International Journal of Traditional and Complementary Medicine Research

2026;7(1):6-12
https://doi.org/10.53811/ijtcmr.1730950

e-ISSN: 2717-7491
https://dergipark.org.tr/en/pub/ijtcmr

Research Article

Characterization and Evaluation of In-Vitro Hemostatic Properties of Turkish *Origanum*, *Satureja*, *Thymbra* and *Thymus* spp. Essential Oils

İlker Demirbolat*

Department of Pharmaceutical Chemistry, Istanbul Kent University, Faculty of Pharmacy, Istanbul, Türkiye. ror.org/01w9wgg77

*Corresponding author: demirbolatilker@gmail.com

Received: 30.06.2025
Revision: 24.11.2025
Accepted: 09.01.2026
Online: 27.04.2026

ABSTRACT

Aim: The aim of this study was to determine the in-vitro haemostatic properties of *Origanum*, *Satureja*, *Thymbra* and *Thymus* spp. essential oils by prothrombin time (PT) and activated partial thromboplastin time (aPTT) tests.

Materials and Methods: Essential oils of *Origanum dubium* Boiss., *Origanum majorana* L., *Origanum onites* L., *Origanum syriacum* subsp. *bevanii* (Holmes) Greuter & Burdet., *Origanum vulgare* subsp. *hirtum* (Link) Ietsw., *Satureja thymbra* L., *Thymbra spicata* L., *Thymbra capitata* (L.) Cav., *Thymus longicaulis* C.Presl and *Thymus vulgaris* L. were obtained by hydro distillation and analyzed for their chemical composition by gas chromatograph consisting of a mass spectrometer and a flame ionization detector (GC-MS/FID). Citrated human plasma were incubated with different concentration of essential oils. Prothrombin time and activated partial thromboplastin time tests were measured using citrated human plasma with a Diagnostica Stago Start coagulation analyzer.

Results: All tested essential oils exhibited varying degrees of inhibition on clotting factors, leading to prolonged prothrombin time and activated partial thromboplastin time. Notably, essential oils containing carvacrol demonstrated a greater inhibition of coagulation, even at lower concentrations compared with species containing thymol, which showed comparatively less pronounced effects.

Conclusion: It has been established that these essential oils possess anticoagulant properties, inhibiting blood clotting. Consequently, it is important to consider that the topical application of these essential oils, or formulations containing these oils, to open and bleed wounds for their antiseptic benefits may exacerbate bleeding due to their anticoagulant effects.

Keywords: Essential oil, chemical composition, anticoagulant, hemostatic properties.

INTRODUCTION

Anything that smells like carvacrol or thymol is commonly named as "kekik" in Turkiye. Species are sometimes titled kekik because they resemble the appearance of other species formerly called kekik whether they have the pungent scent of carvacrol-thymol or not. Turkish kekiks are the members of *Origanum*, *Satureja*, *Thymbra* and *Thymus* genera (Lamiaceae). Species of the Origanum genus is mostly distributed in the Mediterranean and Middle East regions with 22 species consisting of 32 taxa in Turkiye (1). Over 70 species of *Satureja* genus grow naturally in the Mediterranean region of Europe while Turkiye has 15 known taxa (2,3). *Thymus* and *Thymbra* are also Mediterranean genera represented by 57 and 4 taxa respectively in Turkiye (4).

Origanum onites (O. oni.), *Origanum vulgare (O. vul.)* and *Origanum majorana (O. maj.)* are the well-known members of the genus *Origanum*. *Origanum dubium (O. dub.)* and *Origanum syriacum (O. syr.)* also grow wild and are harvested in Turkiye. Carvacrol was found to be the major component of *O. oni.* (66.5-80.5%), *O. vul.* subsp. *hirtum* (23.4-78.7%), *O. syr.* var. *bewanii* (42.5%) and *O. maj.* (78.3-79.5%) essential oils (4). *O. maj.* has also been reported to have a 4-terpineol chemotype (5). *O. dub.* populations are diversified with carvacrol, carvacrol-linalool and linalool chemotypes (6). The carvacrol chemotype *O.dub.* was reported to have 78.0-86.8% (7) carvacrol whereas the carvacrol-linalool chemotype has an essential oil profile of 46.5% carvacrol and 48.3% linalool (8). Linalool content of linalool-rich chemotypes has previously found to be 91.6% (9).

Satureja thymbra (S. thy.) is the most common wild growing *Satureja* species in Turkiye. Essential oil was previously reported to contain 75.5% carvacrol (10). *Thymbra spicata (T. spi.)* and *Thymbra capitata (T. cap.)* (formerly *Coridothymus capitatus*) are two important species from genus *Thymbra*. *T. spi.* essential oil is used in traditional herbal medicine in Turkiye and should contain minimum 65% carvacrol as specified in the Turkish Pharmacopoeia (11). *T. cap.* essential oil is also rich in carvacrol with a reported amount of 73.2% (12). *Thymus vulgaris (T. vul.)* and *Thymus longicaulis (T. lon.)* both grow naturally in Turkiye. In comparison with the above-mentioned species, the essential oils of these two species contain thymol as the main component with the reported values of 46.2% for *T. vul.* and 21.73% for *T. lon.* (13,14).

Prothrombin time (PT) and activated partial thromboplastin time (aPTT) are coagulation assays commonly used to assess the blood's ability to clot, serving as fundamental indicators in the evaluation of hemostatic function. They help diagnose bleeding disorders and monitor anticoagulant therapy. The PT test serves as a key diagnostic tool for evaluating the extrinsic and common

Cite as: Demirbolat İ. Characterization and evaluation of *in-vitro* hemostatic properties of Turkish *Origanum*, *Satureja*, *Thymbra* and *Thymus* spp. essential oils. Int J Trad Complement Med Res. 2026;7(1):6–12. doi:10.53811/ijtcmr.1730950.

pathways of the coagulation cascade. This pathway is initiated by the exposure of tissue factor (Factor III), which, in the presence of calcium ions, activates Factor VII. The extrinsic cascade then merges with the common pathway through the activation of Factor X to Xa, which subsequently activates Factor V and converts prothrombin (Factor II) into thrombin (Factor IIa). Thrombin then facilitates the transformation of fibrinogen (Factor I) into fibrin, after which Factor XIII cross-links the fibrin strands to stabilize the clot. Clinically, PT is widely used to monitor vitamin K antagonist therapy (e.g., warfarin), evaluate hepatic synthetic function, and detect deficiencies in clotting factors involved in these pathways, particularly Factors I, II, V, VII, and X (15).

In contrast, the aPTT test is used to assess the intrinsic and common coagulation pathways. Activation is initiated in vitro by contact with negatively charged surfaces, which activate Factor XII and subsequently trigger the activation of Factors XI and then IX. In conjunction with Factor VIII and calcium ions, Factor IXa activates Factor X, thereby entering the common pathway. This leads to the generation of thrombin through the activation of prothrombin and ultimately results in the formation of a fibrin clot. The aPTT test is particularly important for monitoring unfractionated heparin therapy, identifying inherited or acquired deficiencies in intrinsic factors (notably Factors VIII, IX, XI, and XII), and detecting circulating coagulation inhibitors such as lupus anticoagulants (15).

The increasing popularity of complementary and alternative medicine has led to a rise in the use of aromatherapy essential oils for a variety of health concerns, such as anxiety, insomnia, skin conditions, respiratory issues, and musculoskeletal pain. Essential oils, particularly those rich in bioactive compounds like carvacrol and thymol, have gained attention for their antibacterial and antimicrobial properties. As a result, numerous topical products containing these oils, such as creams, ointments, and carrier-oil blends, are marketed as natural antiseptics to support wound healing and prevent infection.

While antibacterial agents are essential in the wound healing process, their potential effects on other physiological factors, such as coagulation, remain less explored. Specifically, the impact of essential oils on coagulation parameters -such as PT and aPTT- is not well understood. This gap in knowledge is significant, as any alteration in coagulation could have implications for wound healing, particularly in patients with compromised hemostatic function.

Given the widespread use of essential oils in wound care, this study aims to address this gap by investigating, for the first time, the in vitro hemostatic effects of ten different essential oils. The study will measure their impact on PT and aPTT in citrated human plasma, focusing on oils derived from species commonly found in Turkish flora, collectively known as *kekik*; *O. dub.* Boiss., *O. maj.* L., *O. oni.* L., *O. syr.* subsp. *bevanii* (Holmes) Greuter & Burdet., *O. vul.* subsp. *hirtum* (Link) Ietsw., *S. thy.* L., *T. spi.* L., *T. cap.* (L.) Cav., *T. lon.* C.Presl and *T. vul.* L.

By evaluating the hemostatic properties of these oils, this research aims to provide valuable insights into their potential effects on coagulation, which is critical for understanding their role in wound healing and infection control.

MATERIAL AND METHODS

Reagents

n-Hexane for gas chromatography was purchased from Merck (Darmstadt, Germany). C_7-C_{40} alkane series, α-pinene, carvacrol, eugenol, β-caryophyllene and β-caryophyllene oxide (enantiomeric form) were obtained from Sigma-Aldrich (St. Louis, MO, USA). Neoplastine Cl (PT reagent), C.K. Prest (aPTT reagent) and STA $CaCl_2$ were provided by Diagnostica Stago (Asnières sur Seine, France).

Collection of Plant Material

Cultivated samples of *O. dub.* Boiss., *O. maj.* L., *O. oni.* L., *O. syr.* subsp. *bevanii* (Holmes) Greuter & Burdet., *O. vul.* subsp. *hirtum* (Link) Ietsw., *S. thy.* L., *T. spi.* L., *T. cap.* (L.) Cav., *T. lon.* C.Presl and *T. vul.* L. were collected from Aegean Agricultural Research Institute of Ministry of Agriculture and Forestry experimental farms (GPS coordinates 38°33'58.2"N 27°03'10.1"E). Voucher specimens were deposited to Aegean Agricultural Research Institute herbarium with the following numbers TR-77670, 73007, 54558, 76973, 73523, 53198, 53196, 71556, 73022 and 76837 respectively.

Isolation of Essential Oils

Air dried leaves of each plant materials (500 grams) were grounded for coarse particles and subjected to hydro distillation by using a Clevenger apparatus described in European Pharmacopoeia for 3 hours in triplicate. The essential oils were isolated and dried over anhydrous sodium sulphate. Samples were kept in amber vials at 4 °C for further analysis

Analysis of Essential Oils

Solutions of 10% (v/v) essential oils in *n*-hexane were subjected to GC-FID/MS analysis. An Agilent 7890B GC-FID (Santa Clara, CA, USA) coupled to an Agilent 5977E electron impact mass spectrometer (Santa Clara, CA, USA) via a two-way capillary splitter was employed to identify and quantify essential oil components. An Agilent G4513A (Santa Clara, CA, USA) auto injector was utilised for injections of 1 µL sample solutions. HP-5MS column (30 m, 0.25 mm, 0.25 µm) was maintained with the following temperature program; 60 ^{0}C isothermal for 5 minutes then raised to 180 ^{0}C (3 ^{0}C/min) and finally held isothermal for 5 minutes at 180 ^{0}C. Total analysis time was 50 minutes. Helium was used as a carrier gas with a constant flow of 1.5 mL/min. Split ratio was set to 1:50. Temperatures of injector port, ion source, quadrupole, MSD transfer line and FID were as follows respectively; 250 ^{0}C, 230 ^{0}C, 150 ^{0}C, 250 ^{0}C and 220 ^{0}C. FID air flow was 400 mL/min and H_2 flow was adjusted to 30 mL/min. Mass detector scan range was set to 45-450 m/z.

Compounds were identified by comparing their spectral data obtained from the Wiley Registry of Mass Spectral Data 9th edition (April 2011) with NIST 11 Mass Spectral Library (NIST11/2011/EPA/NIH) and by co-injected authentic samples. Retention indices were calculated from co-injected alkane series (C_7-C_{40}) then compared with NIST webbook data. Quantification was carried out utilizing an external standard method based on calibration curves derived from GC-FID analyses of representative compounds.

In vitro haemostatic activities

Blood samples were collected from 5 healthy volunteers with the same blood type (0-positive) in BD Vacutainer tubes containing 3.8% trisodium citrate. Tubes were gently inverted for a few times immediately after collection and then centrifuged at 1500g for 15 minutes. Platelet poor plasmas (calculated with a Sysmex KX 21 Hematology Analyzer) were separated and pooled together. Overall thrombocyte count was less than 10000/µL. Citrated human plasma samples (250 µL) were transferred into polypropylene tubes. Different volumes of each essential oils (1, 2.5, 5, 10 and 20 µL) were added into the plasmas. Test samples were inverted for a few times and incubated for 15 minutes at 37 ^{0}C.

For PT tests, 50 µL test samples were added to coagulation cuvettes. Clotting was induced by introducing 100 µL PT reagent (Neoplastin Cl, previously incubated at 37 ^{0}C). Clotting times were recorded automatically with a Diagnostica Stago Start (Asnières sur Seine, France) haemostasis analyser. For aPTT assays, 100 µL of test samples and 50 µL aPTT reagent (C.K. Prest) were transferred to measurement cuvettes and incubated at 37 ^{0}C. Clotting was induced by adding $CaCl_2$ solution (STA $CaCl_2$ 0.025M). Citrated plasma without essential oil served as negative control for both tests.

The study protocol received approval from the Non-Interventional Research Ethics Committee of Bezmialem University (Approval No: 2021/352). Participants were provided with information regarding the study's purpose, scope, and confidentiality measures, and verbal informed consent was obtained in accordance with the principles of the Declaration of Helsinki (2013 revision). Participation was completely voluntary. No personal identifiers were collected, and all data were anonymized for exclusive use in scientific research. The study did not involve any medical or interventional procedures on the participants.

RESULTS

Given that certain plant species exhibit multiple chemotypes -individuals that are morphologically indistinguishable yet differ in the qualitative and quantitative composition of their essential oil constituents- the precise characterization of plant materials is essential for ensuring their consistency, efficacy, and safety in both traditional and clinical applications. In the present study, the selected species were systematically evaluated for essential oil yields, chemical compositions, and anticoagulant activities. Table 1 provides a detailed summary of the essential oil yields, along with the corresponding densities of the extracted oils.

Table 1. Yields and densities of essential oils.

Species	% Yield (ml/100 g)	% Yield (g/100 g)	Densities (g/ml)
O. dub.	6.5±0.2	5.53±0.17	0.8710±0.0013
O. maj.	2.3±0.1	2.13±0.09	0.8890±0.0009
O. oni.	3.2±0.3	2.96±0.28	0.9262±0.0021
O. syr.	4.8±0.3	4.41±0.28	0.9186±0.0017
O. vul.	5.4±0.4	4.98±0.37	0.9215±0.0015
S. thy.	6.3±0.5	5.75±0.46	0.9126±0.0011
T. spi.	5.7±0.4	5.25±0.37	0.9208±0.0017
T. cap.	4.9±0.5	4.50±0.46	0.9197±0.0022
T. lon.	1.2±0.2	1.09±0.18	0.9071±0.0016
T. vul.	4.2±0.6	3.83±0.55	0.9118±0.0012

Essential oils are complex mixtures of bioactive compounds, which vary in both qualitative and quantitative composition. These compounds, often present in trace amounts, can exhibit a wide range of biological activities. Given the variability in composition, a thorough understanding of the complete chemical profile of an essential oil is crucial for accurately assessing its pharmacological and therapeutic effects. In this context, the essential oils investigated in this study were analyzed for their full chemical composition. Tables 2 and 3 provide a detailed breakdown of the chemical constituents of the essential oils, which were further evaluated for their effects on PT and aPTT.

Table 2. Chemical compositions of essential oils.

No	Compound	R.T	R.I^{L}	*R.I^{C}*	Composition (%)			
					O. dub.	*O. maj.*	*O. oni.*	*O. syr.*
1	α-Thujene	6.65	927	926	ND	0.493	1.013	1.138
2	α-Pinene#	6.89	936	934	0.023	0.602	0.498	0.556
3	Camphene	7.43	950	955	ND	0.288	0.251	0.204
4	Sabinene	8.42	973	972	ND	5.396	0.035	0.054
5	β-Pinene	8.50	978	981	ND	0.363	0.109	0.156
6	1-Octen-3-ol	8.93	986	985	0.072	ND	0.096	0.335
7	3-Octanone	9.09	987	986	ND	ND	ND	ND
8	Myrcene	9.20	989	987	0.058	0.643	1.379	2.727
9	3-Octanol	9.59	994	990	ND	ND	ND	0.08
10	α-Phellandrene	9.72	1004	1001	ND	0.234	0.108	0.190
11	δ-3-Carene	9.94	1007	1012	ND	ND	0.07	0.067
12	α-Terpinene	10.27	1017	1020	ND	6.244	1.214	1.145
13	p-Cymene	10.69	1024	1022	0.062	0.564	4.181	4.500
14	β-Phellandrene	10.81	1027	1028	ND	2.937	0.396	0.514

Table 2 (cont.). Chemical compositions of essential oils.

15	1,8-Cineole	10.92	1031	1030	ND	ND	ND	0.081
16	cis-Ocimene	11.26	1039	1041	ND	0.036	ND	0.068
17	trans-Ocimene	11.74	1054	1056	ND	0.066	0.052	ND
18	γ-Terpinene	12.27	1059	1062	0.047	10.899	6.006	6.083
19	trans-Sabinen Hydrate	12.76	1066	1069	ND	2.954	0.230	0.348
20	cis-Linalool Oxide	12.96	1075	1081	0.200	ND	ND	ND
21	α-Terpinolene	13.51	1086	1089	ND	2.558	0.145	0.105
22	trans-Linalool Oxide	13.70	1091	1093	0.333	ND	ND	ND
23	cis-Sabinene Hydrate	14.21	1099	1096	ND	12.116	0.186	0.095
24	Linalool	14.34	1100	1103	96.997	ND	0.900	0.253
25	1-Terpineol	15.27	1136	1131	ND	2.266	0.060	ND
26	Pinocarveol	16.03	1143	1145	ND	0.081	0.040	ND
27	Camphor	16.17	1145	1149	ND	ND	ND	ND
28	Borneol	17.36	1166	1171	0.086	1.180	1.379	0.636
29	4-Terpineol	17.84	1177	1179	0.077	25.274	1.120	0.744
30	p-Cymene-8-ol	18.45	1182	1186	ND	ND	ND	ND
31	α-Terpineol	18.70	1190	1189	ND	5.387	0.131	0.152
32	Dihydrocarvone	19.06	1194	1198	ND	0.065	ND	ND
33	Piperitol	19.37	1204	1207	ND	0.680	ND	ND
34	Nerol	20.31	1230	1233	ND	0.077	ND	ND
35	Thymol Methyl Ether	20.82	1235	1238	ND	0.090	0.049	0.693
36	Neral	21.09	1242	1244	ND	ND	ND	0.114
37	Carvone	21.28	1243	1249	ND	ND	0.094	ND
38	Carvenone	21.33	1252	1250	ND	ND	ND	ND
39	Linalyl Acetate	21.45	1253	1255	ND	3.474	ND	ND
40	Geraniol	21.52	1267	1263	0.047	ND	ND	ND
41	Geranial	22.66	1269	1271	ND	ND	ND	ND
42	Bornyl Acetate	22.66	1287	1292	ND	0.790	ND	ND
43	Thymol	23.73	1302	1301	0.41	0.076	0.740	8.425
44	Carvacrol#	24.57	1317	1322	0.634	5.418	75.759	69.103
45	Eugenol#	26.11	1358	1349	ND	ND	ND	ND
46	Neryl Acetate	26.18	1363	1367	ND	0.081	ND	ND
47	α-Copaene	26.45	1376	1381	ND	ND	ND	ND
48	β-Bourbonene	26.81	1384	1386	ND	ND	ND	ND
49	Geranyl Acetate	27.01	1392	1390	ND	0.170	ND	ND
50	Caryophyllene#	28.23	1420	1425	ND	2.396	0.711	0.682
51	α-Humulene	29.68	1452	1463	ND	0.113	ND	ND
52	Germacrene	30.81	1480	1472	0.075	0.030	0.379	ND
53	Bicyclogermacrene	31.41	1499	1493	0.197	1.367	0.101	0.144
54	β-Bisabolene	31.94	1506	1511	ND	0.17	1.221	0.141
55	δ-Cadinene	32.51	1523	1527	ND	ND	0.070	ND
56	Spathulenol	34.72	1577	1572	0.045	0.100	0.138	0.123
57	Caryophyllene Oxide#	34.83	1583	1581	ND	0.087	0.272	0.173

#; Co-injected authentic samples of reference substances for identification and quantitation, R.T; Retention time (min), R.I^L; Retention indices derived from literature and NIST database, R.I^C; Calculated retention indices, ND; Not detected.

Table 3. Chemical compositions of essential oils.

No	Compound	Composition (%)					
		O. vul.	*S. thy.*	*T. spi.*	*T. cap.*	*T. lon.*	*T. vul.*
1	α-Thujene	1.348	1.449	1.269	1.088	1.414	1.080
2	α-Pinene#	0.550	0.936	0.435	0.478	0.525	1.042
3	Camphene	0.066	0.289	0.057	0.126	0.508	0.942
4	Sabinene	0.072	0.11	ND	0.029	0.091	ND
5	β-Pinene	0.152	0.426	0.136	0.132	0.212	0.292
6	1-Octen-3-ol	0.077	0.196	0.207	0.296	0.415	0.976
7	3-Octanone	ND	ND	ND	ND	0.942	0.105
8	Myrcene	2.731	1.608	1.380	1.462	0.544	1.903
9	3-Octanol	ND	ND	0.048	0.035	0.128	0.089
10	α-Phellandrene	0.211	0.264	0.169	0.208	0.115	0.192
11	δ-3-Carene	0.074	0.081	0.068	0.065	0.063	0.107
12	α-Terpinene	1.819	2.888	1.105	1.547	1.153	1.869
13	p-Cymene	3.508	2.492	4.218	4.174	15.519	22.644
14	β-Phellandrene	0.385	0.555	0.343	0.431	0.717	0.809
15	1,8-Cineole	ND	ND	ND	ND	0.069	0.634
16	cis-Ocimene	ND	ND	ND	ND	ND	ND
17	trans-Ocimene	0.204	0.104	0.052	0.066	0.069	0.062
18	γ-Terpinene	11.446	35.673	7.436	8.215	3.828	14.621

Table 3 (cont.). Chemical compositions of essential oils.

19	trans-Sabinen Hydrate	0.280	0.227	0.078	0.253	1.220	0.889
20	cis-Linalool Oxide	ND	ND	ND	ND	ND	ND
21	α-Terpinolene	0.101	0.089	0.071	0.098	0.211	0.133
22	trans-Linalool Oxide	ND	ND	ND	ND	ND	0.059
23	cis-Sabinene Hydrate	0.062	0.076	ND	0.088	0.258	0.355
24	Linalool	0.092	0.938	0.107	0.404	0.167	0.353
25	1-Terpineol	ND	ND	ND	0.046	0.178	2.474
26	Pinocarveol	ND	ND	ND	ND	0.077	ND
27	Camphor	ND	ND	ND	ND	0.230	0.358
28	Borneol	0.118	0.494	0.131	0.547	1.642	1.121
29	4-Terpineol	0.733	0.481	0.438	0.689	1.747	0.687
30	p-Cymene-8-ol	ND	ND	ND	ND	0.204	ND
31	α-Terpineol	ND	ND	0.092	0.100	0.092	ND
32	Dihydrocarvone	ND	ND	ND	ND	ND	ND
33	Piperitol	ND	ND	ND	ND	ND	ND
34	Nerol	ND	ND	ND	ND	ND	ND
35	Thymol Methyl Ether	2.061	0.059	ND	0.050	1.370	0.582
36	Neral	ND	ND	ND	0.110	ND	ND
37	Carvone	ND	ND	ND	0.066	0.163	ND
38	Carvenone	ND	ND	ND	0.041	ND	ND
39	Linalyl Acetate	ND	ND	ND	ND	ND	ND
40	Geraniol	ND	ND	ND	ND	ND	ND
41	Geranial	ND	ND	ND	0.176	0.062	ND
42	Bornyl Acetate	ND	ND	ND	ND	ND	0.230
43	Thymol	0.255	0.053	0.382	0.421	49.609	35.093
44	Carvacrol#	72.117	44.282	80.340	73.933	10.139	2.629
45	Eugenol#	ND	ND	ND	0.063	ND	ND
46	Neryl Acetate	ND	ND	ND	ND	ND	ND
47	α-Copaene	ND	ND	ND	ND	ND	0.075
48	β-Bourbonene	ND	ND	ND	ND	ND	0.122
49	Geranyl Acetate	ND	ND	ND	ND	ND	ND
50	Caryophyllene#	0.762	4.606	0.614	3.670	1.681	3.620
51	α-Humulene	0.087	0.222	ND	0.163	0.092	0.128
52	Germacrene	ND	ND	ND	0.028	ND	0.555
53	Bicyclogermacrene	0.073	0.207	ND	ND	ND	ND
54	β-Bisabolene	0.477	ND	ND	ND	0.325	0.065
55	δ-Cadinene	ND	ND	ND	0.03	ND	0.607
56	Spathulenol	ND	0.102	ND	ND	ND	ND
57	Caryophyllene Oxide#	0.077	0.180	0.118	0.346	1.305	0.439

#; Co-injected authentic samples of reference substances for identification and quantitation, ND; Not detected.

PT and aPTT are hematological assays employed to assess the coagulation cascade and the overall hemostatic function of blood. These tests are critical for evaluating both intrinsic and extrinsic pathways of coagulation and for detecting coagulopathies. Table 4 presents the effects of the tested essential oils on hemostatic parameters.

Table 4. PT and aPTT test results of the essential oils.

Sample	1 µl		2,5 µl		5 µl		10 µl		20 µl	
	PT	aPTT	PT	aPTT	PT	aPTT	PT	aPTT	PT	aPTT
O. dub.	15.6	47.1	21.8	88.1	22.5	91.7	22.8	93.6	23.2	94.7
O. maj	12.8	43.4	25.4	98.5	26.7	102.3	28.3	107.2	29.5	124.8
O. oni	27.2	83.6	73.0	247.5	NC	NC	NC	NC	NC	NC
O. syr.	21.7	68.7	67.6	213.0	NC	NC	NC	NC	NC	NC
O. vul.	25.1	78.3	59.6	198.5	NC	NC	NC	NC	NC	NC
S. thy.	19.4	68.3	47.6	124.3	49.1	139.2	70.3	156	NC	NC
T. spi.	27.6	72.4	76.1	256.8	NC	NC	NC	NC	NC	NC
T. cap.	26.3	76.4	69.2	243.4	NC	NC	NC	NC	NC	NC
T. lon.	18.0	55.2	36.6	152.0	42.1	176.3	59.8	223.7	NC	NC
T. vul.	18.2	57.6	38.9	156.5	49.3	187.6	67.8	252.1	NC	NC

PT and aPTT for blank citrated human plasma were 12.6 and 37.7 seconds respectively. NC; indicates that the test was automatically terminated after 500 seconds, signifying the absence of clot formation within the measurement period.

DISCUSSION

Based on the compositional analyses, *O. oni., O. syr., O. vul., T. spi. and T. cap.* were classified as carvacrol chemotypes, reflecting a predominance of this monoterpenoid phenol in their essential oil profiles. *O. maj.* exhibited a chemical profile largely dominated by 4-terpineol, whereas *T. lon.* and *T. vul.* were identified as thymol-dominant species, indicative of a high concentration of this bioactive phenolic compound. *O. dub.*

was characterized as an almost pure linalool chemotype, with linalool constituting the overwhelming majority of its essential oil composition. The essential oil yields obtained in this study, along with their corresponding chemical compositions, are consistent with previously reported values and chemotypic profiles (4-14,16).

As illustrated in Table 4, the addition of even 1 µL of essential oil resulted in a measurable prolongation of both PT and aPTT, indicating a dose-dependent anticoagulant activity. Notably, species in which carvacrol was the predominant constituent exhibited anticoagulant effects approximately four times greater than those dominated by thymol. In contrast, *O. maj*., which is relatively rich in 4-terpineol, and *O. dub*., which contains a high concentration of linalool, demonstrated comparatively lower levels of anticoagulant activities. These findings suggest that the specific chemical profile of the essential oils plays a critical role in determining their anticoagulant potency.

Previous studies with same species have investigated the hemostatic potential of plant-derived extracts, particularly those obtained from *S. thy.* and *T. spi.*, using in vitro assays. These investigations demonstrated that the extracts possess measurable anticoagulant activity, indicative of their capacity to modulate coagulation pathways (17). Corroborating these in vitro findings, an in vivo study in murine model showed that administration of *T. vul.* extract led to prolonged coagulation times, thereby confirming the anticoagulant effects (18). Furthermore, clinical evidence indicates that oral administration of high doses of carvacrol in healthy human subjects significantly prolongs PT, supporting its anticoagulant property (19). Notably, the magnitude of anticoagulant effects reported in these prior studies is lower than that observed with essential oils in the present investigation, likely reflecting the higher concentrations of active anticoagulant constituents in essential oils relative to crude extracts. Although systematic investigations into the anticoagulant properties of essential oils and plant-derived extracts remain limited, the available evidence is consistent with and reinforces the findings of this study, highlighting the potential of these essential oils as modulators of hemostasis.

Although the precise mechanism remains unclear, the pronounced anticoagulant activity observed in plants rich in carvacrol and thymol suggests that these phenolic compounds are likely responsible for the observed effects. It is well established that polyphenol-rich plants can enhance bleeding tendencies, and their use prior to surgical procedures is generally contraindicated due to the risk of excessive hemorrhage. It is hypothesized that carvacrol and thymol may exert their anticoagulant effects by interacting with key clotting factors, potentially inactivating them through a similar biochemical mechanism.

It is important to note that this study was conducted in vitro, and as such, the applicability of these findings to in vivo systems remains uncertain. The potential anticoagulant effects of carvacrol and thymol observed in this controlled environment may not necessarily reflect the same potency or mechanism when these compounds enter systemic circulation. Further research is needed to determine whether these phenolic compounds maintain their anticoagulant activity in vivo and at therapeutic concentrations.

CONCLUSION

Although the use of natural products for therapeutic purposes and general health maintenance has become increasingly popular, scientific research on their pharmacological effects remains insufficient. The present study demonstrates that essential oils rich in carvacrol and thymol inhibit coagulation pathways and significantly prolong bleeding time. Numerous commercially available topical formulations -ranging from semi-solid preparations such as creams and ointments to diluted carrier oil blends and hydrosols- incorporate these essential oils and are commonly employed as natural antiseptics, particularly in the context of wound healing and infection prevention. Given their anticoagulant potential, such formulations should be applied with caution, especially to open wounds, where impaired hemostasis may lead to adverse outcomes.

DECLARATIONS

Acknowledgements: The author does not wish to acknowledge any individual or institution.

Author Contributions: All parts of the study were conducted by the author.

Conflict of Interest: The author declares no conflict of interest.

Supporting Institutions: This research received no external funding.

Ethical Approval: This study was conducted ethically in accordance with the World Medical Association Declaration of Helsinki and authorized by Bezmialem Vakif University Non-Interventional Research Ethics Committee with the approval number of 2021/352. All participants signed the consent form after having received an explanation about the purpose of the research, which was conducted ethically in accordance with the World Medical Association Declaration of Helsinki.

Plagiarism Statement: This article has been evaluated for plagiarism, and no instances of plagiarism were detected.

Use of AI Tools: The author declare that no Artificial Intelligence (AI) tools were used in the creation of this article.

REFERENCES

1. Kizil S, Ipek A, Arslan N, Khawar KM. Some agronomical characteristics and essential oil content of oregano (*Origanum onites* L.) as influenced by planting densities. Journal of Essential Oil Bearing Plants. 2009;12(2):172-80. doi:10.1080/0972060X.2009.10643707.
2. Fırat M. *Satureja avromanica* Maroofi (*Lamiaceae*): an addition to flora of Turkey with contributions to its taxonomy. Issues in Biological Sciences and Pharmaceutical Research. 2015;3(12):123-8. doi:10.15739/ibspr.022.
3. Tümen G, Kirimer N, Ermin N, Baser KHC. The essential oils of two new *Satureja* species from Turkey: *Satureja pilosa* and *S. icarica*. J Essent Oil Res. 1998;10(5):524-6. doi:10.1080/10412905.1998.9700959.
4. Baser KHC, Özek T, Tümen G, Sezik E. Composition of the essential oils of Turkish *Origanum* species with commercial importance. J Essent Oil Res. 1993;5(6):619-23. doi:10.1080/10412905.1993.9698294.

5. Raina AP, Negi KS. Essential oil composition of *Origanum majorana* and *Origanum vulgare* ssp. *hirtum* growing in India. Chem Nat Compd. 2012;47(6):1015-7. doi:10.1007/s10600-012-0133-4.
6. Turgut K, Özyigit Y, Tütüncü B, Sözmen EU. Agronomic and chemical performance of selected *Origanum dubium* Boiss. clones for industrial use. Turk J Agric For. 2017;41(4):272-7. doi:10.3906/tar-1612-75.
7. Kaplan B, Sözmen EU, Turgut K. Chemical diversity of essential oils within the population of *Origanum dubium* Boiss. Nat Volatiles Essent Oils. 2019;6(4):6-12. izlik:JA85DT26RB.
8. Türkmen M, Kara M, Maral H, Soylu S. Determination of chemical component of essential oil of *Origanum dubium* plants grown at different altitudes and antifungal activity against *Sclerotinia sclerotiorum*. J Food Process Preserv. 2012;46:e15787. doi:10.1111/jfpp.15787.
9. Figuérédo G, Cabassu P, Chalchat JC, Pasquier B. Studies of Mediterranean oregano populations. VII: chemical composition of essential oils of carvacrol-rich oregano of various origins. J Essent Oil Res. 2006;18(3):244-9. doi:10.1080/10412905.2006.9699077.
10. Baydar H, Sağdiç O, Özkan G, Karadoğan T. Antibacterial activity and composition of essential oils from Origanum, *Thymbra* and *Satureja* species with commercial importance in Turkey. Food Control. 2004;15(3):169-72. doi:10.1016/S0956-7135(03)00028-8.
11. Demirbolat İ, Kartal M. Zahter uçucu yağı. Türk Farmakopesi Dergisi. 2021;6(1):13-5.
12. Gagliano Candela R, Maggi F, Lazzara G, Rosselli S, Bruno M. The essential oil of *Thymbra capitata* and its application as a biocide on stone and derived surfaces. Plants (Basel). 2019;8(9):300. doi:10.3390/plants8090300.
13. Ozcan M, Chalchat JC. Aroma profile of *Thymus vulgaris* L. growing wild in Turkey. Bulg J Plant Physiol. 2004;30(3-4):68-73.
14. Baser KHC, Özek T, Kürkçüoglu M, Tümen G. Composition of the essential oil of *Thymus longicaulis* C. Presl var. *subisophyllus* (Borbas) Jalas from Turkey. J Essent Oil Res. 1992;4(3):311-2. doi:10.1080/10412905.1992.9698070.
15. Bloom AL. Physiology of blood coagulation. Haemostasis. 1991;20(Suppl 1):14-29. doi:10.1159/000216159.
16. Demirbolat İ, Karık Ü, İşcan İ. Effect of cultivation and geographical location on chemical composition and enantiomeric distribution in linalool-rich *Origanum dubium* Boiss. essential oils obtained at different growth stages. J Essent Oil Res. 2025;37(1):88-94. doi:10.1080/10412905.2024.2404085.
17. Omar G, Abdallah L, Barakat A, Othman R, Bourinee H. In vitro haemostatic efficacy of aqueous, methanol and ethanol plant extracts of three medicinal plant species in Palestine. Braz J Biol. 201980(4):763-68. doi:10.1590/1519-6984.219186.
18. Mashkani ZS, Vatandoost J, Hajjar T, Mahdavi B. *In vivo* coagulation effects of *Thymus vulgaris* leaves extract in mice. Avicenna J Pharm Res. 2023;3(2):74-81.
19. Ghorani V, Alavinezhad A, Rajabi O, Mohammadpour AH, Boskabady MH. Safety and tolerability of carvacrol in healthy subjects: a phase I clinical study. Drug Chem Toxicol. 2021;44(2):177-89. doi:10.1080/01480545.2018.1538233.