97.1	0.000	0.99

Table 2.

Thymus atlanticus activity on the PT and APTT tests according to gender

Test	Concentration, µl	Male (n=24)	Female (n=36)	T	*P*
PT, sec	Control	14.4±1.2	14.8±1.1	1.28	0.203
	0.18 µl	13.0±1.9	13.6±2.2	0.93	0.355
	1.34 µl	25.2±2.2	25.3±1.5	0.30	0.762
	5.17 µl	97.0±1.3	97.6±1.4	1.68	0.099
PTT, sec	Control	23.0±3.1	23.3±2.8	0.47	0.643
	0.18 µl	34.5±6.8	36.7±6.8	1.21	0.233
	1.34 µl	61.1±8.9	64.8±9.8	1.50	0.139
	5.17 µl	110.5±5.6	107.2±6.3	2.09	0.041

When PT and APTT tests were performed in different concentrations of *Thymus atlanticus* leaves among different age groups using the regression test to find a relationship between increasing the dose and age, it was discovered that no significant relationship exists in all concentrations and age groups (Table 3).

Table 3.

Regression analysis for the PT and APTT test and different concentrations of Thymus atlanticus among different age groups

Test	Concentration, µl	19-21 yrs (n=28)	>22 yrs (n=32)	T	*P*	Eta Squared
PT, sec	Control	14.6±0.88	14.7±1.42	0.272	0.786	0.001
	0.18	13.1±2.1	13.5±2.1	0.715	0.477	0.009
	1.34	25.6±1.4	25.0±2.1	1.20	0.234	0.024
	5.17	97.6±1.3	97.2±1.5	1.09	0.282	0.020
APTT, sec	Control	23.0±2.6	23.3±3.1	0.465	0.643	0.004
	0.18	36.4±6.3	35.3±7.3	0.58	0.564	0.006
	1.34	63.2±9.5	63.4±9.7	0.10	0.921	0.000
	5.17	109.2±5.6	108.0±6.7	0.704	0.484	0.008

Table 4.

Regression analysis for the different concentrations of Thymus atlanticus leaves in the PT and APTT tests

Test	Concentration, µl	B	Beta	R	R^2	F	T	*P*
PT, sec	0.18	0.05	0.089	0.089	0.008	0.46	0.68	0.050
	1.34	0.22	0.33	0.33	0.11	6.84	2.62	0.011
	5.17	-0.011	-0.013	0.013	0.001	0.010	-0.10	0.919
APTT, sec	0.18	-0.011	-0.026	0.026	0.001	0.038	-0.196	0.845
	1.34	0.073	0.241	0.241	0.058	3.58	1.89	0.064
	5.17	0.025	0.053	0.053	0.003	0.165	0.407	0.686

Furthermore, when we conducted the regression test to determine the proportion of the dose with the increase in blood fluidity, we discovered that concentrations of 0.18 µl and 1.34 µl were significantly effective in prolonging the time of the PT test, but had no effect on the APTT test (Table 4).

Discussion

Thrombosis is a major cause of cerebral infarction, acute myocardial infarction, and various types of cardiovascular illness.[10] Anticoagulants are drugs that are widely used to avoid and treat thrombotic disorders.[11] In this context, many epidemiological studies have shown that a diet rich in polyphenol compounds may reduce the development of cardiovascular disease.[12] On the other hand, the search for anticoagulant agents from natural herbal medicine is of intense interest because inhibiting blood coagulation through anticoagulant drugs is an important method of avoiding thrombotic disorders.[13]

In this study, different quantities of *Thymus atlanticus* leaves were tested for in vitro anticoagulant action in normal human plasma using two coagulation tests: APTT and PT. The APTT investigates the endogenous pathway of coagulation, whereas the PT investigates the exogenous pathway of coagulation.[14] According to the findings of our investigation, thyme leaves considerably lengthened coagulation time ($P \leq 0.05$) in a concentrated dependent way at all tested concentrations, as compared to controls in the APTT experiment.

Likewise to the APTT assay, thyme leaves inhibited clotting time in a concentration-dependent way. The current study's data clearly show that *Thymus atlanticus* leaves had a significant inhibitory effect on both intrinsic and extrinsic coagulation pathways. These findings are consistent with a recent study by Khouya et al.,[15] who found that *Thymus* plants increased the APTT and PT in human plasma. As a result, its high polyphenol content could be responsible for these effects. All aqueous extracts have significant antioxidant activity and are high in total polyphenols and flavonoids, although they behave differently in the inflammatory and coagulation processes investigated.

Hmidani et al.[6] undertook a follow-up study to assess and compare the anti-inflammatory, antioxidant, and anticoagulant effects of three thyme species grown in southeastern Morocco in vitro. *Thymus atlanticus* species were discovered to have significant antioxidant, anti-inflammatory, and anticoagulant properties. The bioactive compounds in these plants are responsible for the differences in their activities. The researchers believe that thymes have significant anticoagulant properties due to their high polyphenol content.

The study found no significant variation in coagulation time between males and females, except for APTT at a concentration of 5.17µl, when comparing anticoagulant activities of different concentrations of *Thymus atlanticus* leaves on APTT and PT according to gender. Furthermore, there was no correlation between age and anticoagulant activity of *Thymus atlanticus* leaves on PT and APTT in the study.

Polyphenol identified in *Thymus atlanticus* may be regarded as a potential bioactive compound for medicinal reasons, particularly in thrombosis-related illnesses. More research is needed to better understand the active chemicals found in Thymus plants as well as the processes through which they work. Further research with larger sample size is required to gain a deeper understanding of the population.

In conclusion, *Thymus atlanticus* leaves considerably lengthened APTT and PT in all tested concentrations. Furthermore, only the APTT test at a concentration of 5.17 µl revealed a significant difference in the anticoagulant activity of *Thymus atlanticus* leaves between males and females.

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Competing Interests

The authors declare that they have no competing interests.

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