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Research Article

Evaluation of Anti-Coagulant Activity of *Ficus racemosa* Bark Extract in Rats

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ABSTRACT

Coagulation disorders such as thrombosis and stroke are major health concerns, and the use of conventional anticoagulants is often limited by adverse effects. this study evaluated the anticoagulant potential of the ethanolic extract of Ficus racemosa bark (EEFRB). in vitro analysis demonstrated significant prolongation of prothrombin time (pt) at 1 mg/ml and activated partial thromboplastin time (APTT) at 10 mg/ml. in vivo studies in rats, administered EEFRB (100, 200, and 400 mg/kg), showed increased pt (days 3 and 5), significant APTT prolongation (day 5), reduced platelet count, and extended clotting time, indicating effects on both extrinsic and intrinsic pathways. LC–MS analysis identified key bioactive compounds, including coumarin, quercetin, kaempferol, and β-sitosterol. overall, EEFRB exhibited dose-dependent anticoagulant activity comparable to warfarin, with notable efficacy at 200 and 400 mg/kg.

INTRODUCTION

Haemostasis is a vital physiological process that prevents blood loss through coordinated interactions among the vessel wall, platelets, and coagulation factors, leading to stable clot formation[1]. disruption of this balance results in hypercoagulable states, increasing the risk of conditions such as deep vein thrombosis, pulmonary embolism, stroke, and myocardial infarction[2]. although conventional anticoagulants like heparin and warfarin are effective, their use is limited by adverse effects, including bleeding and dermatological reactions[3]. this has driven interest in safer, plant-based alternatives. *Ficus racemosa* (family moraceae), a medicinal plant with diverse pharmacological activities, has been traditionally suggested to possess anticoagulant properties; however, scientific validation remains limited. the plant is rich in bioactive compounds such as coumarin, quercetin, kaempferol, β-sitosterol, lupeol, and ellagic acid, which are associated with anticoagulant effects. therefore, the present study aims to evaluate the anticoagulant activity of *Ficus*

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racemosa bark extract using both *in vitro* and *in vivo* models[4,5,6].

MATERIALS AND METHODS

Ficus racemosa bark was collected from the B.K. Mody Government Pharmacy College campus, Rajkot, and authenticated at Saurashtra University (Ref. No. SU/BIO/2516692). The bark was washed, shade-dried, powdered, and extracted using the maceration method[7,8]. The dried powder was subjected to extraction with Ethanol, and the extract was concentrated and stored for further analysis. Preliminary Phytochemical screening confirmed the presence of flavonoid, Phenolic, Coumarin, alkaloids, Terpenoids, and steroids, while quantitative estimation of total Phenolic and flavonoid content was performed using spectrophotometric methods[9,10,11,12]. Phytoconstituents were further analyzed using LC–MS[13].

In vitro anticoagulant activity was evaluated using platelet-poor plasma obtained from rat blood, with Prothrombin Time (PT) and Activated Partial Thromboplastin Time (APTT) as key parameters[13]. The extract was tested at varying concentrations. For *in vivo* studies, Sprague Dawley rats (180–200 g) were divided into five groups: normal control, standard (warfarin 0.2 mg/kg), and three treatment groups receiving Ethanolic extract (100, 200, and 400 mg/kg, Oral.) for 3 days, Blood samples were collected on days 3rd, 5th, and 7th, and evaluated for PT, APTT, clotting time, and platelet count[13,14,15]. Statistical analysis was performed using ANOVA followed by Tukey's test, with significance set at $p < 0.05$[13].

RESULTS AND DISCUSSION:

1. **Yield %**

The percentage yield of the Ethanolic extract obtained from 100 grams of *Ficus racemosa* Bark was determined to be 7%w/w.

Figure 1 Extract of racemosa Bark

Figure 2 Tree of Ficus racemosa

Figure 1 bark of Ficus racemosa

2. Phytochemical analysis of Ethanolic extract of Ficus racemosa bark Powder

Table 1 Phytochemical analysis of Ethanolic extract of Ficus racemosa bark Powder

Chemical test	Name of test	Inference	Result
Alkaloids	Mayer's Test	Creamy-white colored precipitate	+
	Dragendorff's Test	Orange-colored precipitate	+
	Wagner's Test	Brown-colored precipitate	+
	Hager's Test	Yellow-colored precipitate	+
Saponins	Foam test	Foam layer (1cm)	+
Protein and Amino acids	Million's Test	White Precipitate turns red upon gentle heating. Not observed	_
Flavonoid	Alkaline reagent Test	Yellow colour become colourless after adding Conc. HCl	+
	Shinoda test	Magnetic red colour	+
	Ferric chloride Test (1%)	Blue-green coloured	+
	Lead acetate Test	Yellow coloured precipitate	+
Phenols	Ferric chloride Test (5%)	Dark green color	+
	Lead acetate Test	White coloured precipitate	+
Glycosides	Keller Killani Test	Reddish-brown ring color at junction and bluish-green colored upper layer	_
	Legal's Test	Pink to red color	+
Steroids	Liebermann – Burchard Reaction	Initially red color, followed by blue color and in last green color appear chloroform layer	+
	Salkowski's test	Red color in chloroform layer and greenish-yellow color in acid layer	+
Tannins	Ferric chloride solution (5%)	Dark green color	+
	Lead acetate solution	White precipitate	+
	Potassium dichromate test	Yellow-colored precipitate	+
Carbohydrates	Molisch's test	Purple to violet ring at junction of two layers	+
	Benedict's test	Reddish-brown precipitate	+
Coumarin test	10% NaoH	Yellow Color	+

3. Total Flavonoid Content (TFC)

Table 2 Conc. and absorbance at 510 nm of Quercetin and EEFRB

Conc.(µg/ml)	Abs (nm)
10	0.172
20	0.234
40	0.387
60	0.523
80	0.722
100	0.895

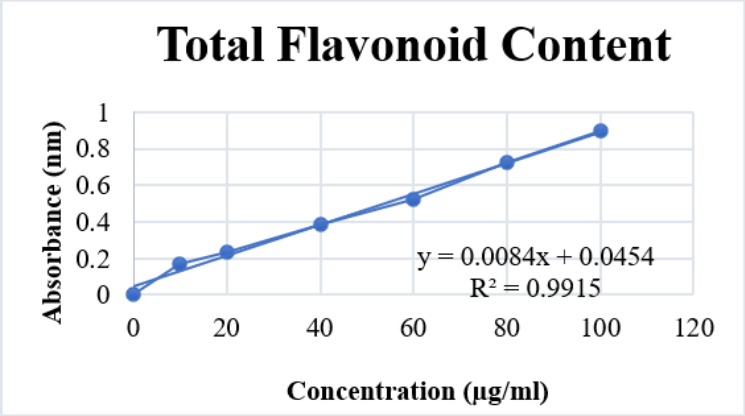

Figure 4 standard curve of Quercetin

4. Total Phenolic Content (TPC)

Table 3 Conc. and absorbance at 765 nm of gallic acid and EEFRB

Conc.(µg/ml)	Abs (nm)
10	0.196
20	0.265
40	0.464
60	0.636
80	0.919
100	1.214
120	1.345

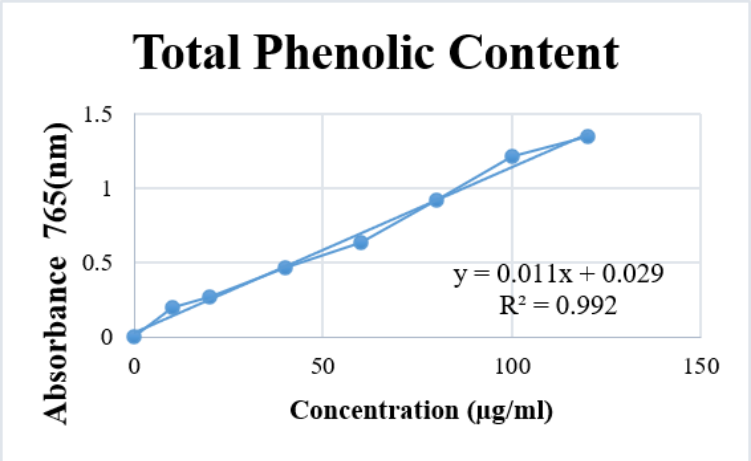

Figure 5 standard curve of Quercetin

5. Phytochemical profile of the Ficus racemosa Bark Ethanolic extract of by LC-MS

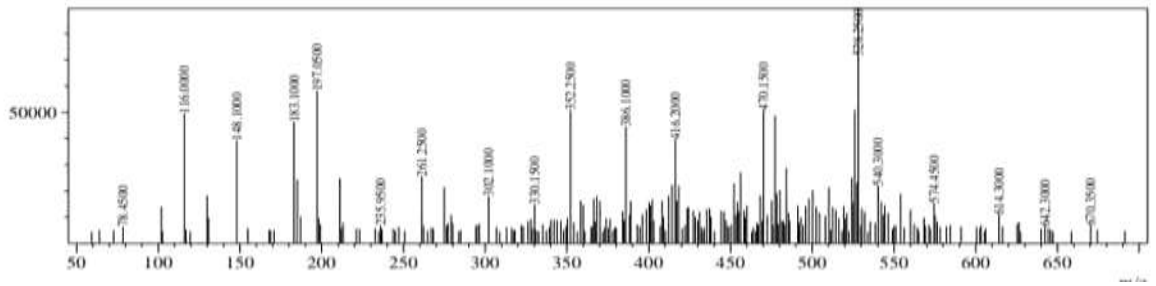

Figure 2 ion mass spectra reveal major ion product of Quercetin at m/z -302.10(303.05g/mol)

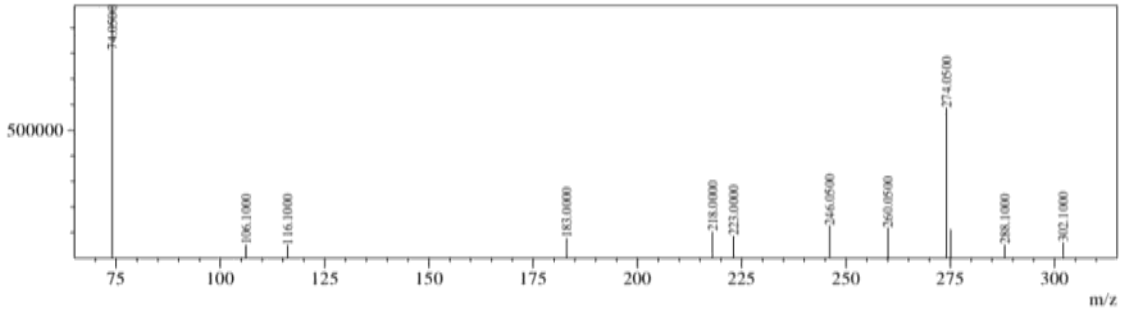

Figure 7 ion mass spectra reveal major ion product of Kaempferol at m/z -288.10 (287.06g/mol)

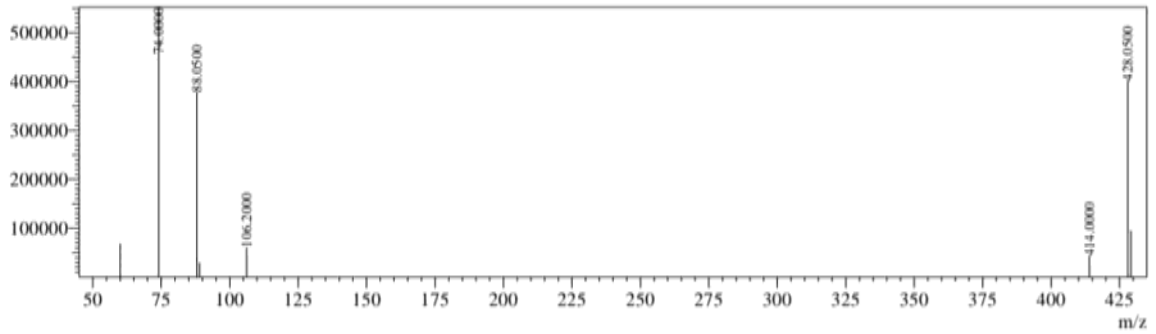

Figure 8 ion mass spectra reveal major ion product of β-Sitosterol at m/z -414.00 (415.40g/mol)

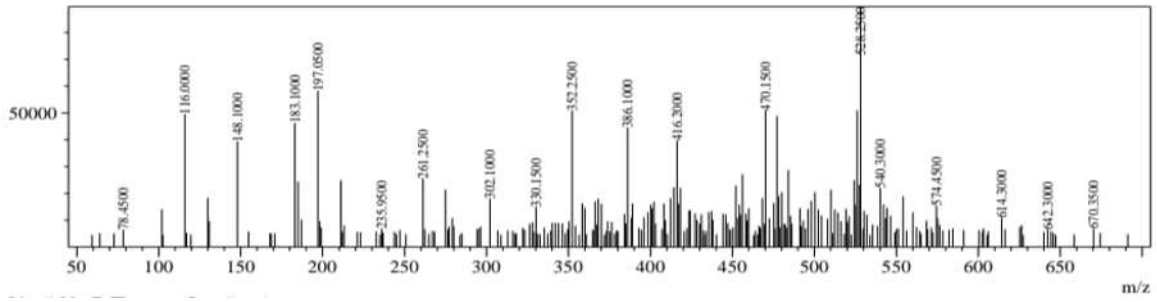

Figure 9 ion mass spectra reveal major ion product of Coumarin at m/z -148.10 (147.05g/mol)

6. *In vitro* study Prothrombin time:

Table 4 Effect of EEFRB on Prothrombin time (PT)

Sr. No	Conc. (mg/ml)	Prothrombin time (s) Mean ± SEM	Prothrombin time in INR unit
1	Blank	11.00 ± 0.58	1
2	0.2	20.18 ±0.43*	1.83
3	0.3	21.81 ±1.01*	1.98

4	0.4	22.61 ±0.76*	2.06
5	0.5	24.29 ±0.39*	2.21
6	0.6	26.56 ±1.05*	2.41
7	0.7	29.75 ±0.47*	2.7
8	0.8	32.29 ±0.22*	2.94
9	0.9	33.43 ±0.71*	3.04
10	1	34.84 ±0.26*	3.17
11	1.2	29.96 ±0.53*	2.72
12	10	28.61 ±0.26*	2.6
13	100	26.02 ±0.47*	2.37
14	500	25.36 ±0.46*	2.31

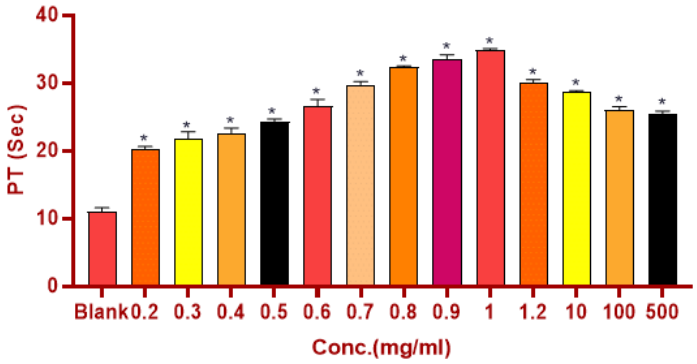

Figure 10 Effect of EEFRB on Prothrombin

Table 5 Activated Partial Thromboplastin time test (APTT)

Sr. No	Conc.(mg/ml)	Effect of EEFRB APTT (s)
1	Blank	32.33 ± 0.88
2	1	33.52 ± 0.65
3	2	34.51 ± 0.68
4	3	36.43 ± 0.14*
5	4	39.95 ± 0.43*
6	5	41.84 ± 0.34*
7	6	46.64 ± 0.18*
8	7	51.72 ± 0.39*
9	8	58.38 ± 0.33*
10	8.5	58.19 ± 0.37*
11	9	63.15 ± 0.96*
12	9.5	66.31 ± 0.76*
13	10	69.75 ± 0.18*
14	11	59.03 ± 0.35*
15	12	57.40 ± 0.70*
16	13	54.52 ± 0.82*
17	14	53.02 ± 0.05*
18	15	53.49 ± 0.21*

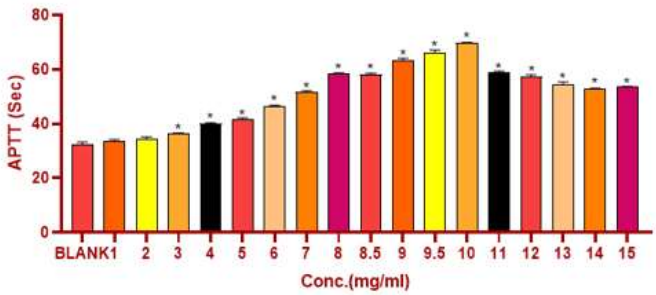

Figure 11 Activated Partial Thromboplastin time test (APTT)

7. In vivo study Prothrombin time:

Table 6 Prothrombin time at different days

Group	Day 3 PT time (s)	Day 5 PT time (s)	Day 7 PT time (s)
Control	15.02± 0.40	15.30±0.21	16.65±0.31
Standard (0.2mg/kg Warfarin)	27.98±0.60*	34.80±0.32*	31.91±0.28*
100mg/kg EEFRB	21.64±0.38*	25.27±0.50*	23.64±0.62*
200mg/kg EEFRB	23.61±0.53*	30.81±0.25*	26.6±0.67*
400mg/kg EEFRB	27.10±0.57*	31.12±0.87*	31.08±0.24*

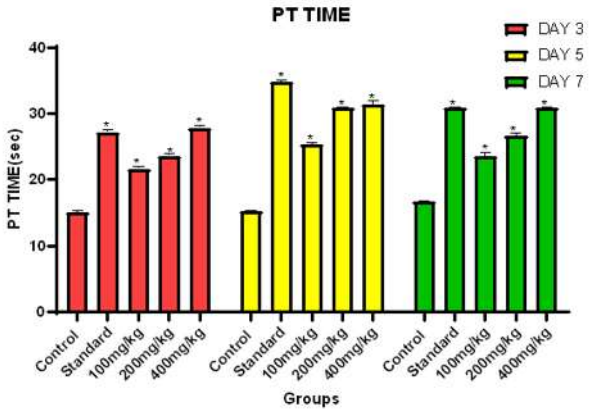

Figure 12 Prothrombin time at different days

Table 7 APTT Time at different days

Group	Day 3 APTT time (s)	Day 5 APTT time (s)	Day 7 APTT time (s)
Control	14.36±0.57	15.30±0.21	16.65±0.31
Standard(0.2mg/kg Warfarin)	22.51±0.61*	34.80±0.32*	30.82±0.28*
100mg/kg EEFRB	17.97±0.56*	25.27±0.50*	23.64±0.62*
200mg/kg EEFRB	18.95±0.50*	30.81±0.25*	26.6±0.67*
400mg/kg EEFRB	20.16±0.53*	31.32±0.87*	30.23±0.24*

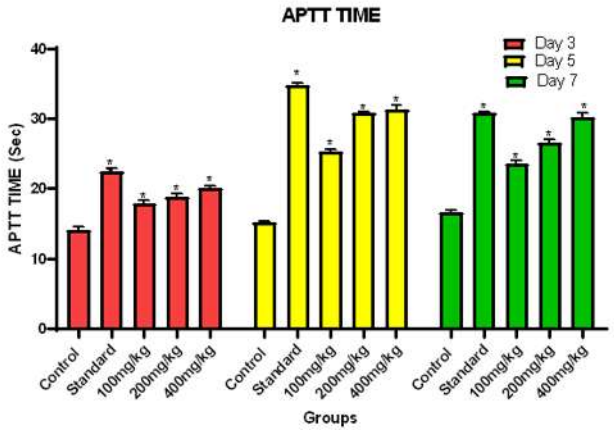

Figure 13 APTT Time at different days

Table 8 Clotting time

Group	Day 3 clotting time (s)	Day 5 clotting time (s)	Day 7 clotting time (s)
Control	38.81±0.71	37.68±0.66	38.1±0.72
Standard(0.2mg/kg Warfarin)	55.45±0.79*	75.02±0.74*	71.87±0.68*
100mg/kg EEFRB	48.46±0.68*	61.08±0.69*	59.13±0.79*
200mg/kg EEFRB	52.43±0.74*	66.73±0.78*	65.2±0.83*
400mg/kg EEFRB	54.58±0.82*	72.08±0.84*	70.38±0.76*

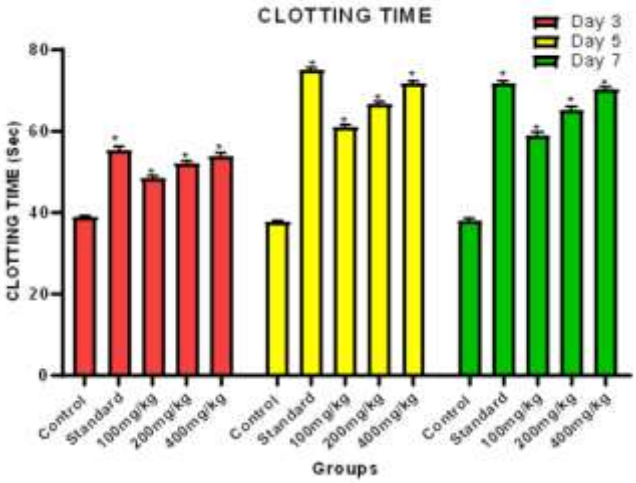

Figure 14 clotting time

Table 9 Platelet Count

Group	Day 3 Platelet count	Day 5 Platelet count	Day 7 Platelet count
Control	865.40±25.10	37.68±0.66	38.1±0.72
Standard(0.2mg/kg Warfarin)	55.45±0.79*	75.02±0.74*	71.87±0.68*
100mg/kg EEFRB	48.46±0.68*	61.08±0.69*	59.13±0.79*
200mg/kg EEFRB	52.43±0.74*	66.73±0.78*	65.2±0.83*
400mg/kg EEFRB	54.58±0.82*	72.08±0.84*	70.38±0.76*

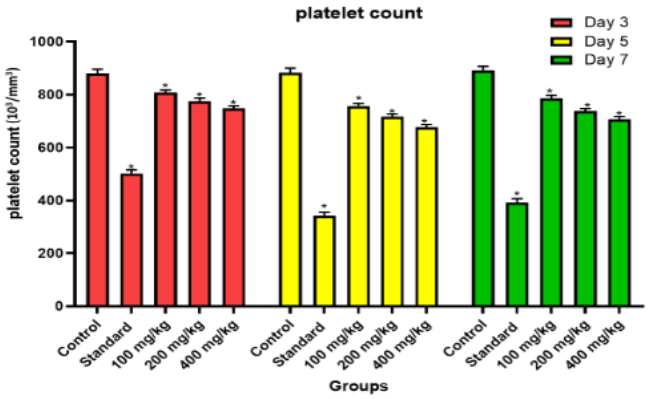

Figure 15 Platelet Count

Table 10 CBC on 3rd day

PARAMETER	Control Group	Standard Group	100mg/kg	200mg/kg	400mg/kg
HBG g/dL	14.81±0.73	20.21±0.95	20.31±0.49	18.09±0.39	20.56±0.61
RBCmill./Cmm	8.20±0.19	9.51±0.84	7.74±0.25	7.44±0.80	7.65±0.40
WBC $10^3/mm^3$	7.69±0.32	8.38±0.19	9.01±0.55	7.66±0.40	7.22±0.81
PLT$10^3/mm^3$	865.40±25.10*	501.67 ±5.58*	807.50 ± 4.1*	775.00 ± 4.83*	748.33 ±3.66*
HCT %	53.21±0.51	54.06±0.35	55.9±0.64	51.33±0.29	48.53±0.37
MCV fL	62.36±2.98	68.19±2.81	69.18±2.331	67.386±2.77	63.15±2.75
MCH pg	23.91±0.59	21.30±0.20	24.23±0.46	21.56±0.35	25.93±0.67
MCHC g/dL	34.25±0.87	37.15±0.32	36.25±0.91	31.85±0.63	36.06±2.77
RDW-CV %	14.21±0.12	14.25±0.36	14.23±0.78	14.1±0.24	14.23±0.43
NEU %	39.76±0.43	33.66±0.75	36.36±0.58	27.46±0.85	35.73±2.50
LYM %	56.16±0.75	60.66±0.58	57.83±0.15	61.16±0.579	58.5±0.88
EOS %	1±0	1±0	1±0	1±0	1±0
MON %	8.86±0.59	5.63±0.38	8±0.66	7±0.47	7.9±0.52

Table 11 CBC on 5th day

PARAMETER	Control Group	Standard Group	100mg/kg	200mg/kg	400mg/kg
HBG g/dL	18.65±0.60	19.75±0.88	18.85±0.31	19.60±0.35	2.95±1.60
RBCmill./Cmm	8.55±0.18	9.30±0.85	8.40±0.28	7.15±0.15	7.50±1.00
WBC $10^3/mm^3$	7.65±0.40	8.35±1.10	9.00±0.55	7.65±0.40	7.20±0.38
PLT$10^3/mm^3$	882.50 ±7.39*	340.83 ±5.83*	757.17 ±3.96*	717.50 ±3.82*	676.67 ±4.41*
HCT %	55.00±2.40	52.90±1.60	55.10±0.45	52.90±1.40	49.40±0.90
MCV fL	62.00±2.70	62.00±2.70	69.20±2.00	66.00±2.80	63.90±4.80
MCH pg	21.10±0.70	21.70±1.50	25.00±0.80	21.10±0.70	25.30±0.85
MCHC g/dL	35.10±0.35	38.00±0.90	37.00±0.85	33.00±0.90	38.80±2.70
RDW-CV %	14.20±0.05	14.20±0.02	14.20±0.03	14.05±0.04	14.20±0.03
NEU %	35.00±1.80	33.00±1.70	36.50±1.60	27.50±0.90	35.50±2.90
LYM %	55.00±2.00	61.50±1.60	56.50±2.10	61.00±1.60	58.20±2.80
EOS %	1±0	1±0	1±0	1±0	1±0
MON %	8.00±1.10	5.00±0.35	7.90±1.40	6.80±1.50	4.40±0.20

Table 12 CBC on 7th day

PARAMETER	Control Group	Standard Group	100mg/kg	200mg/kg	400mg/kg
HBG g/dL	18.70±0.58	19.85±0.92	18.95±0.28	19.70±0.34	2.90±1.65
RBCmill./Cmm	8.58±0.17	9.35±0.88	8.50±0.29	7.20±0.13	7.55±1.02
WBC $10^3/mm^3$	7.70±0.43	8.38±1.12	9.05±0.58	7.70±0.43	7.25±0.40

PLT10^3/mm^3	890.83 ±6.51*	391.67 ±6.15*	785.83 ±4.73*	737.50 ±3.82*	707.50 ±3.82*
HCT %	55.15±2.50	53.00±1.60	55.25±0.46	53.05±1.42	49.50±0.92
MCV fL	62.10±2.78	62.10±2.78	69.50±2.05	66.30±2.85	64.10±4.90
MCH pg	21.20±0.76	21.85±1.55	25.30±0.86	21.20±0.76	25.45±0.88
MCHC g/dL	35.30±0.36	38.20±0.92	37.30±0.88	33.10±0.90	39.00±2.75
RDW-CV %	14.21±0.04	14.24±0.02	14.24±0.03	14.10±0.04	14.23±0.03
NEU %	35.10±1.82	33.10±1.75	36.70±1.55	27.70±0.92	35.70±2.95
LYM %	55.20±1.95	61.70±1.55	56.90±2.18	61.20±1.58	58.60±2.85
EOS %	1±0	1±0	1±0	1±0	1±0
MON %	8.10±1.12	5.05±0.37	8.00±1.48	6.90±1.55	4.50±0.23

CONCLUSION

The present study provides strong scientific evidence supporting the anticoagulant potential of *Ficus racemosa* bark extract. The ethanolic extract (EEFRB) demonstrated significant anticoagulant activity in both *in vitro* and *in vivo* studies by prolonging Prothrombin Time (PT) and Activated Partial Thromboplastin Time (APTT), indicating effects on the extrinsic and intrinsic coagulation pathways, respectively. In vivo administration at doses of 200 and 400 mg/kg resulted in a marked increase in PT, APTT, and clotting time, along with a reduction in platelet count, suggesting inhibition of platelet function. These effects may be attributed to bioactive constituents such as coumarin, quercetin, kaempferol, and β-sitosterol. Overall, the findings highlight the ethanolic extract of *Ficus racemosa* bark as a promising natural anticoagulant, warranting further pharmacological and clinical investigations to establish its safety and therapeutic potential.

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