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Research

In-Vitro Evaluation of the Anticoagulant Activity of Methanolic Leaf Extract and Fractions of *Ficus Benjamina* On Blood

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Abstract:

Background: Thromboembolic disorders, including stroke, deep vein thrombosis, pulmonary embolism, and myocardial infarction, remain major contributors to global morbidity and mortality. Although currently available synthetic anticoagulants are effective in the prevention and treatment of these conditions, their clinical use is often limited by adverse effects, including bleeding complications, drug interactions, and the need for regular monitoring. These limitations have prompted increasing interest in the identification of safer and more effective anticoagulant agents from natural sources. *Ficus benjamina*, a medicinal plant widely used in traditional medicine, is rich in bioactive phytochemicals such as flavonoids, phenolic compounds, tannins, and triterpenoids, which have demonstrated diverse pharmacological activities. **Aim:** To investigate the *in vitro* anticoagulant potential of the methanolic leaf extract of *Ficus benjamina* using Prothrombin Time (PT) and Activated Partial Thromboplastin Time (aPTT) assays. **Methodology:** Shade-dried leaves of *Ficus benjamina* were extracted with methanol using the Soxhlet extraction method. The methanolic extract was subjected to preliminary phytochemical screening and evaluated for *in vitro* anticoagulant activity using citrated goat plasma. Anticoagulant activity was assessed by measuring Prothrombin Time (PT) and Activated Partial Thromboplastin Time (aPTT) at extract concentrations of 4,000, 8,000, 12,000, and 16,000 µg/mL. Heparin (100 IU/mL) was used as the positive control. **Results:** The methanolic leaf extract of *Ficus benjamina* yielded 10.31% (w/w) and was found to contain alkaloids, flavonoids, phenolic compounds, tannins, cardiac glycosides, quinones, resins, reducing sugars, and gums and mucilage. The extract exhibited significant *in vitro* anticoagulant activity by prolonging both Prothrombin Time (PT) and Activated Partial Thromboplastin Time (aPTT) in a concentration-dependent manner. At the highest tested concentration (16,000 µg/mL), PT increased from 12.80 to 18.60 s, while aPTT increased from 31.50 to 51.80 s, indicating marked anticoagulant activity. **Conclusion:** The methanolic leaf extract of *F. benjamina* exhibited significant concentration-dependent *in-vitro* anticoagulant activity,

Keywords: *F. benjamina*; Anticoagulant activity; Methanolic leaf extract; Prothrombin Time; Activated Partial Thromboplastin Time; Phytochemicals; Heparin.

INTRODUCTION: Blood is a specialized connective tissue that plays a vital role in maintaining physiological homeostasis. An average healthy adult possesses approximately 5–6 Liters of blood, which transports oxygen and nutrients to body tissues while removing metabolic waste products. In addition, blood contributes to immune defence by carrying leukocytes and helps maintain haemostasis through platelets, which form plugs at sites of vascular injury to prevent excessive blood loss. The major cellular components of blood include red blood cells (RBCs), white blood cells (WBCs), and platelets, suspended in plasma [1].

Haemostasis is a complex physiological process that prevents excessive bleeding following vascular injury. The coagulation cascade consists of intrinsic and extrinsic pathways that ultimately converge into a common pathway, leading to the formation of fibrin. Fibrin stabilizes the initial platelet plug by forming a mesh-like network, thereby ensuring effective clot formation and wound healing while preventing spontaneous bleeding [2]. Anticoagulants are a class of drugs that inhibit blood clot formation and are widely used in the prevention and treatment of thromboembolic disorders. These medications reduce the risk of life-threatening cardiovascular complications, including myocardial infarction, stroke, deep-vein thrombosis (DVT), pulmonary embolism (PE), and systemic embolism [3]. Acute pulmonary embolism, caused by obstruction of the pulmonary arteries by thrombi originating from distant veins, is commonly treated with oral anticoagulants such as apixaban, warfarin, and dabigatran [4]. Deep-vein thrombosis, characterized by clot formation within the deep venous system, is frequently managed using heparin therapy [5]. Ischemic stroke, resulting from obstruction of cerebral blood vessels, is commonly treated with antiplatelet agents such as aspirin to reduce the risk of recurrent thrombotic events [6].

Despite their clinical efficacy, anticoagulant and antiplatelet therapies are associated with several adverse effects, including hemorrhage, hematoma, anaphylaxis, heparin-induced thrombocytopenia (HIT), bullous hemorrhagic lesions, and phlebitis. These complications highlight the need for safer and more effective therapeutic alternatives, particularly those derived from natural sources. Cardiovascular diseases continue to represent a major global health challenge. Although mortality from acute myocardial infarction (AMI) has declined considerably over the past two decades due to advances in diagnosis and treatment, the worldwide burden of cardiovascular disease remains substantial. It is estimated that approximately 64 million individuals will be living with heart failure by 2026 as a result of improved survival and an aging population [7]. Likewise, stroke remains one of the leading causes of mortality and long-term disability worldwide. Cardioembolic stroke accounts for approximately 20–30% of ischemic strokes, with atrial fibrillation being the predominant underlying cause. Appropriate anticoagulant therapy has been shown to reduce the risk of stroke in patients with atrial fibrillation by approximately 60–70% [8].

Medicinal plants have long served as valuable sources of bioactive compounds for the development of novel therapeutic agents. *Ficus benjamina* L. (Family: Moraceae), commonly known as the weeping fig, is a widely distributed evergreen tree native to India, southern China, Southeast Asia, Malaysia, the Philippines, northern Australia, and several Pacific islands. It is also cultivated extensively in many tropical and subtropical regions around the world. The plant grows as a large evergreen shrub or tree reaching approximately 8 m in height with a broad spreading crown and characteristic drooping branches. *Ficus benjamina* possesses considerable ethnomedicinal importance. Various parts of the plant, including its leaves, roots, and stem bark, have been traditionally used for the treatment of numerous ailments. Scientific investigations have demonstrated that the plant exhibits diverse pharmacological properties, including antimicrobial, antioxidant, anti-inflammatory, antipyretic, antidysenteric, and antidiabetic activities [9-11]. These biological activities suggest that *F. benjamina* is a promising source of phytochemicals with potential therapeutic applications.

MATERIALS AND METHODOLOGY

SOLVENTS AND CHEMICALS

Methanol was purchased from SUMISONS SCIENTIFIC PRIVATE LIMITED, Chennai.

AUTHENTICATION

The leaves of *Ficus benjamina* were collected from ACP medical garden, Tiruvannamalai. The herbarium was prepared and authenticated by Dr. J. Suresh Kumar, Dept of Botany, Kalaignar Karunanidhi Government Arts College, Tiruvannamalai.

METHODOLOGY

EXTRACTION [12]

The methanolic extract of *Ficus benjamina* leaves was prepared using the Soxhlet extraction method. Approximately 110 g of shade-dried, coarsely powdered leaf material was packed into a cellulose extraction thimble and placed in the Soxhlet extractor. The extractor was connected to a round-bottom flask containing methanol as the extraction solvent and fitted with a reflux condenser. The extraction was carried out by heating the solvent to reflux, allowing the methanol vapours to condense and continuously percolate through the plant material. The extraction cycle of reflux, percolation, and siphoning was continued until the solvent in the siphon tube became nearly colourless, indicating exhaustive extraction of the phytoconstituents. Following completion of the extraction, the methanolic extract was concentrated by evaporating the solvent on a hot plate until a semisolid mass was obtained. The dried extract was weighed to determine the percentage yield and stored in an airtight container at 4°C until further phytochemical and pharmacological analyses.

PHYTOCHEMICAL SCREENING [13]

he methanolic leaf extract of *Ficus benjamina* was subjected to preliminary phytochemical screening using standard qualitative phytochemical methods to determine the presence or absence of major classes of secondary metabolites. The extract was evaluated for secondary metabolites and it's the tests were performed according to established laboratory procedures, and the results were recorded based on the development of characteristic color changes or precipitates indicative of the respective phytochemical constituents.

BLOOD SAMPLE COLLECTION AND PLASMA PREPARATION [14]

Blood should be collected under an approved Institutional Animal Ethics Committee (documented or equivalent) protocol and by a trained veterinarian or qualified personnel. Use a healthy adult goat that has been ethically approved for the study. Properly restrain the goat in a standing position. Clip and disinfect the skin over the jugular vein. Collect blood aseptically from the jugular vein using a 20–22 G sterile needle attached to a syringe. For anticoagulant studies, immediately transfer the blood into tubes containing 3.2% sodium citrate (0.109 M) at a 9:1 blood-to-anticoagulant ratio (9 parts blood: 1 part sodium citrate). Gently invert the tube 4–5 times to mix do not shake vigorously. Centrifuge in 1500 × g for 10–15 minutes to obtain plasma is required. Use the plasma immediately for clotting time, PT and aPTT for in vitro anticoagulant assays, or store aliquots at −80°C until analysis.

ANTI COAGULANT ASSAY METHODS:

ACTIVATED PARTIAL THROMBOPLASTIN TIME (aPTT) [15-19]

Pre -warm well mixed Erba Actime (a specialised *in-vitro* diagnostic (IVD) reagent) and Calcium Chloride to 37°C. Add 100 µl of the patient or control plasma into a reaction tube and incubate at 37°C for 2 minutes. Add 4000 -16000 µg/ml of *Ficus benjamina* extract (depends on the concentration). Add 100 µl of prewarmed Calcium Chloride. Start simultaneously a timer. The time required for clot formation is the APTT, gently rock the reaction tube back and forth in the 37°C-water bath, continually looking for clot formation. Perform all tests in duplicate.

$$\% \text{ Increase in APTT} = \frac{\text{APTT Value} - \text{APTT control}}{\text{APTT control}} \times 100$$

Were,

APTT Value = Clotting time (seconds) of the test sample containing the extract/drug.

APTT Control = Clotting time (seconds) of the control sample without the extract/drug.

% Increase in APTT = The percentage by which the clotting time has increased compared to the control.

PROTIME LS PROTHROMBIN TIME(PT) [20-23]

Mix sufficient Protime LS reagent to complete the anticipated testing for the day and incubate reagent at 37°C no more than 4hours. Add 50 µl of the plasma or control plasma into a reaction tube and incubate at 37°C for 2 minutes. Add 4000 -16000 µg/ml of *Ficus benjamina* extract (depends on the concentration). Add 100 µl of freshly mixed reagent and start simultaneously a timer. Note the time for clot formation to the nearest 0.1 seconds.

$$\% \text{ Increase in PT} = \frac{\text{PT Value} - \text{MnPT Value}}{\text{MnPT Value}} \times 100$$

$$\text{INR Value} = \frac{\text{PT Value}}{\text{MnPT Value}} \times 100$$

Were,

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PT Value = Prothrombin Time of the test sample (seconds)
MnPT Value = Mean Normal Prothrombin Time (average PT of healthy control plasma, in seconds)
INR = International Normalized Ratio (To measure how long it takes blood clot)
PT = Patient/Test sample Prothrombin Time
MnPT = Mean Normal Prothrombin Time

RESULT AND DISCUSSION

Fresh leaves of *Ficus benjamina* were collected, shade-dried for 3 days, and pulverized into a coarse powder. The powdered leaf material was extracted with methanol using a Soxhlet extraction apparatus. The resulting extract was concentrated by evaporating the solvent on a hot plate to obtain a semisolid residue. The extraction process is illustrated in Figures 1 and 2**.** The percentage yield of the methanolic extract was calculated based on the weight of the dried extract and is presented in Table 1**.**

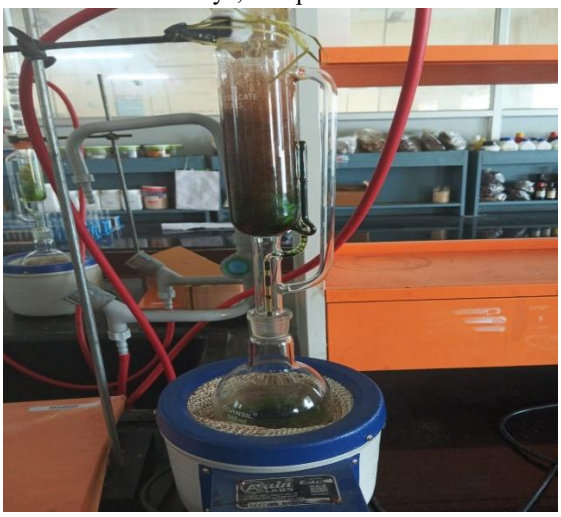

Figure 1: Methanolic extraction

Figure 2: Extract residue

Table 1: Percentage yield of methanolic extract of leaves.

Name of the plant	Percentage yield (%)
Ficus benjamina	10.31gm

PHYTOCHEMICAL SCREENING Phytochemical screening was performed buy chemical tests using the extract and respective reagents, and inference of phytoconstituent.

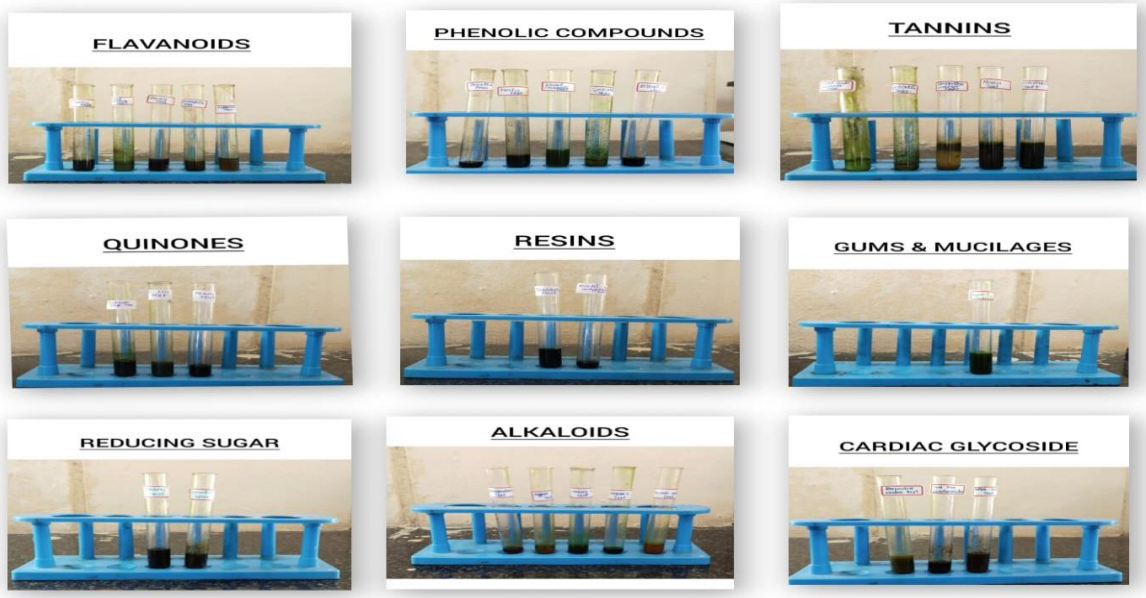

Figure 3: Presence of phytochemical screening of the residue

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Figure 4: Absence of phytochemical screening of the residue

Table 2: Presence of Phytoconstituents

PHYTOCONSTITUENTS	CHEMICAL TEST	INFERENCE
Alkaloids	Dragendroff's test	+
	Wager's test	+
	Picric acid test	+
Reducing sugar	Benedict's test	+
	Fehling's test	+
Cardiac glycosides	Bromine water test	+
	Test for cardenolides	+
	Keller – killani test	+
Phenolic components	Iodine test	+
	Ferric chloride test	+
	Gelatin test	+
	Ellagic test	+
Tannins	Gelatin test	+
	Braymer's test	+
	Sodium hydroxide	+
	Bromine water test	+
	Lead sub acetate	+
Flavonoids	Alkaline reagent test	+
	Ammonia test	+
	Lead acetate test	+
	Ferric chloride test	+
Resins	Acetic anhydride test	+

	Turbidity test	+
Gum and mucilage's	Alcohol test	+
Quinones	Alcoholic KOH test	+
	Conc.Hcl test	+
	Sulphuric acid test	+

Table 3: Absence of phytoconstituents

Phytoconstituents	**Chemical test**	**Inference**
Protein and amino acid	Biuret test	-
	Millon's test	-
	Ninhydrin test	-
	Xanthoprotein test	-
Anthraquinones	Bornthager's test	-
	Ammonium hydroxide test	-
Anthocyanins	Hydrochloride test	-
Glycosides	Aqueous NaOH test	-
	Legal test	-
Cholesterol	Detection of cholesterol	-
Emodin	Detection of emodin's	-
Carbohydrates	Saliwonaff's test	-
	Resorcinol test	-

Anticoagulant activity of the methanolic leaf extract of *Ficus benjamina*

The anticoagulant activity of the methanolic leaf extract of *Ficus benjamina* was evaluated using Prothrombin Time (PT) and Activated Partial Thromboplastin Time (aPTT) assays, with heparin (100 IU/mL) serving as the standard drug.

Table 4: ACTIVATED PARTIAL THROMBOPLASTIN TIME TEST

S.no	Sample	Con (µg/ml)	aPTT Value	Control value	Difference between APTT & CONT	% Of value difference
1.	Control	0	31.50 Sec's	31.5 Sec's	0.00 Sec's	0.00
2.	*F.benjamina*	4000	34.20 Sec's	31.5 Sec's	2.70 Sec's	8.57
3.	*F.benjamina*	8000	38.80 Sec's	31.5 Sec's	7.30 Sec's	23.17
4.	*F.benjamina*	12000	44.60 Sec's	31.5 Sec's	13.10 Sec's	41.59
5.	*F.benjamina*	16000	51.80 Sec's	31.5 Sec's	20.30 Sec's	64.44
6.	Heparin	100 iu/ml	60.90 Sec's	31.5 Sec's	29.40 Sec's	93.33

Control value = 31.5 Sec's

Reference value =24-36 Sec's

The methanolic leaf extract of *Ficus benjamina* exhibited concentration-dependent anticoagulant activity by prolonging the aPTT. The control plasma showed an aPTT of 31.50 s. Treatment with the extract increased the aPTT to 34.20 s (8.57%), 38.80 s (23.17%), 44.60 s (41.59%), and 51.80 s (64.44%) at concentrations of 4000, 8000, 12000, and 16000 µg/mL, respectively.

In comparison, heparin (100 IU/mL) prolonged the aPTT to 60.90 s (93.33%). Although the extract showed similar activity than heparin, it produced a significant dose-dependent increase in aPTT, with the highest concentration achieving 51.80 s, indicating marked anticoagulant activity. The greater prolongation of aPTT suggests that *F. benjamina* primarily affects the intrinsic and common coagulation pathways.

Table 5: PROTHROMBIN TIME TEST

S.no	Sample	Con(µ/ml)	PT Value	INR Value	Difference between PT & MnPT	% of value difference
1.	Control	0	12.80 Sec's	1.00	0.00 Sec's	0.00
2.	*F.benjamina*	4000	13.60 Sec's	1.06	0.80 Sec's	6.25
3.	*F.benjamina*	8000	14.95 Sec's	1.17	2.15 Sec's	16.80
4.	*F.benjamina*	12000	16.75 Sec's	1.30	3.95 Sec's	30.86
5.	*F.benjamina*	16000	18.60 Sec's	1.45	5.80 Sec's	45.31
6.	Heparin	100 iu/ml	24.30 Sec's	1.90	11.50 Sec's	89.84

MnPT Value = 12.8 Sec's
Reference Value = 9.25 – 13. 1 Sec's

The methanolic leaf extract of *Ficus benjamina* exhibited concentration-dependent anticoagulant activity by prolonging the PT. The control plasma showed a PT of 12.80 s. Treatment with the extract increased the PT to 13.60 s (6.25%), 14.95 s (16.80%), 16.75 s (30.86%), and 18.60 s (45.31%) at concentrations of 4000, 8000, 12000, and 16000 µg/mL, respectively.

In comparison, heparin (100 IU/mL) prolonged the PT to 24.30 s (89.84%). Although the extract showed similar activity than heparin, it produced a significant dose-dependent increase in PT, indicating moderate anticoagulant activity through the extrinsic and common coagulation pathways.

DISCUSSION

The present study demonstrated that the methanolic leaf extract of *Ficus benjamina* possesses dose-dependent anticoagulant activity, as evidenced by significant prolongation of both Prothrombin Time (PT) and Activated Partial Thromboplastin Time (aPTT). The baseline coagulation values were within the normal reference range reported for healthy goats by Zersen et al., indicating that the coagulation assessment was reliable. At the highest extract concentration (16,000 µg/mL), PT increased from 12.80 to 18.60 s and aPTT from 31.50 to 51.80 s, confirming a concentration-dependent anticoagulant effect.

These findings are consistent with previous reports on the anticoagulant properties of *Ficus* species. Ambreen et al. demonstrated significant prolongation of PT and aPTT in six *Ficus* species, while Kumar et al. highlighted the antithrombotic potential of *Ficus benghalensis*. Similarly, Hamed et al. reported marked prolongation of PT and aPTT with *Ficus carica* latex, suggesting potent anticoagulant and fibrinolytic activity. Although the anticoagulant effect of *F. benjamina* was lower than that of heparin, the observed increase in clotting times indicates promising biological activity, which may be attributed to phytochemicals such as flavonoids and phenolic compounds reported in *Ficus* species. Overall, the findings support the potential of *F. benjamina* as a natural source of anticoagulant agents, warranting further isolation of active constituents and in vivo studies to confirm its therapeutic efficacy.

CONCLUSION:

The present study demonstrated that the methanolic leaf extract of *Ficus benjamina* possesses significant ***in-vitro*** anticoagulant activity, as evidenced by the concentration-dependent prolongation of Prothrombin Time (PT) and Activated Partial Thromboplastin Time (aPTT). The observed anticoagulant effect, although lower than that of the standard drug heparin, indicates the potential of the extract to interfere with the coagulation cascade. This activity may be attributed to the presence of bioactive phytochemicals, including flavonoids, phenolic compounds, and tannins, which have been reported to exhibit anticoagulant and antithrombotic properties. These findings suggest that *F. benjamina* is a promising source of natural anticoagulant compounds with potential therapeutic applications. However, further studies are necessary to isolate and characterize the active constituents, elucidate their underlying mechanisms of action, and establish their efficacy and safety through ***in-vivo*** studies and clinical evaluation.

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