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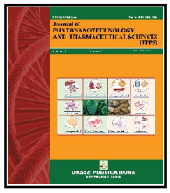

Original Article : Open Access

Thrombolytic activity of *Drosera peltata* Thunb. and *Yucca aloifolia* L.: A comparative *in vitro* evaluation

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Abstract

Thrombosis, the pathological formation of blood clots within vessels, is a leading cause of cardiovascular diseases, stroke, and pulmonary embolism. Disruption in the balance between coagulation and fibrinolysis underlies thrombotic disorders. While synthetic anticoagulants and thrombolytics are available, their use is often limited by adverse effects such as haemorrhage and resistance. This study explores the thrombolytic potential of *Drosera peltata* Thunb. and *Yucca aloifolia* L., medicinal plants traditionally used for their anti-inflammatory and analgesic properties. Ethanolic extracts of *D. peltata* leaves and *Y. aloifolia* roots were prepared *via* maceration and analyzed for phytochemical composition using gas chromatography-mass spectrometry (GC-MS). Thrombolytic activity was evaluated *in vivo* using albino Wistar rats divided into nine groups. Parameters such as bleeding time, clotting time, thrombus length, platelet count, leukocyte count, haemoglobin levels, and C-reactive protein (CRP) levels were measured. Histopathological analysis of thrombotic tissue was conducted to assess structural changes. Phytochemical screening revealed the presence of flavonoids, alkaloids, phenols, and saponins in both extracts. GC-MS identified bioactive compounds, including dimethyl sulfoxide, benzoic acid, coumarin, and hexadecanoic acid, known for their anti-inflammatory and anticoagulant properties. *In vivo* results showed significant reductions in thrombus length, prolonged bleeding time, and enhanced fibrinolytic activity in treated groups compared to controls. Reduced CRP levels and platelet counts indicated anti-inflammatory and antithrombotic effects. Histopathological analysis demonstrated decreased fibrin deposition and improved vascular integrity. The findings suggest that *D. peltata* and *Y. aloifolia* possess significant thrombolytic potential, likely mediated by flavonoids and other phytoconstituents that modulate coagulation pathways. These plants represent promising natural alternatives for thrombolytic therapy. Further studies, including molecular docking and clinical trials, are needed to elucidate mechanisms and validate therapeutic efficacy.

1. Introduction

Thrombosis, a pathological condition characterized by the formation of blood clots within the vasculature, is a major contributor to cardiovascular diseases (CVDs), stroke, and pulmonary embolism (Ahmed, 2011). The physiological balance between coagulation and fibrinolysis is critical in maintaining hemostasis, and any disruption in this balance can lead to thrombotic disorders (Chen *et al.,* 2018; Banu *et al.,* 2020). Antithrombotic therapy, including anticoagulants and thrombolytic agents, remains the mainstay of treatment. However, these pharmacological interventions are often associated with adverse effects such as haemorrhage, drug resistance, and systemic complications (Stone and Burns, 2018). Virchow's Triad, first described by Rudolf Virchow in 1856, outlines three primary factors contributing to thrombosis: endothelial injury, abnormal blood flow, and hypercoagulability (Harsh, 2005). Endothelial injury may result from infections, trauma, or catheter placement, while alterations in blood flow due to venous congestion or cardiovascular disorders further exacerbate thrombotic risk. Hypercoagulability, characterized by elevated levels of fibrinogen, prothrombin, and clotting factors, plays a pivotal role in thrombus formation (Vane, 1971). In recent years, plant-based thrombolytic agents have gained attention due to their bioactive compounds, including flavonoids and phenolics, which exhibit anti-inflammatory, antioxidant, and fibrinolytic properties (Yam *et al.,* 2018). *D. peltata* and *Y. aloifolia* have been traditionally used for their medicinal properties, including anti-inflammatory and analgesic effects (Vickers, 1999). Studies suggest that flavonoids present in these plants may contribute to fibrinolysis by modulating coagulation pathways (Jones *et al.,* 1993). Thus, the present study aims to evaluate the thrombolytic potential of ethanolic extracts of *D. peltata* and *Y. aloifolia*, analyzing their phytochemical composition and biological activity in an animal model.

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2. Materials and Methods

2.1 Collection and authentication of plant materials

The dried leaves of *Drosera peltata* Thunb. and dried roots of *Yucca aloifolia* L. were obtained and authenticated by Dr. K. Madhav Chetty, Professor and Head, Department of Botany, Tirupathi University, Andhra Pradesh, India. The plant specimens were deposited in the herbarium for reference (362-*D. peltata* and 361-*Y. aloifolia*).

2.2 Preparation of plant extracts

The collected plant materials were shade-dried, ground into fine powder, and stored in airtight containers. The ethanolic extract was

prepared using the maceration method. Briefly, 500 g of powdered plant material was soaked in ethanol (1:2 ratio) for seven days at room temperature with periodic agitation. The extract was filtered using muslin cloth and evaporated under reduced pressure to obtain a concentrated residue (Ahmed, 2011).

2.3 Phytochemical screening

Phytochemical screening was conducted using standard qualitative tests to detect alkaloids, flavonoids, phenols, saponins, glycosides, and tannins (Satoskar *et al.,* 2007; Naaz *et al.,* 2024).

2.4 Specification and procedure of GC-MS analysis

The gas chromatography-mass spectrometry (GC-MS) analysis was performed using a SHIMADZU QP-2010 GC-MS system. The instrument was equipped with a fused silica column packed with Elite-5 ms, composed of 5% diphenyl and 95% dimethylpolysiloxane, with dimensions of 30 mm × 0.25 mm × 0.25 µm. Helium was used as the carrier gas at a constant flow rate of 1 ml/min. A 2 µl volume of the seed extract was injected into the system for analysis. The detector employed was a Turbo gold mass detector operated with TurboMass 5.2 software. The GC oven temperature was initially set at 110°C with a holding time of 2 min. The injector temperature was maintained at 250°C, while the inlet line and source temperatures were both set at 200°C. Mass spectra were obtained under ionization energy of 70 eV, with a scan period of 0.5 seconds, covering a fragment range of 45 to 450 Da. The MS detection time was 31.2 min. Data interpretation was conducted using the National Institute of Standards and Technology (NIST) database, where the spectra of unknown components were compared with the NIST library for identification. GC-MS analysis was performed to identify the bioactive constituents present in the ethanolic extracts of *D. peltata* and *Y. aloifolia* (Liu, 2011; Banu *et al.,* 2025).

2.5 Experimental animals and grouping

A total of 54 male albino wistar rats (150-200 g) were used for *in vivo* thrombolytic evaluation. The study was approved by the Institutional Animal Ethics Committee (IAEC-02/0068/SES/2024/41/106). The animals were randomly divided into nine groups (n = 6 per group):

- **Group 1:** Normal control
- **Group 2:** Disease control (induced thrombosis)
- **Group 3:** Standard thrombolytic drug (positive control)
- **Groups 4-9:** Different doses of *D. peltata* and *Y. aloifolia* extracts

2.6 Evaluation of hemostatic and thrombolytic parameters in rats

The study involved a comprehensive assessment of hemostatic and thrombolytic parameters in rats, including bleeding time, clotting time, thrombus induction, biochemical and haematological analysis.

2.6.1 Bleeding time and clotting time

Bleeding time was determined by making a small incision on the rat's tail and recording the duration until the bleeding stopped, following the method described by Tripathi (2013). This test provided insights into the vascular response and platelet function. Clotting time was assessed by collecting blood in a capillary tube and breaking it at regular intervals to observe the formation of fibrin threads. The time taken for fibrin formation indicated the efficiency of the coagulation cascade.

2.6.2 Thrombus induction and measurement

Thrombus formation was induced in the rat tail, and the extent of clot development was evaluated at 24, 48, and 72 h post-treatment using a digital caliper, as described by Jones *et al.* (1993). This method allowed for precise measurement of thrombus length, providing crucial data on the progression and resolution of the thrombus under experimental conditions.

2.6.3 Biochemical and hematological analysis

To evaluate the anti-inflammatory and fibrinolytic activity, various biochemical and hematological parameters were measured, including haemoglobin levels, platelet count, leukocyte count, and C-reactive protein (CRP) levels. These markers provided insights into the systemic inflammatory response and the ability of the test substance to modulate hemostatic function (Yam *et al.,* 2018).

3. Results and Discussion

3.1 Phytochemical analysis

Preliminary phytochemical analysis of *D. peltata and Y. aloifolia* indicated that the plants are rich in various primary and secondary metabolites, as outlined in Table 1.

Table 1: Preliminary phytochemical analysis of *D. peltata and Y. aloifolia*

S.No.	Phytoconstituents	Tests	*D. peltata*	*Y. aloifolia*
1.	Alkaloid	Dragendroff's test	++	++
		Hager's test	++	+
		Mayer's test	++	+
		Wagner's test	++	++
		Barfoed's test	+	+
		Molish's test	-	+
2.	Carbohydrates	Seliwanoff's test	+	++
3.	Reducing sugars	Benedict's test	++	+
		Fehling's test	++	+

4.	Flavanoids	Alkalinereagenttest	++	+
		Shinoda test	++	+
	Ferric	chloridetest	++	+
5.	Glycosides	Borntrager's test	++	+
		Legal's test	++	+
		10% NaOH test	++	+
6.	Tannins	Bromine watertest	+	-
7.	Phenols	Iodine test	++	+
		Lead acetate test	++	+
	Ferric	chlorideTest	++	+
8.	Sterols	Salkowski's test	+	++
		Libermann burchard'sTest	+	+
9.	Saponins	Foam test	++	++
10.	Coumarins	NaOH test	++	++
11.	Terpenoids		-	++

(+) indicates presence, (-) indicates absence

Figure 1: Phytochemical analysis of extracts.

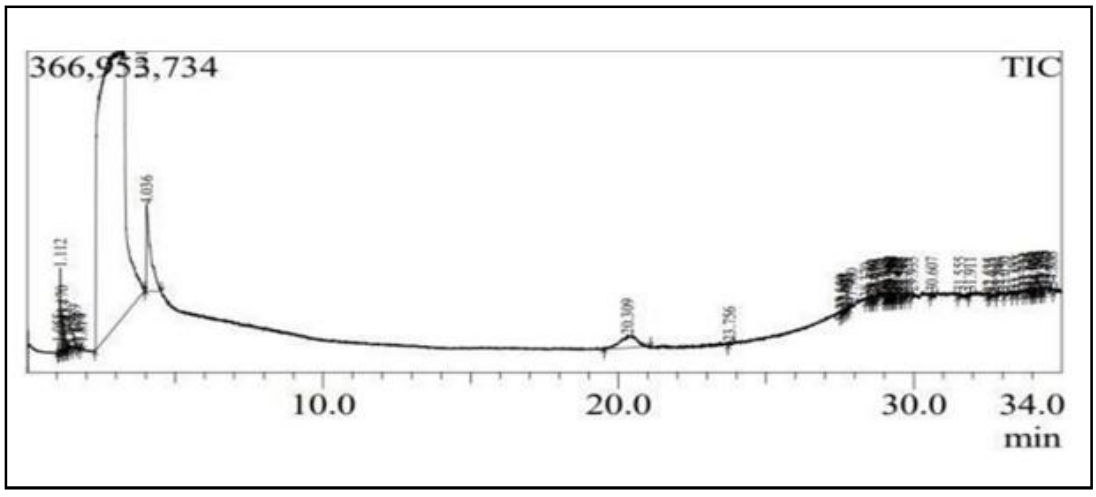

Figure 2: GC-MS chromatogram of *D. peltata*.

3.2 GCMS analysis of ethanolic extract of *D. peltata*

The GC-MS analysis of *D. peltata* revealed major peaks as shown in Figure 2 and compound identified in Table 2.

3.3 GC-MS analysis of ethanolic extract of *Y. aloifolia*

The GC-MS analysis of *Y. aloifolia* revealed major peaks as shown in Figure 3 and the compound identified in Table 3.

Table 2: GC-MS analysis results of *D. peltata*

S.No.	Retention time	Chemical constituents	Area %	Uses
1	34.039	Benzoic acid	0.09	Treat skin irritation and inflammation caused by burns
2	1.055	1,1-Cyclopropanedicarbonitrile	0.07	Decreases pain
3	20.309	Dimethyl sulfoxide	2.40	Treats painful bladder syndrome, and decreases topical pain. Treats inflammation, headache,osteoarthritis, rheumatoid arthritis, and severe facial pain.
4	34.304	Pentasiloxane	0.10	Anti-inflammatory removes wrinkles and skin blemishes and irritation, prevents scaling
5	34.405	Hexadecanoic acid	0.13	Anti-inflammatory
6	32.625	Hydroxybutyric acid	0.07	Analgesic
7	29.365	Phenothiazone 32	0.09	Treats moderate to severe pain.
8	33.535	Thiatriazole	0.07	Anti-inflammatory
9	31.055	Pyridine-2	1.02	Relieve symptoms caused by irritation of the urinary tract such as pain, burning, and the feeling of needing to urinate urgently or frequently.
10	34.20	Dimethoxyamine	2.80	Analgesic, Anti-inflammatory,

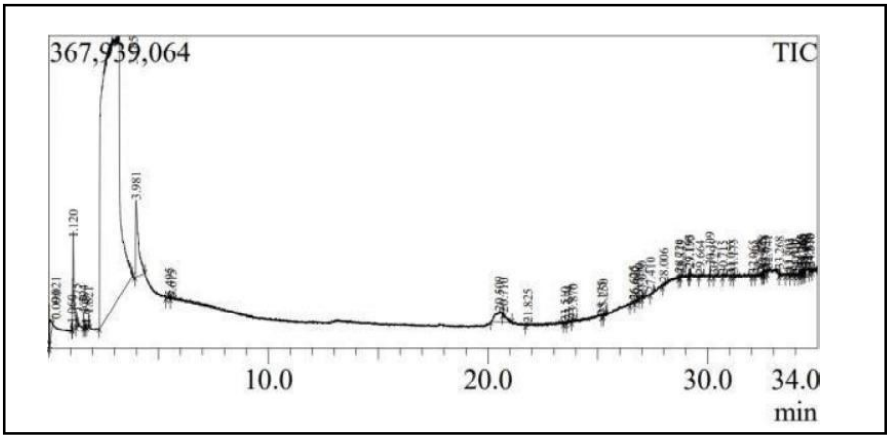

Figure 3: GC-MS Chromatogram of *Y. aloifolia*.

Table 3: GC-MS analysis results of *Y. aloifolia*

S.No.	Retention time	Chemical constituents	Area %	Uses
1	20.509	Dimethyl sulfoxide	1.75	Decreases pain and inflammation.
2	22.450	Sulfamide	1.02	Anti-inflammatory,Treats bronchitis, bacterial meningitis, ear and eye infections, UTI Infections, and severe burns.
3	26.890	Benzoic acid	0.80	Treat skin irritation and inflammation caused by burns
4	29.150	Phenol	0.05	Used as an oral analgesic, relieves itching, and treats pharyngitis.
5	24.063	Methyl ester	1.20	Anti-inflammatory
6	33.703	Pentasiloxane	0.17	Anti-inflammatory removes wrinkles and skin blemishes and irritation, prevents scaling
7	32.760	Butyric acid	0.20	Treats inflammatory conditions (non-specific bowel inflammation, diverticulitis, diversion colitis)
8	23.870	2-Propanol	1.34	Used to prevent migraine headaches and chest pain(angina)
9	25.175	Propanoic acid	1.02	Treatment of inflammation associated with tissue injury.
10	25.250	Coumarin-6-ol	1.07	Anti-inflammatory and antipyretic

3.4 Evaluation of hemostatic and thrombolytic parameters in rats

3.4.1 Length of thrombus in tail

The measurement of thrombus length in the tail was conducted to assess the extent of thrombosis progression over time. A calibrated scale marked in centimetres was used to ensure accuracy in measuring the thrombus formation at different time points. To monitor the dynamic changes, the thrombus was photographed at 24, 48, and 72 h following induction. These images provided a visual representation of thrombus development and allowed for comparative analysis over time (Figure 4). Additionally, a final observation and measurement were performed on the 29th day of the study, which marked the conclusion of the experimental period.The recorded data facilitated the evaluation of thrombus progression, regression, or stabilization, offering insights into the effectiveness of therapeutic interventions or the natural course of thrombosis under experimental conditions.

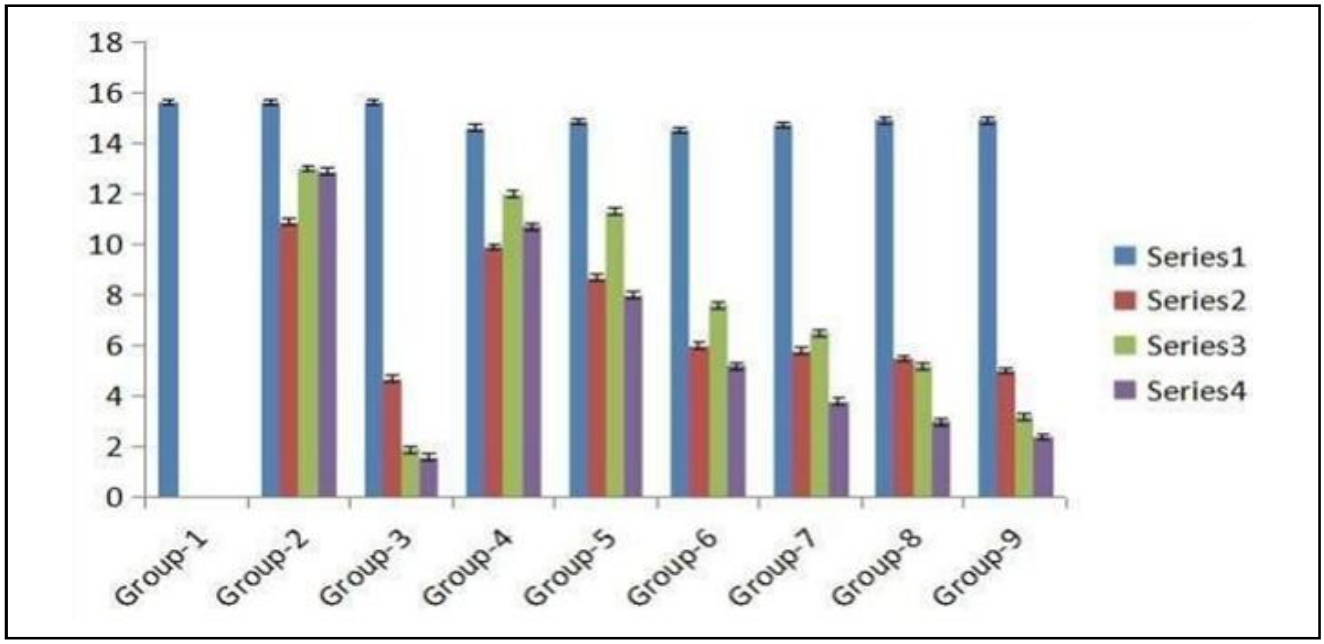

Figure 4: Length of thrombus in tail.

3.4.2 Bleeding time assessment in experimental groups

The bleeding time assessment was conducted by warming the rat's tail in 40°C water for approximately one min to enhance blood circulation. A small slit was then made in the middle of the tail using a scalpel, and the bleeding time was recorded from the moment the first drop of blood touched a filter paper until the bleeding completely stopped. Observations continued for up to 30 seconds after each drop to ensure accuracy in measuring the cessation of bleeding. The results showed significant variations in bleeding time across different experimental groups. Group 1 (Control) exhibited the longest bleeding time (96 ± 0.92 sec), indicating an unaltered coagulation process. In contrast, Group 2 had the shortest bleeding time (32.3 ± 0.10 sec), suggesting enhanced coagulation, possibly due to pro-coagulant treatment or physiological changes. Groups 3 to 9 displayed intermediate bleeding times, reflecting varying degrees of clotting efficiency influenced by different experimental interventions. The recorded bleeding times may correlate with platelet function, clotting factor activity, or pharmacological effects of test substances. These findings are critical for evaluating the impact of experimental conditions on hemostatic function and understanding potential therapeutic agents that modulate coagulation.

Table 4: Bleeding time across different groups

Treatment groups	Bleeding time (sec)
Group-1	96.0 ± 0.92
Group-2	32.3 ± 0.10
Group-3	59.5 ± 0.12
Group-4	35.5 ± 0.14
Group-5	42.7 ± 0.12
Group-6	45.5 ± 0.13
Group-7	53.9 ± 0.16
Group-8	55.5 ± 0.12
Group-9	56.6 ± 0.15

3.4.3 Blood clotting time

Blood clotting time refers to the duration required for blood to form a stable clot. In this procedure, a bold prick was made in the vein of the rat's tail, and the blood was collected in a 15 cm-long capillary tube. To determine the clotting time, the capillary tube was broken at 15-second intervals until a fine fibrin thread appeared between the fragmented sections. The time elapsed from the first appearance of blood from the vein to the formation of fibrin was recorded as the

blood clotting time. The results demonstrated noticeable differences in clotting time across the experimental groups. Group 1 exhibited the shortest clotting time (10 ± 0.75 min), indicating faster coagulation, while Group 2 had the longest clotting time (25 ± 0.72 min), suggesting delayed clot formation. Groups 3 to 9 showed varying clotting times, reflecting differences in coagulation potential influenced by experimental conditions. The clotting time may be affected by platelet function, fibrinogen levels, coagulation factor activity, or pharmacological interventions. These findings are essential for understanding hemostatic variations and evaluating the effects of different treatments on blood coagulation.

Table 5: Clotting time across different groups

Treatment groups	Clotting time (sec)
Group-1	10.0 ± 0.75
Group-2	25.0 ± 0.72
Group-3	12.5 ± 0.93
Group-4	22.3 ± 0.87
Group-5	20.0 ± 1.30
Group-6	18.5 ± 0.12
Group-7	17.5 ± 0.12
Group-8	15.6 ± 0.15
Group-9	13.3 ± 0.08

3.4.4 C-Reactive protein (CRP) levels

C-reactive protein (CRP) is an inflammatory biomarker used to assess systemic inflammation and the severity of physiological stress or disease conditions. Elevated CRP levels indicate an active inflammatory response, which can be associated with infections, tissue damage, or thrombotic events. In this study, CRP levels (measured in mg/dl) were analyzed across different experimental groups to evaluate inflammatory variations under different conditions.The results revealed a significant difference in CRP levels among the groups. Group 1 (Control) had the lowest CRP level (0.3 ± 0.03 mg/dl), indicating a normal inflammatory state. In contrast, Group 2 exhibited the highest CRP level (2.84 ± 0.17 mg/dl), suggesting a strong inflammatory response. Other groups showed intermediate CRP levels, with values ranging from 0.89 ± 0.17 mg/dl in Group 9 to 2.55 ± 0.25 mg/dl in Group 4. These variations reflect differences in immune responses, disease progression, or the effects of pharmacological interventions. The CRP levels observed in this study provide critical insights into the inflammatory status and potential therapeutic outcomes of experimental conditions.

Table 6: C-reactive protein (CRP) levels across different groups

Treatment groups	C-reactive protein levels (mg/dl)
Group-1	0.3 ± 0.03
Group-2	2.84 ± 0.17
Group-3	1.0 ± 0.08
Group-4	2.55 ± 0.25
Group-5	2.4 ± 0.27
Group-6	2.0 ± 0.29
Group-7	1.8 ± 0.28
Group-8	1.53 ± 0.15
Group-9	0.89 ± 0.17

3.4.5 Haemoglobin levels

Haemoglobin (Hb) is a crucial component of red blood cells responsible for oxygen transport throughout the body. Measuring haemoglobin levels provides insight into anaemia, oxygen-carrying capacity, and overall blood health. In this study, haemoglobin levels (g/dl) were assessed across different experimental groups to evaluate potential variations due to physiological or pharmacological influences. The results indicate significant differences in haemoglobin levels among the groups. Group 1 (Control) exhibited a normal haemoglobin level of 13.2 ± 0.20 g/dl. However, Group 2 showed the lowest haemoglobin level (9.01 ± 0.23 g/dl), suggesting potential anaemia or hemodilution. Other groups displayed haemoglobin levels ranging from 10.3 ± 0.12 g/dl in Group 4 to 14.0 ± 0.05 g/dl in Group 9, with Groups 8 and 9 showing the highest values. These variations may be attributed to differences in erythropoiesis, nutritional status, disease conditions, or treatment interventions. Monitoring haemoglobin levels in experimental models is essential for assessing oxygen transport efficiency, hematopoietic function, and overall physiological well-being.

Table 7: Hemoglobin levels across different groups

Treatment groups	Hemoglobin level (g/dl)
Group-1	13.2 ± 0.20
Group-2	9.01 ± 0.23
Group-3	13.2 ± 0.24
Group-4	10.3 ± 0.12
Group-5	11.3 ± 0.14
Group-6	12.9 ± 0.22
Group-7	13.2 ± 0.23
Group-8	13.7 ± 0.04
Group-9	14.0 ± 0.05

3.4.6 Platelet count

Platelets play a crucial role in hemostasis and thrombosis, contributing to blood clot formation and wound healing. Measuring platelet count ($\times 10^3/\mu l$) helps assess coagulation efficiency, bleeding disorders, and thrombotic risks. In this study, platelet levels were analyzed across different experimental groups to determine variations influenced by physiological or pharmacological factors.The results revealed notable differences in platelet counts among the groups. Group 2 exhibited the highest platelet count (455.59 ± 0.02 $\times 10^3/\mu l$), indicating a possible hypercoagulable state or reactive thrombocytosis. In contrast, Group 1 (Control) had a normal platelet count of 150.77 ± 0.01 $\times 10^3/\mu l$, while Groups 3 and 9 showed similar values (150.43 ± 0.03 and 150.5 ± 0.03 $\times 10^3/\mu l$, respectively). Intermediate platelet counts were observed in Group 4 (300.36 ± 0.08 $\times 10^3/\mu l$), Group 5 (245.74 ± 0.15 $\times 10^3/\mu l$), and Group 6 (203.9 ± 0.14 $\times 10^3/\mu l$), suggesting varying degrees of platelet activation or depletion. The decreasing trend in Groups 7 and 8 (175.3 ± 0.03 and 166.93 ± 0.07 $\times 10^3/\mu l$, respectively) may indicate reduced platelet production or increased consumption.

These findings provide valuable insights into platelet dynamics, clotting potential, and the impact of experimental conditions on hemostatic function.

Table 8: Platelet count across different groups

Treatment groups	Platelet (x10³/µl)
Group-1	150.77 ± 0.01
Group-2	455.59 ± 0.02
Group-3	150.43 ± 0.03
Group-4	300.36 ± 0.08
Group-5	245.74 ± 0.15
Group-6	203.9 ± 0.14
Group-7	175.3 ± 0.031
Group-8	66.93 ± 0.07
Group-9	150.5 ± 0.03

3.4.7 Leukocyte count

Leukocytes, or white blood cells (WBCs), play a vital role in the immune response, infection control, and inflammation regulation. Evaluating leukocyte count (×10 y /l) helps in understanding immune system activation, disease progression, and potential inflammatory responses. In this study, leukocyte levels were analyzed across different experimental groups to assess immune variations.The results indicate substantial differences in leukocyte counts among the groups. Group 2 exhibited the highest leukocyte count (20.86 ± 0.05 ×10 y /l), suggesting a strong immune or inflammatory response. Similarly, Group 4 (19.01 ± 0.03 ×10 y /l) and Group 5 (17.49 ± 0.03 ×10 y /l) also showed elevated leukocyte levels, indicating potential leukocytosis. Group 1 (Control) had a normal leukocyte count of 8.82 ± 0.02 ×10 y /l, while other groups demonstrated intermediate values, with a gradual decline observed from Group 6 (15.57 ± 0.23 ×10 y/l) to Group 9 (10.54 ± 0.13 ×10 y/l).The varying leukocyte levels across the groups may be attributed to differential immune activation, infections, systemic inflammation, or the effects of therapeutic interventions. Elevated WBC counts could indicate an inflammatory response or infection, while lower counts may suggest immune suppression or resolution of inflammation. These findings provide crucial insights into the immunological status and physiological responses under different experimental conditions.

Table 9: Leukocyte count across different groups

Treatment groups	Leukocyte (x10⁹/µl)
Group-1	8.82 ± 0.02
Group-2	20.86 ± 0.05
Group-3	10.89 ± 0.06
Group-4	19.01 ± 0.03
Group-5	17.49 ± 0.03
Group-6	15.57 ± 0.23
Group-7	14.70 ± 0.18
Group-8	12.07 ± 0.17
Group-9	10.54 ± 0.13

4. Conclusion

The study demonstrates the significant thrombolytic potential of *D. peltata* and *Y. aloifolia,* highlighting their ability to modulate coagulation and fibrinolysis. The phytochemical analysis confirmed the presence of flavonoids, alkaloids, phenols, and saponins, which contribute to their anti-inflammatory and anticoagulant properties. GC-MS analysis identified bioactive compounds like benzoic acid, coumarin, and hexadecanoic acid, known for their thrombolytic effects. The *in vivo* evaluation using Wistar rats revealed reduced thrombus length, prolonged bleeding time, enhanced fibrinolysis, and decreased CRP levels, indicating significant anti-thrombotic and anti-inflammatory effects. Histopathological analysis further confirmed reduced fibrin deposition and improved vascular integrity. These findings suggest that *D. peltata* and *Y. aloifolia* could serve as natural alternatives for thrombolytic therapy, offering potential benefits over synthetic agents that often cause adverse effects. However, further research, including molecular docking and clinical trials, is necessary to elucidate the exact mechanism and validate their therapeutic efficacy.

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Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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