RESEARCH PAPER

Comparative In-Vitro Anticoagulant Activity Of *Ixora Coccinea* Plant Parts

Urmistha Sarkar[1*], Mrinmoy Manna[2], Shribus Karar[3], Kousik Saha[4], Krishanu Pramanick[5], Mouma Mondal[6], Suman Pramanik[7]

[1*]Assistant Professor, Department of Pharmacology, Netaji Subhas Chandra Bose Institute of Pharmacy, Chakdaha, West Bengal, India, Orcid id: 0009-0009-3303-696X, urmisthasarkar@gmail.com
[2]Student, Master of Pharmacy, Department of Pharmacology, Netaji Subhas Chandra Bose Institute of Pharmacy, Chakdaha, West Bengal, India, Orcid id: 0009-0006-4317-6657, mrinmoymanna08@gmail.com
[3]Student, Bachelor of Pharmacy, Department of Pharmacy, Netaji Subhas Chandra Bose Institute of Pharmacy, Chakdaha, West Bengal, India, kararshribus@gmail.com
[4]Assistant Professor, Department of Pharmacology, Netaji Subhas Chandra Bose Institute of Pharmacy, Chakdaha, West Bengal, India, Orcid id: 0000-0001-5314-6757, kausiksaha1024@gmail.com
[5]Assistant Professor, Department of Pharmacology, Netaji Subhas Chandra Bose Institute of Pharmacy, Chakdaha, West Bengal, India, Orcid id: 0009-0001-8943-9225, krishanupramanick97@gmail.com
[6]Student, Bachelor of Pharmacy, Department of Pharmacy, Netaji Subhas Chandra Bose Institute of Pharmacy, Chakdaha, West Bengal, India, Orcid id: 0009-0009-5601-2303, moumamondal2004@gmail.com
[7]Student, Bachelor of Pharmacy, Department of Pharmacy, Netaji Subhas Chandra Bose Institute of Pharmacy, Chakdaha, West Bengal, India, sp3520115@gmail.com

Abstract

Background: Natural products are increasingly explored as safer alternatives to synthetic anticoagulants. *Ixora coccinea*, a medicinal plant rich in phytochemicals, was evaluated for its in vitro anticoagulant potential.

Methods: Flower and stem extracts were tested using coagulation time assays. Control and standard drug groups established baseline and reference values.

Results: The stem extract demonstrated moderate, dose-dependent activity (117 ± 0.22 s) at low dose; (130 ± 0.44 s) at high dose. The flower extract showed stronger effects, with the high-dose group (158 ± 0.45 s) nearly equivalent to the standard drug (159 ± 0.35 s).

Conclusion: *Ixora coccinea* exhibits significant anticoagulant activity, particularly in its floral components, likely due to flavonoids, phenolics, and terpenoids. These findings support its potential as a source of natural anticoagulant agents. Further phytochemical isolation and in vivo validation are recommended.

Keywords: *Ixora coccinea*, anticoagulant activity, phytochemicals, coagulation assay, thrombotic disorders

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Introduction

Anticoagulants and coagulants are essential chemicals that regulate blood clotting, preventing excessive bleeding and blood clots. Blood clot development is triggered by coagulants, commonly referred to as blood thinners.[1] They are used to treat ailments like stroke, pulmonary embolism, and deep vein thrombosis. Anticoagulants disintegrate pre-existing clots or stop the clotting cascade. Anticoagulants are used for long-term prevention, while coagulants are usually utilized for acute bleeding disorders.

The intrinsic and extrinsic pathways are the two primary pathways involved in the blood coagulation system.[2] The extrinsic process is started by tissue factor, a protein secreted by injured tissues, whereas the intrinsic pathway is started by contact with negatively charged surfaces and involves several clotting factors. The body's ability to regulate bleeding, known as hemostasis, depends on both channels being intact. Bleeding problems can result from deficiencies in any one of these clotting factors. The components X, V, II (prothrombin), and I (fibrinogen) activate the common pathway, which is also referred to as the "thrombin generation" pathway.[3]

One of the main drawback of anticoagulants [4] are the elevated risk of bleeding. Certain anticoagulants, like warfarin, need to be taken with food restrictions to keep blood levels steady. To guarantee the medication's efficacy, routine observation, and dosage modifications are required. Dermatological issues, hair loss, diarrhea, and nausea are other possible adverse effects.[5]

Herbal anticoagulants have many advantages, such as a wide range of bioactive substances, fewer adverse effects, increased cost -effectiveness and safety, and the potential to treat several illnesses. [6]

The plant *Ixora coccinea*, occasionally referred to as "Jungle Geranium," has a long history of use in Indian traditional medicine, especially in Ayurveda and folk medicine. Its stem, leaves, blooms, and roots are all used to cure a variety of illnesses, including skin conditions like ulcers, scabies, and abscesses [7]. While the stem is used as an oral decoction for hypertension, amenorrhea, irregular menstrual cycles, and catarrhal bronchitis, the leaves and roots are used as antiseptics [8].

Materials And Methods

Preparation of Extract

Fresh stems and flowers of *Ixora coccinea* were collected and washed with clean water and shed dried. Then dried flowers were taken for the maceration extraction process for 72 hours and dried stems were placed into the soxhlation chamber for the soxhlation process .The whole soxhlation process took 120 hours to complete. Mixture of 70% ethanol and 30% water was used as solvents for both process. The extracts were collected, filtered and kept inside a vacuum desiccator for evaporation. The dried extracts were kept at 4°C temperature in a refrigerator.

Drugs and Chemicals Used

Warfarin (Cipla Limited), Ethanol (Loba chemicals), (S.N. Chemical-thane), Distilled water, calcium chloride ($CaCl_2$).

Preliminary Phytochemical Investigations of the Extract

The phytochemical analysis of the hydroalcoholic extract of the Ixora Coccinea flowers and stems were conducted to determine the presence of several phytoconstituents, including proteins, sterols, carbohydrates, saponin, flavonoids, glycosides, alkaloids, tannin and starch [9].

Blood Collection

2 ml of blood was collected from brachial wing vein of chicken (*Gallus gallus*) under standard settings of temperature (23±2) °C and relative humidity (55±10%). The collected blood was kept in a test tube.

Blood serum preparation

A covered test tube, preferably red-topped, was used to collect whole blood, which was then left to coagulate for 15 to 30 minutes at room temperature. A serum was produced by centrifuging the clot at (2,000–3,000) rpm for ten minutes. After that, the serum was carefully handled while being put into a clean polypropylene tube. In order to prevent freeze-thaw cycles, the serum was transported and stored in 0.5 ml aliquots at -20°C or lower if it wasn't analysed right away[10].

Saline solution (0.9%) preparation

The 0.9 grams of sodium chloride were dissolved in 100 milliliters of sterile water and then thoroughly mixed until equality was achieved. For further usage, the 0.9% saline solution was stored in a clean, sterile container to avoid contamination throughout the preparation process [11].

$CaCl_2$ (25mM) solution preparation

To make a 25 mM $CaCl_2$ solution in 100 mL, the target molarity and molar mass were determined. The total molar mass of 110.98 g/mol was obtained by calculating the molecular masses of $CaCl_2$ as 40.08 g/mol and 70.90 g/mol. using the molarity formula, the mass of $CaCl_2$ required for 100 mL of a 25 mM solution was determined. After that, the mass of $CaCl_2$ was measured and dissolved in water. To ensure uniformity the solution was immediately moved to a 100 mL volumetric flask and dilute with distilled water.[12]

Dose preparation

Based on the final volume of the solution, the desired concentration [0.2mg/ml (low dose), 0.4mg/ml (high dose)] of *Ixora coccinea* stem and flower extracts were determined using the formula Mass of extract = Concentration (mg/ml) × Volume (ml). The planned quantity was modified by the intended end volume. An appropriate solvent, such as water, was added to the extract and thoroughly mixed until the extract was entirely dissolved. If there were any insoluble particles, the extract was dissolved in water after being treated with POLYSORBATE-80 (TWEEN-80). To preserve its integrity, the solution was stored according to the right procedures, which included refrigeration or room temperature storage as well as light protection. Weighing the right amount of extract to correspond with the intended concentration and final volume was the process.

Prothrombin Time Test

The purpose of the experiment was to compare clotting times across several groups and investigate the impact of a drug on them. One such testable hypothesis may be, "Exposure to Substance X would prolong prothrombin time compared to the control group"[13]. 0.2 ml plasma, 0.1 ml of hydroalcoholic extract of *Ixora coccinea* stems and flowers at different concentration [0.2 mg/ml for *Ixora coccinea* stem Low Dose (ICSLD), *Ixora coccinea* flower Low Dose (ICFLD) and 0.4 mg/ml for *Ixora coccinea* stem High Dose (ICSHD), *Ixora coccinea* flower High Dose (ICFHD)] and 0.3 ml of $Cacl_2$ (25mM) were added together in a test tube and incubated both the mixture at 37ºC in water bath. For the control, the extract solution was substituted with an equal volume of 0.9% saline solution. The clotting time was recorded with stopwatch by tilting the test-tube every 5 sec. This coagulation time is called the prothrombin time [14].

Table:1 Groups and their measurement

Groups	Drug present	Amount of plasma(ml)	Amount of Extract(ml)	$CaCl_2$ solution(ml)
I	Control (0.9% saline solution)	0.2	0.1	0.3
II	Standard (0.1mg/ml warfarin solution)	0.2	0.1	0.3
III	ICSLD(0.2mg/ml)	0.2	0.1	0.3
IV	ICSHD(0.4mg/ml)	0.2	0.1	0.3

V	ICFLD(0.2mg/ml)	0.2	0.1	0.3
VI	ICFHD(0.4mg/ml)	0.2	0.1	0.3

Results

Preliminary Phytochemical Investigation

After conducting a preliminary phytochemical analysis on the extract from the *Ixora Coccinea* flower, the following observations were identified.

Table:2 A preliminary phytochemical investigation of *Ixora coccinea* flower and stem extract

SL. NO.	TEST NAME	RESULT
1	Test for alkaloids	Positive
2	Test for Carbohydrates	Positive
3	Test for Glycosides	Positive
4	Test for Protein and Amino acids	Negative
5	test for Flavonoids	Positive
6	Test for Saponins	Negative
7	Test for Steroids	Positive
8	Test for Lipid or Fat	Negative
9	Test for Tannins	Positive
10	Test for Starch	Positive

Prothrombin Time Test

The study was carried out to evaluate the effect of stem and flower extract of *Ixora coccinea* as an anticoagulant in blood samples by using the principles of coagulation time.

Table:3 Coagulation assay of different treatment group

Groups	Drug present	Time of coagulation(seconds)
I	Control (0.9% saline solution)	67±0.23✶
II	Standard (0.1mg/ml warfarin solution)	159±0.35✶
III	ICSLD(0.2mg/ml)	117±0.22✶
IV	ICSHD(0.4mg/ml)	130±0.44✶
V	ICFLD(0.2mg/ml)	106±0.27✶
VI	ICFHD(0.4mg/ml)	158±0.45✶

All values are mean± SEM, n=3, *p<0.01 significantly

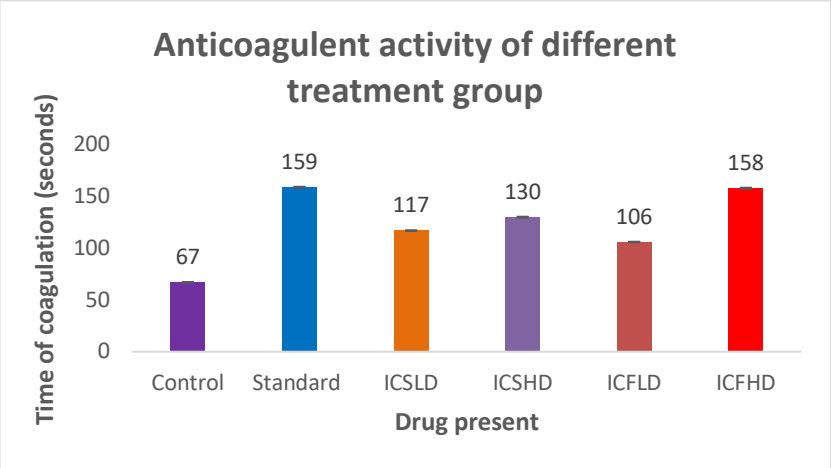

Figure:1 Graphical presentation of anticoagulant activity of different treatment group

From the above table and graphical presentation both the low and high dose of *Ixora coccinea* stem extract were shown significant anticoagulant activities, which took (117 ± 0.22) sec and (130 ± 0.44) sec for coagulation of blood plasma. At the same time *Ixora coccinea* flower extract at both low and high doses required (106 ± 0.27) and (158 ± 0.45) seconds, respectively, for blood plasma to coagulate. When it compared to the standard which took (159 ± 0.35) sec for plasma coagulation, while

control group took (67 ± 0.23) sec for coagulation of blood plasma.

Discussion

The present study evaluated the anticoagulant potential of *Ixora coccinea* flower and stem extracts using in vitro coagulation assays. The results demonstrated a clear variation in activity among the different treatment groups when compared to the control. The control group exhibited a coagulation time of (67 ± 0.2) seconds, representing the baseline physiological clotting response. In contrast, the standard drug significantly prolonged coagulation (159 ± 0.35 seconds), thereby validating the sensitivity of the assay and serving as a benchmark for comparison.

Among the test groups, the stem extract at low dose (ICSLD) extended coagulation time to (117 ± 0.22) seconds, while the high dose (ICSHD) further increased it to (130 ± 0.44) seconds. This dose-dependent enhancement suggests that bioactive constituents within the stem extract contribute to anticoagulant activity, though the effect remains moderate compared to the standard.

The flower extract at low dose (ICFLD) showed relatively weaker activity (106 ± 0.27 seconds), indicating limited efficacy at lower concentrations. Flowers often contain anthocyanins and phenolic compounds, but their anticoagulant effect requires higher availability. However, the flower extract at high dose (ICFHD) produced a coagulation time of (158 ± 0.45) seconds, nearly equivalent to the standard drug. This striking result highlights the potent anticoagulant potential of the flower extract, particularly at higher concentrations, and suggests that phytochemicals present in the floral components may play a dominant role in modulating clotting mechanisms such as:

Chelation of calcium ions by polyphenols [15] and terpenoids [16], preventing activation of clotting factors.

Inhibition of thrombin activity by flavonoids [17].

Antioxidant action reducing oxidative stress [18], which otherwise promotes platelet activation.

Overall, the findings reveal that both flower and stem extracts of *Ixora coccinea* possess anticoagulant properties, with the flower extract at high dose demonstrating activity comparable to the standard. The dose-dependent trends observed in both extracts support the hypothesis that increasing concentrations enhance the inhibitory effect on coagulation pathways. These results underscore the pharmacological relevance of *Ixora coccinea* and warrant further investigation into its phytochemical constituents, mechanism of action, and potential therapeutic applications in the prevention or management of thrombotic disorders.

Conclusion

The purpose of this investigation was to determine whether the chemical components of *Ixora Coccinea* stem and flower might improve the anticoagulant effects. Overall, the findings highlight the pharmacological relevance of *Ixora coccinea* as a potential source of natural anticoagulant agents. The dose-dependent trends observed reinforce the therapeutic promise of its extracts, especially the flowers, in the prevention or management of thrombotic disorders. Further studies focusing on isolation of active constituents, mechanistic pathways, and in vivo validation are warranted to establish its clinical applicability.

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