



## Invitro Anticoagulant Activity of Monoon longifolium Leaf Extract on Normal Healthy blood plasma

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Type of Publication: Original Research Paper

Conflicts of Interest: Nil

### Abstract

Haemostasis is a process involving in clot formation in the walls of ruptured or damaged blood vessels in order to prevent abnormal bleeding as well as to keep intravascular blood in fluid form. Platelet hyperactivity and platelet interaction with endothelial cells contribute to the development and progression of many cardiovascular diseases such as Atherosclerosis and Thrombosis. The impact of platelet activity with different pharmacological agents such as Acetylsalicylic acid and Coumarin derivatives, has been shown to be effective in the prevention of cardiovascular diseases. In this study we aimed to evaluate the possible anticoagulant effect of methanolic extract of leaves of Monoonlongifolium. The methanolic extract of Monoonlongifolium was tested for invitro prothrombin time (PT) test.

**Keywords:** Hemostasis, Platelet, Anticoagulant, Methanolic extract, Monoonlongifolium

### Introduction

India has a huge history for the treatment of various diseases using medicinal plants. Indian plants show widespread bioactivity with minimum side effects. In contrast to synthetic compounds, herbal products are safer and hence it is preferred for treatment of various ailments (C.K. Kokate et al., 2008). Hemostasis and blood coagulation involves complex interactions between the injured vessel walls, platelets and coagulation factors. It involves positive feedback system and various clotting factors (Clotting factor I-XIII). These numbers represent the order in which they were discovered and not the order of participation in the clotting process. Blood clotting results in formation and insoluble thread like mesh of fibrin which traps blood cells and is much stronger than the rapidly formed platelet plug (K.D. Tripathi 2013). In the final stages of this process Prothrombin activator acts on the plasma protein prothrombin converting it into thrombin. Thrombin can act on another plasma protein Fibrinogen and converts it into fibrin.

Prothrombin activator can be formed by two process (Intrinsic and Extrinsic pathway) which often occur together. The extrinsic pathway occurs rapidly (within seconds) when there is tissue damage outside the circulation (Ross and Wilson et, al 2001). Damaged tissue releases a complex of chemicals called thromboplastin or tissue factor, which initiates coagulation. The intrinsic pathway is slower (3-6min) and is confined to the circulation. It is triggered by damage to a blood vessel lining (endothelium) and the effects of platelets adhering to it. After a time, the clot shrinks, squeezing out serum, a clear sticky fluid that consist of plasma from which clotting factors have been removed. In general blood clotting occurs in three stages (K. Sembulingam et, al 2012):

1. Formation of prothrombin activator. (Intrinsic and Extrinsic pathway)
2. Conversion of prothrombin into thrombin.
3. Conversion of fibrinogen into fibrin

**Fig 1: Anticoagulant activity**

## Materials And Methods

### Collection Of Plant Material

The leaves of *Monoonlongifolium* had been collected from wild developing tree with inside the botanical garden, Pharmacognosy department, in Vaageswari college of Pharmacy, Thimmapur, Karimnagar, Telangana, India. Identification and authentication had been performed by a certified taxonomist (BSI/DRC/2025-26/Tech./Identification 27).

Leaves had been collected, separated, shade dried and powdered with laboratory mixer and sieved. Preparation of plant extract: The leaves of *Monsoon longifolium* was collected, shade dried and powdered using laboratory mixer which is then sieved. 50g of powder was weighed and kept for the soxhlation process using 500ml of methanol as a solvent for 6hrs. Then the extract was collected in a China dish and kept for evaporation. After evaporating the extract was stored under room conditions till further use.

### Blood Collection And Plasma Sample Preparation

Blood sample were drawn via vein puncture from healthy volunteer donor. The blood placed separately in containers containing EDTA to prevent the clotting process. Centrifugation (15min at rate 3000rpm) was carried out to separate the blood cells from plasma in order to obtain Pure Platelet Plasma (PPP) for prothrombin time test (fig:1, 2,3 & table:1).

### 3 .Anticoagulation Assay

Group I: Negative control (0.2 ml plasma + 0.1 ml saline + 0.3 ml  $\text{CaCl}_2$ )

Group II: Positive control (0.2 ml plasma + 0.1 ml EDTA 50 mg/ml + 0.3 ml  $\text{CaCl}_2$ )

Group III: Test group (0.2 ml plasma + 0.1 ml 0.125 g/ml extract + 0.3 ml  $\text{CaCl}_2$ )

Group IV: Test group (0.2 ml plasma + 0.1 ml 0.25 g/ml extract + 0.3 ml  $\text{CaCl}_2$ )

Group V: Test group (0.2 ml plasma + 0.1 ml 0.5 g/ml extract + 0.3 ml  $\text{CaCl}_2$ )

Group VI: Test group (0.2 ml plasma + 0.1 ml 1 g/ml extract + 0.3 ml  $\text{CaCl}_2$ )



**Fig2: Anticoagulant activity of different concentrations of plant extract**

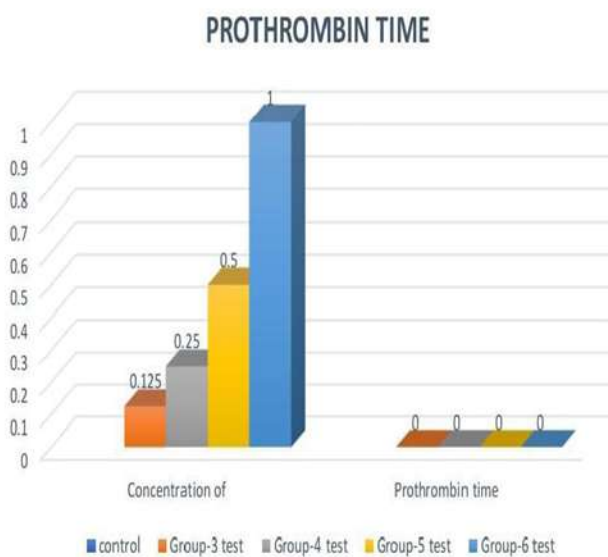


**Table 1: The average prothrombin times (PT) observed for each group are as follows:**

Test tubes	Concentration of extract (g/ml)	Prothrombin time
Group I	-	10 sec
Group II	-	15 sec
Group III	0.125	1min

Group IV	0.25	3min
Group V	0.5	4.7min
Group VI	1	5.5min

**Fig:3 prothrombin time**



## Results And Discussion

### Anticoagulant Evaluation by Leaves Extract of Monoon longifolium

Abnormalities of coagulation are frequently present in seriously sick patients and usually result in disability and death, hence requires prompt diagnosis and treatment (Marcel and Steven 2006) Acute platelet thrombus formation leads to the development of atherosclerosis, followed by embolization and of stenosed vessels (Lou et al 1989) platelets and thrombin are interdependent since thrombin induced activation of platelets is an important as platelets availability for Thrombus formation (Riaz, et al 2009).

As tannins and phenolics phytoconstituents are present in Methanolic extract of Monoon longifolium. These Tannins and Phenolic compounds helps to prevent

blood clots by stopping platelets from becoming active, sticking together, and forming clots. They also increase PT time, showing a strong effect against clot(thrombus) formation.

Prothrombin test on normal healthy plasma using Methanolic extract of Monoon longifolium leaves for anticoagulant activity.

### Conclusion

Pharmacological screening of comparative study of different concentrations of plant leaf extract. Soxhlation of methanol solvents of Monsoon longifolium had shown interesting results. Methanolic solution extracts exhibited the dose dependent anticoagulant activity.

The methanolic extract of Monsoon Longifolium leaves exhibits significant anticoagulant activity,

evident from the prolonged prothrombin time in a dose dependent manner. These results suggest that Monsoon Longifolium could serve as a potential source of natural anticoagulant agent.

### Acknowledgment

Authors are thankful to Ms. B. Sowjanya, Vaageswari College of Pharmacy Karimnagar India for her valuable suggestions in improving the contents of this research article.

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