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Research Article

Evaluation of the potential anticoagulant properties of medicinal plants' extracts

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Abstract
Plant-derived phenolics are widely recognized for their therapeutic properties**.** Therefore, the present study aimed to evaluate medicinal plant extracts' anticoagulant activities to develop a safe and effective strategy for controlling thrombotic disorders. Total phenolic content (TPC) from 120 dried powders of different medicinal plants purchased from the local market of Pune, India, were extracted separately in 70% ethanol. Their contents were determined using the Folin-Ciocalteu assay. Anticoagulant activity was initially assessed using the clotting time (CT) assay. The highest phenolic content (8.3 mg/mL) was estimated in *Woodfordia fruticosa* flower. Out of 120 plant extracts, 47 exhibited anticoagulant activities with a significant prolongation of clotting time (CT) (More than control time i. e. 8 min). From these plant extracts, 36, 29 and 21 plants showed anticoagulant activities performed by activated partial thromboplastin time (APTT), prothrombin time (PT), and thrombin time (TT) tests, respectively. The plant extracts from *W. fruticosa* flower*, Terminalia chebula* fruit*, Punica granatum* peel*, Tecoma undulata* bark*, Tedrminalia bellirica* fruit*, Zingiber officinale* rhizome*,* and *Curcuma zedoaria* rhizome exhibited prominent anticoagulant activities with a significant prolongation of CT, APTT, PT and TT tests. Linear regression revealed a weak positive correlation between plant phenolic concentrations and anticoagulant activities, as evaluated by CT, APTT, PT and TT tests. ANOVA test showed significant differences ($P < 0.05$) in the anticoagulant activities of the plant phenolics. The plant extracts showing very high anticoagulant activity could be useful for preparing herbal drugs to control thrombotic disorders.

Keywords: Anticoagulant activity, *Punica granatum, Terminalia chebula,* Total phenolics, *Woodfordia fruticosa*

INTRODUCTION

Blood coagulation is an important physiological process that stops bleeding by forming a blood clot. Thrombosis is a pathological condition of a blood clot inside the blood vessel that hampers blood flow and results severe clinical consequences such as cardiovascular disorders (stroke, heart attack) and vascular damage, myocardial infarction, and venous thromboembolism (Kuriakose and Xiao, 2020; Feigin *et al*., 2021). This condition is often formed by endothelial injury, stasis of blood flow, or hypercoagulability; these factors are collectively known as Virchow's triad (Kumar *et al*., 2010). Recently, the incidence of heart attacks and thrombosis has increased due to high dietary consumption of cholesterol and triglycerides. As per World Health Organization (WHO) report July 2024, the cardiovascular diseases is the main leading cause of death globally. Antiplatelet agents, anticoagulants, and thrombolytics are a common and effective strategy to inhibit blood clot

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initiation and formation (Furie and Furie, 2008).

Despite the huge developments in the treatment of thrombosis, its consequences persist as a leading cause of morbidity and mortality. It encourages the need of research on therapeutic targets for the ultimate solution to control thrombosis without any side effects (Rosenberg and Aird, 1999). Venous thromboembolism is a critical condition where blood clots form in veins and transfer to the lungs. About one or two in 1000 people are estimated to suffer from this condition (Lutsey and Zakai, 2023). This condition often recurs and can cause permanent issues such as pain, swelling in the legs, and post-thrombotic syndrome (Heit *et al*., 2005). Arterial thrombosis promotes acute coronary syndrome and cerebrovascular events is accountable for a significant proportion of cardiovascular deaths (Mozaffarian *et al*., 2016). Effective regulation and prevention of thrombosis are vital for minimizing a significant health burden worldwide.

Plant extracts and their constituents have been utilized significantly as an effective strategy to control various pathological disorders and thrombotic disorders due to their effectiveness and minimum side effects. Plants synthesize various components such as phenolic acids, flavonoids, tannins, terpenoids, and alkaloids for their defensive action and regulation of other physiological processes. Some serve as anticoagulants, antiplatelet agents, and fibrinolytics (Middleton *et al*., 2000). For example, green tea, grapes and berries-derived polyphenols have the ability to inhibit platelet aggregation and progress the endothelial function by the minimizing the risk of clot formation (Duffy *et al*., 2001). Curcumin is a secondary metabolite in turmeric, exhibits anti-inflammatory and antioxidant properties and effectively reduces blood clot formation in vessels.

Studies have revealed that curcumin inhibits platelet aggregation and can control the expression of clotting factors, making it a possible therapeutic candidate for thrombosis prevention (Srivastava *et al*., 1995). Similarly, ginsenosides from ginseng perform antithrombotic effects by inhibiting platelet aggregation and promoting fibrinolytic activity while contributing to control of cardiovascular health (Fan *et al*., 2024). Thus, plant-based compounds effectively control thrombotic disorders with minimal side effects. Many synthetic anticoagulants and antiplatelet drugs are available in the market for treatment, but they have been avoided in some cases because of their adverse effects, including bleeding complications (Middleton *et al*., 2000). Rutin is a flavonoids in different vegetables and fruits that exhibits antithrombotic effects without noteworthy toxicity, making it a safe option for long-term use for health (García-Lafuente *et al*., 2009). Additionally, plant-based therapies provide multidimensional health benefits beyond thrombosis inhibition. It has been explored that phenolics from natural sources possess prominent antioxidant and anti-inflammatory properties, exhibiting efficacy in exerting beneficial effects on the vascular system by inhibiting the oxidation of low-density lipoprotein (LDL), blocking platelet aggregation, reducing blood pressure, and maintaining endothelial function (Behl *et al*., 2020). Thus, combining plant-derived antithrombotic candidates in therapeutic regimens is an ultimate and effective approach for preventing and managing thrombosis-associated diseases with low side effects. The study aimed to screen different medicinal plant extracts for their anticoagulant activities to identify effective extracts that could accomplish effective anticoagulant characteristics and promote the development of novel therapy.

MATERIALS AND METHODS

Chemicals and reagents

Lyophilized plasma, Liquicelin-E, Uniplastin and Fibroscreen reagents were purchased from Tulip Diagnostics Pvt. Ltd. Polyvinylpyrrolidine (PVP), calcium carbonate, sodium phosphate, acetone, phosphoric acid, calcium chloride, hydrochloric acid, sodium acetate, and sodium phosphate were obtained from RANKEM Pvt. Ltd. Trypsin and bovine serum albumin (BSA fraction V) were purchased from Sisco Research Laboratories (SRL), Pvt. Ptd. N-p-tosyl-gly-pro-arg-p-nitroanilide acetate and N-benzoyl-DL-arginine-p-nitroanilide (BAPNA) were obtained from Sigma-Aldrich, India. All chemicals were analytical grade.

Collection of plant samples

A total of 130 well-authenticated dried powders of different medicinal plants were purchased from Manakarnika Aushadhalaya, Shedge Building, Padwal Lane, Gandhipeth, Prabhat Colony, Chinchwadgaon, Pimpri-Chinchwad, Pune (MH), India. For further study, all powder samples were preserved at room temperature in a moisture-free compartment.

Preparation of phenolic extracts

Extracts from the procured powders were prepared using 70% ethanol as the solvent, following the procedures described by Singleton and Rossi (1965) and Waterhouse (2002). Briefly, 1 gram of each powder was suspended in 10 ml of solvent (1:10 w/v) and stirred for up to 2 hours at room temperature. Thereafter, each suspension was filtered through Whatman filter paper No. 1, and the filtrate of each sample was preserved at 4°C in a refrigerator for further use.

Determination of phenolic content

The estimation of phenolics in plant extracts was performed using the Folin-Ciocalteu reagent (FCR) method, as described by Singleton and Rossi (1965) and Waterhouse (2002). Gallic acid was used as the standard reference for plotting the calibration curve. Twenty

microliters of each plant extract sample were mixed with 0.5 ml of FCR and allowed to react for 5 minutes at room temperature. Then, 2 ml of 7.5% sodium carbonate solution was added to the mixture and incubated at 37°C for 30 minutes. After incubation, the intensity of the developed colour was measured spectrophotometrically at 760 nm. The total phenolic concentration of each sample was calculated using the standard curve of gallic acid and expressed as mg/ml GAE (Gallic Acid Equivalent).

Human ethic approval

This study was approved by the Institutional Review Board (IRB) a**t** Shree Clinical Laboratory, Arvind Hospital, Durga Chowk, Akola (M. S.) India. For blood collection, 10 healthy volunteers (ages 20-35 years old) were selected, with no medication history for at least two weeks before blood sample collection. The volunteers were informed about the research, and their willingness to participate was documented by signing written informed consent.

Preparation of blood plasma

Human blood was drawn via venepuncture, usually from volunteers by a needle and stored in an air-tight syringe. To prevent natural coagulation, 1 ml of 3.2% tri -sodium citrate was added to 9 ml of blood and centrifuged at 3000 rpm at 15 min. The top yellowish supernatant as blood plasma was taken by micropipette and preserved at 4 ^{0}C for further study of coagulation assay.

Clotting time (CT) test

The screening of plant extracts for blood anticoagulant activities was performed using the procedure reported in the previous study with minor modifications (Dapper *et al*., 2007). The blood plasma (100 µl) was mixed with plant ethanolic extracts (100 µl) at room temperature. Thereafter, recalcification was done by adding 100 µl of $CaCl_2$ (0.025 mol/L) to the blood plasma extract mixture. The visualization of clot formation confirmed the presence of anticoagulant activity in the plant extract. A control was prepared by mixing the blood plasma with a 0.85% saline solution. The active plant extracts (anticoagulants) were further processed to evaluate anticoagulant activity using the same procedure. The prolongation time of clot formation was recorded on a coagulometer. The anticoagulant activity was considered by subtracting the control clotting time from the test clotting time.

Activated partial thromboplastin time test (APTT) of plant extracts

The APTT test of anticoagulant activity showing plant extracts was assessed using the procedure described by Ramachandraiah *et al*. (2019). Briefly, 90 µL of normal citrated blood plasma and 10 µL plant extract was pre-incubated for 1 minute then, 100 µL of LIQUICELIN -E was added and incubated at 37°C for 5 min. Coagulation was initiated by adding 100 µL of 0.02 M CaCl□, and the clotting time was measured using a coagulometer. Simultaneously, an aliquot was prepared without plant extract by mixing 10 µl of phosphate buffer, 90 µl of plasma, 100 µl of Liquicelin-E reagent and 100 µl of calcium chloride as control. The increase in clotting time of the test sample compared to the control assumed the extract had anticoagulant activity. The difference in clotting time was calculated by subtraction of control clotting time from test clotting time.

Prothrombin time (PT) test

Prothrombin time test of active plant extracts was performed similarly to APTT test in a coagulation test tube containing 10 µl plant extract and 90 µl of plasma mixed and incubated at 37^{0}C for 5 minutes, followed by the addition of 200 µl of Uniplastin. The control tube was prepared by adding phosphate buffer instead of plant extract. The clotting time of plasma was recorded on a coagulometer. The difference in clotting time was calculated by subtraction of control clotting time from test clotting time.

Thrombin time (TT) test

In a coagulation test tube, 10 µl active plant extract was mixed with 100 µl of blood plasma and incubated at 37^{0}C for 5 minutes, followed by the addition of 200 µl of FIBROSCREEN reagent. The control tube was prepared by adding 100 µl of phosphate buffer instead of plant extract. The clotting time of plasma was recorded on coagulometer. The difference in clotting time was calculated by subtracting control clotting time from test clotting time.

Statistical Analysis

One-way ANOVA test was conducted in MS Excel to compare the prolongation times of the CT, APTT, PT, and TT tests. Similarly, the linear regression analysis of plant phenolics with prolongation times was also performed using MS Excel.

RESULTS

Total phenolic concentration in plant extracts

The total phenolic concentration in plant extracts was estimated using the Folin-Ciocaltue assay procedure. The details of phenolic concentration are mentioned in Table 1. Among 120 plants the highest phenolic content (8.3 mg GAE/ml) was observed in *W. fruticosa* flower, while the lowest phenolic content (0.075 mg GAE/ml) was found in *B. arundinacea* leaf. Based on phenolic concentration 120 plants were categorized into five groups such as very high (6 to 9 mg GAE /ml) three plants, high (4 to 6 mg GAE/ml) six plants, mod-

erate (2 to 4 mg GAE/ml) thirty-two plants, low (1 to 2 mg GAE /ml) thirty-seven plants, and very low (1> mg GAE/ml) forty-two plants. The high phenolic content was observed in plants such as *T. bellirica* fruit (6.225 mg GAE/ml) and *T. arjuna* bark (6 mg GAE/ml). The maximum phenolic content was observed in plants such as *E. officinalis* fruit (4.725 mg GAE/ml), *T. chebula* fruit (4.55 mg GAE/ml), *C. papaya* leaf (4.5 mg GAE/ml), *P. granatum* peel (4.45 mg GAE/ml), *T. undulata* bark (4.35 mg GAE/ml) and *A. arabica* bark (4.3 mg GAE/ml) as shown in Table 1.

Anticoagulant activities of plant extracts

Anticoagulant activity in plant extracts was tested by a simple and predominantly available clotting test. Among 120 different plant extracts, 47 exhibited anticoagulant activity with significant plasma clotting time (CT) prolongation (Blood clot formation required more time than control i. e. 8 min in presence of plant extract). Of these active plant extracts, 36, 29 and 21 plants exhibited anticoagulant activities with prominent prolongation time of APTT, PT, and TT tests, respectively (Table 1). Plants showing all anticoagulant activities (CT, APTT, PT and TT) were further selected for assessment of coagulation time using the same procedures (Table 2). *W. fruticosa* flower extract exhibited the highest anticoagulant effects, with significant prolongation time in all coagulation tests: CT (>30 min), APTT (>10 min), PT (>10 min), and TT (1.32 min). Six plant extracts such as *T. chebula* fruit, *P. grantum* fruit peel, *T. undulate* bark, *T. bellirica* fruit, *Z. officinale* rhizome, and *C. zedoaria* rhizome exhibited the maximum anticoagulant activities, with substantial prolongation time in CT, APTT, PT, and TT tests compared to other plants. Conversely, *P. tuberosa* tuber demonstrated the lowest anticoagulant activity across all tests (CT, APTT, PT, and TT). *C. papaya* fruit, *M. esculenta* fruit, *R. centifolia* flower, and *S. asoca* bark showed notable anticoagulant activity, primarily in CT (>30 min), with less pronounced prolongation in APTT, PT, and TT tests. *R. centifolia* flower and *B. ligulata* exhibited minimal anticoagulant activity, affecting only the TT test. *M. esculenta* showed minimal anticoagulant activity, specifically in PT (0.44 min), while *P. tuberosa* demonstrated minimal activity in both PT (0.30 min) and APTT (0.88 min) tests. Based on the one-way ANOVA test analysis, there were significant mean differences in the prolongation time of all tests ($P < 0.01$). The linear regression analysis revealed a weak positive correlation (R^2 = 0.172 CT, R^2 = 0.0395 APTT, R^2 = 0.0563 PT and R^2 = 0.6384 TT) between plant phenolic concentrations and anticoagulant activities (Fig 1).

DISCUSSION

The formation of blood clots in vessels leads to thrombotic disorders such as deep vein thrombosis (DVT) and pulmonary embolism (PE), with severe complications of vessel damage if it is not regulated properly (Lichota *et al*., 2020). Thrombotic disorders cause genetic predispositions and mutations that are responsible for prolonged malignancy (Nicholson *et al*., 2020). Anticoagulant therapy is a keystone treatment for avoiding clot propagation and preventing cardiovascular disorders (Simon and Jeffrey, 2005). Plant derived compounds including phenolic acids, flavonoids and tannins, are important in human health due to their powerful antioxidant, anti-inflammatory, and antimicrobial

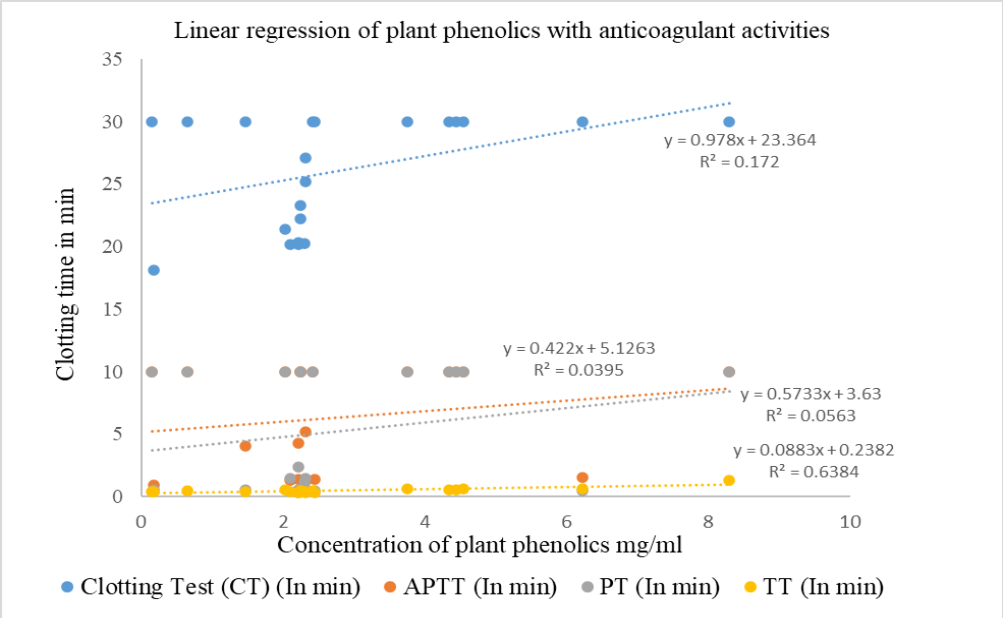

Fig. 1. *Linear regression of plant phenolics with anticoagulant activities was analyzed using MS Excel, examining the relationship between plant phenolic concentrations and the prolongation times of clotting time (CT), activated partial thromboplastin time (APTT), prothrombin time (PT) and thrombin time (TT) tests*

Table 1. List of medicinal plants used for screening of anticoagulant activities with clotting time (CT) test and their total phenolic contents (TPC) mg GAE/ml. Screening of anticoagulant activity showing plants for activated partial thromboplastin time (APTT), prothrombin time (PT) and thrombin time (TT) tests to confirm the intrinsic, extrinsic and other pathway inhibition. Serial number of plants is mentioned in superscript (+ indicates the anticoagulant activity present in plant extract, - indicates the anticoagulant activity absent in plant extract)

Plant	TPC mg GAE/ ml	Plant	TPC mg GAE/ml	Plant	TPC mg GAE/ml	Plant	TPC mg GAE/ ml
[1]*Woodfordia fruticosa* flower CT+,APTT+,PT+,TT+	8.30	[31]*Mammea longifolia* bark CT+,APTT+,PT-,TT-	2.2	[61]*Adhatoda vasica* leaf CT-	1.35	[91]*Sida cordifolia* root CT-	0.67
[2]*Terminalia bellirica* fruit CT+,APTT+,PT+,TT+	6.22	[32]*Pistacia integerrima* gall CT+,APTT-,PT-,TT-	2.2	[62]*Sphaeranthus indicus* whole plant CT-	1.32	[92]*Hedychium spicatum* rhizome CT-	0.65
[3]*Terminalia arjuna* bark CT+,APTT+,PT+,TT-	6.03	[33]*Holarrhena antidysente* seed CT+,APTT+, PT-,TT-	2.17	[63]*Trachyspermum ammi* seed CT-	1.3	[93]*Zingiber officinale* rhizomes CT+, APTT+,PT+,TT+	0.65
[4]*Emblica officinalis* fruit CT+,APTT+,PT+,TT-	4.72	[34]*Lawasonia inermis* leaf CT+, APTT-,PT-,TT-	2.15	[64]*Mallotus philippinensis* fruit CT-	1.28	[94]*Momordica charantia* fruit CT-	0.6
[5]*Terminalia chebula* fruit CT+,APTT+,PT+,TT+	4.55	[35]*Butea monosperma* bark CT+,APTT-,PT-,TT-	2.12	[65]*Ocimum sanctum* leaf CT+,APTT+,PT+, TT+	1.27	[95]*Operculina turpethum* root CT-	0.6
[6]*Carica papaya* leaf CT+, APTT+, PT+,TT+	4.51	[36]*Phyllanthus amarus* whole plant CT+ APTT-,PT-,TT-	2.12	[66]*Psoralia corylifolia* seed CT-	1.26	[96]*Dioscorea bulbifera* tuber CT-	0.57
[7]*Punica granatum* peel CT+, APTT+, PT+,TT+	4.45	[37]*Laccifer lacca* resin CT+, APTT+, PT+,TT+	2.1	[67]*Ricinus communis* seed CT-	1.275	[97]*Asparagus racemosus* root CT-	0.55
[8]*Tecoma undulata* bark CT+,APTT+,PT+,TT+	4.35	[38]*Curcuma amada* rhizomes CT-	2.05	[68]*Symplocos racemosa* bark CT-	1.25	[98]*Solanum xantho-carpum* whole plant CT-	0.525
[9]*Acacia arabica* bark CT+, APTT+,PT-,TT-	4.3	[39]*Prunus cerasoides* bark CT-	2.05	[69]*Piper longum* leaf CT-	1.225	[99]*Acorus calamus* rhi-zome CT-	0.51
[10]*Jatropha curcas* latex CT+, APTT-,PT+,TT-	3.25	[40]*Aegle marmelos* leaf CT+,APTT+,PT-,TT-	2.02	[70]*Wrightia tinctoria* bark CT-	1.225	[100]*Berberis aristata* root CT-	0.50
[11]*Mimusops elengi* bark CT+,APTT+, PT+,TT-	3.25	[41]*Cinnamomum tamala* leaf CT+, APTT+,PT-,TT-	2.07	[71]*Valeriana wallichii* leaf CT-	1.175	101*Fumaria vaillantii* whole plant CT-	0.50
[12]*Myrica esculenta* fruit CT+, APTT+, PT+,TT+	2.45	[42]*Swertia chirayita* whole plant CT+, APTT+, PT-,TT-	1.925	[72]*Cassia angustifolia* leaf CT-	1.15	[102]*Boerhavia diffusa* whole plant CT-	0.47
[13]*Rosa centifolia* flower CT+, APTT+,PT+,TT+	2.425	[43]*Clerodendrum serratum* root CT+, APTT-,PT-,TT-	1.85	[73]*Cedrus deodara* bark CT-	1.15	[103]*Eclipta alba* leaf CT-	0.47
[14]*Tectaria cicutaria* whole plant CT+, APTT+,PT+,TT-	2.425	[44]*Glycyrrhiza glabra* roots CT+, APTT-,PT-,TT-	1.825	[74]*Embelia ribes* fruit CT-	1.15	[104]*Pongamia pinnata* seed CT-	0.48
[15]*Carum roxburghianum* Seed CT+, APTT+,PT+,TT-	2.35	[45]*Azadirachta indica* leaf CT-	1.775	[75]*Alpinia galanga* rizhome CT-	1.125	[105]*Coriandrum sativum* seed CT-	0.45

Contd.....

Table 1. Contd...

[16]*Gardenia gummifera* gum CT+, APTT+,PT+,TT-	2.35	[46]*Convolvulus pluricaulis* whole plant CT-	1.775	[76]*Garcinia pedunculata* fruit CT-	1.125	[106]*Crataeva nurvala* bark CT-	0.45
[17]*Bauhinia variegata* bark CT+, APTT+,PT+,TT+	2.325	[47]*Gymnema sylvestre* leaf CT-	1.75	[77]*Randia spinosa* fruit CT-	1.075	[107]*Grewia hirsuta* whole plant CT-	0.4
[18]*Decalepis hamiltonii* root CT+, APTT+,PT+,TT+	2.325	[48]*Bacopa monnieri* leaf CT-	1.7	[78]*Andrographis paniculat* leaf CT-	1.025	[108]*Tinospora cordifolia* bark CT-	0.4
[19]*Syzygium cumini* bark CT+,APTT+,PT-,TT-	2.325	[49]*Centratherum anthelmi* leaf CT-	1.7	[79]*Aloe vera* leaf CT-	0.95	[109]*Abutilon indicum* root CT-	0.35
[20]*Hibiscus rosa-sinensis* flower CT+,APTT-,PT-,TT-	2.3	[50]*Vitex negundo* leaf CT-	1.7	[80]*Cissampelos pareira* leaf CT-	0.9	[110]*Barleria prionitis* leaf CT-	0.325
[21]*Quercus infectoria* gall CT+,APTT+,PT+,TT+	2.3	[51]*Argyreia speciosa* root CT-	1.675	[81]*Piper nigrum* fruit CT-	0.875	[111]*Withania somnifera* root CT-	0.325
[22]*Abies webbiana* leaf CT+, APTT+,PT+,TT-	2.25	[52]*Cassia fistula* leaf CT-	1.65	[82]*Acacia catechu* bark CT-	0.85	[112]*Putranjiva roxburghii* leaf CT-	0.3
[23]*Bergenia ligulata* rhizome CT+, APTT+,PT+,TT+	2.25	[53]*Mesua ferrea* seed CT-	1.65	[83]*Anethum graveolens* seed CT-	0.825	[113]*Sapindus trifoliatus* fruit CT-	0.275
[24]*Pluchea lanceolata* root CT+,APTT-,PT-,TT-	2.25	[54]*Centella Asiatica* whole plant CT-	1.55	[84]*Fagonia cretica* whole plant CT-	0.825	[114]*Tribulus terrestris* root CT-	0.275
[25]*Salvia miltiorrhiza* root CT+,APTT+,PT+,TT+	2.25	[55]*Asteracantha longifolia* seed CT-	1.525	[85]*Trichosanthes dioica* fruit CT-	0.8	[115]*Vetiveria zizanioides* root CT-	0.25
[26]*Tephrosia purpurea* whole plant CT+,APTT+,PT-,TT-	2.25	[56]*Cyperus rotundus* rhizomes CT-	1.5	[86]*Enicosteona littorale* whole plant CT-	0.775	[116]*Pueraria tuberosa* tuber CT+,APTT+,PT+, TT+	0.175
[27]*Cinnamomum cassia* bark CT+, APTT+,PT+,TT+	2.225	[57]*Saraca asoca* bark CT+,APTT+, PT+,TT+	1.475	[87]*Piper cubeba* fruit CT-	0.775	[117]*Bryonia laciniosa* seed CT-	0.15
[28]*Mucuna pruriens* seed CT+,APTT-,PT-,TT-	2.225	[58]*Picrorhiza kurroa* root CT-	1.425	[88]*Smilax china* root CT-	0.775	[118]*Caesalpinia crista* seed CT-	0.15
[29]*Plumbago zeylanica* root CT+,APTT+,PT+,TT+	2.225	[59]*Acacia concinna* bark CT-	1.375	[89]*Salmalia malabarica* bark CT-	0.75	[119]*Curcuma zedoaria* rhizome CT+, APTT+,PT+,TT+	0.15
[30]*Commiphora mukul* gum resin CT+,APTT-,PT-,TT-	2.20	[60]*Citrus sinensis* fruit peel CT-	1.375	[90]*Piper retrofractum* fruit CT-	0.675	[120]*Bambusa arundina-cea* leaf CT-	0.075

Table 2. List of anticoagulant activities showing plants with clotting time (CT), activated partial thromboplastin time (APTT), prothrombin time (PT) and thrombin time (TT) tests and their clotting time in min. (Clotting times of all tests statistically analyzed by ANOVA test for comparison of prolongation times)

Sr. No.	Botanical Name	CT (min)	APTT (min)	PT (min)	TT (min)	F Value	P Value
1	*Woodfordia fruticosa* flower	> 30	> 10	> 10	1.32		
2	*Carica papaya* fruit	> 30	1.50	0.46	0.58		
3	*Terminalia chebula* fruit	> 30	> 10	> 10	0.58		
4	*Punica granatum* fruit peel	> 30	>10	>10	0.56		
5	*Tecoma undulata* bark	> 30	>10	> 10	0.54		
6	*Terminalia bellirica* fruit	> 30	> 10	> 10	0.62		
7	*Myrica esculenta* fruit	> 30	1.40	0.44	0.33		
8	*Rosa centifolia* flower	> 30	> 10	> 10	0.47		
9	*Decalepis hamiltonii* root	27.12	1.2	0.5	0.38		
10	*Bauhinia variegata* bark	> 25.20	5.20	1.47	0.31		
11	*Quercus infectoria* gall	> 20.25	1.34	1.15	0.41	181.46	P<0.05
12	*Salvia miltiorrhiza* root	> 23.31	> 10	0.57	0.44		
13	*Bergenia ligulata* rhizome	> 22.24	> 10	> 10	0.46		
14	*Plumbago zeylanica* root	> 20.14	4.30	2.34	0.32		
15	*Cinnamomum cassia* bark	20.35	1.40	0.57	0.31		
16	*Laccifer lacca* resin	20.15	1.31	1.42	0.42		
17	*Ocimum sanctum* leaf	21.40	> 10	> 10	0.54		
18	*Saraca asoca* bark	> 30	4.01	0.57	0.39		
19	*Zingiber officinale* rhizome	> 30	> 10	> 10	0.46		
20	*Pueraria tuberosa* tuber	18.5	0.88	0.55	0.36		
21	*Curcuma zedoaria* rhizome	> 30	> 10	> 10	0.41		

properties, and they have shown efficacy in preventing blood coagulation pathways. These compounds protect against chronic diseases such as cardiovascular diseases, cancer, and neurodegenerative disorders by scavenging free radicals, controlling inflammatory responses, and preventing microbial growth (Pandey and Rizvi, 2009; Middleton *et al*., 2000). They can inhibit platelet aggregation and modulate the activity of coagulation factors to prevent clot formation. Thus, incorporating phenolic-rich foods into the diet is beneficial for controlling overall health and preventing thrombotic diseases (Hubbard *et al*., 2004). To investigate prominent anticoagulant therapy, the present study screened 120 plant phenolic extracts for anticoagulant study using blood plasma CT test. From these plants, 47 exhibited anticoagulant activities. The plant extracts *W. fruticosa, T. chebula*, *P. grantum*, *T. undulata*, *T. bellirica*, *Z. officinale*, and *C. zedoaria* extracts exhibited prominent anticoagulant activities with enough prolongation of CT, APTT, PT, and TT tests (Table 2). APTT was useful for testing the coagulation factors like IX, XI, XII, and prekallikrein in the intrinsic pathway, while PT is used to assess coagulation factors V, VII, and X in the extrinsic coagulation cascade pathway (Azevedo *et al*., 2007). Similarly, TT is used to evaluate the blood coagulation process that converts fibrinogen into fibrin, which is directly activated by the addition of a thrombin factor (Koch and Biber, 2007). The results of the present study showed that active plant extracts significantly prolong the APTT, PT and TT clotting times. For the first time this study reported the anticoagulant activity in three plants: *T. undulate* bark, *L. lacca* resin, and *S. asoca* bark. The high phenolic content was observed in flower extract of *W. fruticosa* extract, which showed the highest anticoagulant activity compared with other plants tested in this study (Table 2). *W. fruticosa* has been recognised for its high phenolic content that exhibits significant biological activities, including antioxidant, antimicrobial, and anti-inflammatory (Das *et al*., 2007). The phenolic compounds of this plant might play a crucial role in anticoagulant activity. Additionally, we found the plant extracts including *T. chebula* fruit*, P. granatum* fruit peel*, T. undulate* bark*, T. bellirica* fruit*, Z. officinale* rhizome, and *C. zedoaria* rhizome the high level of phenolic contents and significant anticoagulant activities. These plants contain phenolic acids, tannins and flavonoids, inhibiting platelet aggregation, increasing fibrinolytic activity, and reducing blood clot development risk (Sharifi-Rad et al., 2021). This research has confirmed that these plants can efficiently serve as natural anticoagulants due to their rich phenolic compounds (Reddy *et al*., 2003). These properties make them valuable for preventing thrombotic disorders and cardiovascular diseases (Bachheti *et al*., 2022).

The extracts from *T. undulate* bark*, L. lacca* resin, and *S. asoca* bark exhibited prominent anticoagulant activities. These plants have been reported to have high phenolic contents and which are responsible for their various biological activities (Rohilla and Garg, 2014). *L. lacca* contains high phenolic contents, contributing to its anti-inflammatory and antimicrobial properties (Reshma *et al*., 2018). Similarly, *S. asoca* has high

phenolic concentrations exhibiting significant antioxidant and anticancer activities (Tewari et al., 2017). ANOVA test analysis revealed significant differences in the prolongation time of all tests ($P < 0.01$). This indicates that plant phenolics possess different mechanisms of anticoagulant activity. The linear regression analysis also exposed a weak positive correlation between plant phenolic concentrations and anticoagulant activities (Fig. 1).

This also indicates that a particular phenolic component or compound other than phenolics might be involved in anticoagulant activity. Previous reports have demonstrated that phenolics inhibit platelet aggregation by interfering in signaling pathways responsible for clot formation, thus minimising the risk of thrombus formation (Pawlaczyk *et al*., 2011). Furthermore, phenolics trigger the activity of endogenous anticoagulant proteins such as tissue plasminogen activator (tPA) and minimize the activity of pro-coagulant factors (Kolodziejczyk-Czepas and Czepas, 2023). The results of the present study demonstrated that the plant extracts showing anticoagulants activity are critical in avoiding and controlling thrombotic disorders by preventing the formation and suspending the blood clots. These plant extracts could be useful as therapeutic solution to reduce the risk of deep vein thrombosis, pulmonary embolism, and stroke formed from abnormal clotting. This study motivates future research to identify novel anticoagulant molecules from these plant extracts and study identified compound mechanisms in anticoagulant activity for their safety importance in modern medical practice.

Conclusion

The present study concluded that *W. fruticosa* flower extract was the rich source of phenolic content among the 120 plant extracts. The extracts from *W. fruticosa* flower, *T. chebula* fruit, *P. granatum* fruit peel, *T. undulate* bark, *T. bellirica* fruit, *Z. officinale* rhizome, and *C. zedoaria* rhizome were the prominent reservoirs of anticoagulants. For the first time, this study reported that significant anticoagulant activities were present in *T. undulate* bark, *L. lacca* resin and *S. asoca* bark. In contrast, *P. tuberosa* tuber extract contained low anticoagulant activity exhibiting compounds. Linear regression analysis demonstrated a weak positive correlation between phenolic content and anticoagulant activity. The ANOVA test revealed significant differences in the anticoagulant efficacy of the plant phenolic extracts ($P < 0.01$). The highest anticoagulant activity exhibiting plant extracts is important for utilization to prevent and treat thrombotic disorders by inhibiting and delaying blood clot formation. This study suggests the importance of research on the purification and characterization of prominent anticoagulants from these plant extracts as potential ingredients for developing herbal anticoagulant drugs.

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Conflict of interest

The authors declare that they have no conflict of interest.

REFERENCES

1. Azevedo, A. P. S., Farias, J. C., Costa, G. C., Ferreira, S. C., Aragao-Filho, W. C., Sousa, P. R., Pinheiro, M. T., Maciel, M. C., Silva, L. A., Lopes, A. S. & Barroqueiro, E. S. (2007). Anti-thrombotic effect of chronic oral treatment with *Orbignya phalerata Mart. J. of Ethnopharmacol*, 111 (1), 155-159. https://doi.org/10.1016/j.jep.2006.11.005
2. Bachheti, R. K., Worku, L. A., Gonfa, Y. H., Zebeaman, M. D., Pandey, D. P. and Bachheti, A. (2022). Prevention and treatment of cardiovascular diseases with plant phytochemicals: A Review. *J. Evid.-based Complement. Altern. Med.*, (1), 01-21. doi: 10.1155/2022/5741198
3. Behl, T., Bungau, S., Kumar, K., Zengin, G., Khan, F., Kumar, A., Kaur, R., Venkatachalam, T., Tit, D. M., Vesa, C. M., & Barsan G. (2020). Pleotropic effects of polyphenols in cardiovascular system. *Biomed & Pharmacother*, 1 (130),1107-1114. https://doi.org/10.1016/j.biopha.2020.110714
4. Dapper, D.V., Achinike, P.N. & Gwotmut, M.D. (2007). The effects of Aloe vera [gel] on clotting time, prothrombin time and plasma fibrinogen concentration in albino Wistar rats. *Port. Harcourt. Med. J*, 2, 56-60. DOI:10.4314/phmedj.v2i1.38894
5. Das, P. K., Goswami, S., Chinniah, A., Panda, N., Banerjee, S., Sahu, N. P. & Achari, B. (2007). Woodfordia fruticosa: Traditional uses and recent findings. *Journal of Ethnopharmacol*, 110(2), 189-199. https://doi.org/10.1016/j.jep.2006.12.029
6. Duffy, S. J., Vita, J. A., Holbrook, M., Swerdloff, P. L. & Keaney Jr, J.F. (2001). Effect of acute and chronic tea consumption on platelet aggregation in patients with coronary artery disease. *Arterioscler. Thromb. Vasc. Biol,* 21 (6), 1084-1089. DOI: 10.1161/01.atv.21.6.1084
7. Fan, W., Fan, L., Wang, Z., Mei, Y., Liu, L., Li, L., Yang, L. & Wang, Z. (2024). Rare ginsenosides: a unique perspective of ginseng research. *J. Adv. Res*, 66, 303-328. https://doi.org/10.1016/j.jare.2024.01.003
8. Feigin, V. L., Stark, B. A., Johnson, C. O., Roth, G.A. , Bisignano, C., Abady, G. G., Abbasifard, M., Abbasi-Kangevari, M., Abd-Allah, F., Abedi, V. & Abualhasan, A. (2021). Global, regional, and national burden of stroke and its risk factors, 1990–2019: a systematic analysis for the Global Burden of Disease Study 2019. *The Lancet, Neurol*, 20(10), 795-820. DOI: 10.1016/S1474-4422(21)00252-0

9. Furie, B. & Furie, B. C. (2008). Mechanisms of thrombus formation. *N, Engl, J, Med*, 359(9), 938-949. DOI: 10.1056/NEJMra0801082
10. García-Lafuente, A., Guillamón, E., Villares, A., Rostagno, M. A. & Martínez, J. A. (2009). Flavonoids as anti-inflammatory agents: implications in cancer and cardiovascular disease. *Inflamm. res*, 58(9), 537-552. DOI: 10.1007/s00011-009-0037-3
11. Heit, J. A., Cohen, A. T. & Anderson Jr, F.A. (2005). Estimated annual number of incident and recurrent, non-fatal and fatal venous thromboembolism (VTE) events in the US. *Blood*, 106(11), 910. https://doi.org/10.1182/blood.V106.11.910.910
12. Hubbard, G. P., Wolffram, S., Lovegrove, J. A. & Gibbins, J. M. (2004). Ingestion of quercetin inhibits platelet aggregation and essential components of the collagen□ stimulated platelet activation pathway in humans. *Journal of Thrombosis and Haemostasis*, *2*(12), 2138-2145. https://doi.org/10.1111/j.1538-7836.2004.01067.x
13. Koch, E. & Biber, A. (2007). Treatment of rats with the *Pelargonium sidoides* extract has no effect on blood coagulation parameters or on the pharmacokinetics of warfarin. *Phytomedicine*, 14, .40-45. https://doi.org/10.1016/j.phymed.2006.11.026
14. Kolodziejczyk-Czepas, J. & Czepas, J. (2023). Plant-Derived Compounds and Extracts as Modulators of Plasmin Activity—A Review. *Molecules*, *28*(4), 1677.
15. Kumar, D. R., Hanlin, E., Glurich, I., Mazza, J. J. & Yale, S. H. (2010). Virchow's contribution to the understanding of thrombosis and cellular biology. *Clin. Med. res*, 8(3-4), 168-172. doi: 10.3121/cmr.2009.866
16. Kuriakose, D. & Xiao, Z. (2020). Pathophysiology and treatment of stroke: present status and future perspectives. *Int. J. mol. Sci,* 21(20), 7609. doi: 10.3390/ijms21207609
17. Lichota, A., Szewczyk, E. M. & Gwozdzinski, K. (2020). Factors affecting the formation and treatment of thrombosis by natural and synthetic compounds. *Int. J. Mol. Sci*, 21(21), 7975. doi: 10.3390/ijms21217975
18. Lutsey, P. L. & Zakai, N. A. (2023). Epidemiology and prevention of venous thromboembolism. *Nat. Rev. Cardiol*, 20(4), 248-262. doi: 10.1038/s41569-022-00787-6.
19. Middleton, E., Kandaswami, C. & Theoharides, T.C. (2000). The effects of plant flavonoids on mammalian cells: implications for inflammation, heart disease, and cancer. *Pharmacol. rev*, 52(4), 673-751. https://pubmed.ncbi.nlm.nih.gov/11121513/
20. Mozaffarian, D., Benjamin, E. J., Go, A. S., Arnett, D. K., Blaha, M. J., Cushman, M., Das, S. R., De Ferranti, S., Després, J. P., Fullerton, H. J. & Howard, V. J. (2016). Heart disease and stroke statistics—2016 update: a report from the American Heart Association. *circ*, 133(4), 338-360. https://doi.org/10.1161/CIR.0000000000000350
21. Nicholson, M., Chan, N., Bhagirath, V. & Ginsberg, J. (2020). Prevention of venous thromboembolism in 2020 and beyond. *J, clin, med,* 9(8), 2467. https://doi.org/10.3390/jcm9082467
22. Pandey, K. B. & Rizvi, S. I. (2009). Plant polyphenols as dietary antioxidants in human health and disease. *Oxid. Med. Cell. Longev, 2*(5), 270-278. DOI:10.4161/oxim.2.5.9498
23. Pawlaczyk, I., Czerchawski, L., Kuliczkowski, W., Karolko, B., Pilecki, W., Witkiewicz, W. & Gancarz, R. (2011). Anticoagulant and anti-platelet activity of polyphenolic-polysaccharide preparation isolated from the medicinal plant Erigeron canadensis L. *Thromb. Res,* 127(4), 328-340. DOI: 10.1016/j.thromres.2010.11.031
24. Ramachandraiah, C., Nandish, S.M., Kengaiah, J., Srinivas, C., Shiviah, A., Hanumegowda, S. M. & Sannaningaiah, D. (2019). Anticoagulant, antiplatelet and fibrinogenolytic activity of serine protease from Cicer arietinumseed extract. *Int J Pharm Biol Sci*, 9(2), pp.863-878.
25. Reddy, L. A. L. I. N. I., Odhav, B. & Bhoola, K. D. (2003). Natural products for cancer prevention: a global perspective. *Pharma. therap*, 99(1), 1-13. DOI: 10.1016/s0163-7258(03)00042-1
26. Reshma, B. V., Manohar, R. N. & Anaha, V .I. (2018). A review on Laccifer lacca. *World J. Pharm. Res, 7*(10), 206-218. DOI:10.20959/wjpr201810-12282
27. Rohilla, R. & Garg, M. (2014). Phytochemistry and pharmacology of Tecomella undulata. *Int. J. of Green Pharm,* 8(1). DOI:10.4103/0973-8258.126811
28. Rosenberg, R.D. & Aird, W.C. (1999). Vascular-bed–specific hemostasis and hypercoagulable states. *N. Engl. J. Med,* 340(20), 1555-1564. DOI: 10.1056/NEJM199905203402007
29. Sharifi-Rad, J., Quispe, C., Zam, W., Kumar, M., Cardoso, S.M., Pereira, O.R., Ademiluyi, A.O., Adeleke, O., Moreira, A.C., Živković, J. & Noriega, F. (2021). Phenolic bioactives as antiplatelet aggregation factors: The pivotal ingredients in maintaining cardiovascular health. *Oxid. Med. Cell. Longev,* (1), 2195902. doi: 10.1155/2021/2195902
30. Simon, J. M. & Jeffrey, S. G. (2005) New Anticoagulants for the Prevention and Treatment of Venous Thromboembolism. *Vasc. Health. Risk. Manag*. 1(1): 41–53.doi: 10.2147/vhrm.1.1.41.58936
31. Singleton, V. L. & Rossi, J. A. (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *Am. J. Enol. Vitic*, 16(3), 144-158. DOI: 10.5344/ajev.1965.16.3.144
32. Srivastava, K. C., Bordia, A. & Verma, S. K. (1995). Curcumin, a major component of food spice turmeric (Curcuma longa) inhibits aggregation and alters eicosanoid metabolism in human blood platelets. *Prostaglandins. Leukot. Essent. Fat. Acids*, 52(4), 223-227. https://doi.org/10.1016/0952-3278(95)90040-3
33. Tewari, R., Gupta, M., Ahmad, F., Rout, P.K., Misra, L., Patwardhan, A. & Vasudeva, R. (2017). Extraction, quantification and antioxidant activities of flavonoids, polyphenols and pinitol from wild and cultivated Saraca asoca bark using RP-HPLC-PDA-RI method. *Ind. Crops Prod*, 103, 73-80. https://doi.org/10.1016/j.indcrop.2017.03.036
34. Waterhouse, A.L. (2002). Determination of total phenolics. *Current Protocols in Food Analytical Chemistry*, 6(1), 1-1 https://doi.org/10.1002/0471142913.fai0101s06