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Assessment of *In-vitro* thrombolytic activity of *Curcuma caesia* and *Coccinia grandis*

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Abstract

Thrombotic disorders are a major global health concern, often treated with synthetic agents like streptokinase, which may lead to adverse effects such as bleeding and hypersensitivity. Exploring natural, plant-based alternatives offers a promising route for safer thrombolytic therapy. The project focuses on evaluating the clot-dissolving potential of *Curcuma caesia* and *Coccinia grandis*, both individually and synergistic effect of combined plant extract, using two methods 1st is by an *in-vitro* clot lysis model and another is by Prothrombine time test. Streptokinase served as the positive control, while normal saline solution served as the negative control, in an *in-vitro* thrombolytic model that assessed the clot lysis impact of an ethanolic extract of *C. caesia* rhizome and *C. grandis* leaf. A thrombolytic effect of 61.4%±1.8 was seen when 75% extract of *Curcuma caesia* rhizome and 25% extract of *Coccinia grandis* leaves were employed together, in comparison to the effects of Streptokinase (64.6±4.11) as a positive control and water (7.5%±0.45) as a negative control. The current study highlights the remarkable thrombolytic activity of *C. caesia* and *C. grandis* extracts, which are utilized to treat cardiovascular disorders. So, it's important to find out what these extracts include that makes them thrombolytic and to monitor there *In-vivo* clot-solving capacity.

Keywords: Thrombolytic Activity; Streptokinase; *In-vitro*; Cardiovascular Diseases; *Curcuma caesia*; *Coccinia grandis*

1. Introduction

Our modern medical system is in desperate need of a panacea—a medicine that would not only treat all of our illnesses but also prevent them from returning and improve our overall health. The current medications may get you through one or more of the criteria. Traditional medicine, however, may be able to meet all of the criteria. The traditional medicines of old offer a vast array of uses in treating various illnesses [1].

By dissolving blood clots, thrombolytic medicines reduce the severity of complications brought on by a blood artery obstruction. They are prescribed to treat a variety of diseases, including myocardial infarction, thromboembolic stroke, pulmonary embolism, and deep vein thrombosis [2].

The creation of blood clots and the accompanying restriction of blood flow in blood vessels can lead to serious problems such stroke, heart attack, pulmonary embolism, or deep vein thrombosis. In essence, if a clot blocks an important artery, it can deprive vital organs of oxygen and cause substantial damage.

- **Normal function:** The formation of a clot at spot of an injury is an essential part of the body's natural ability to control bleeding.
- **Complications arise when:** Blood clots can obstruct blood flow if they develop in the wrong place or are too big.
- **Potential consequences**

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- Stroke: A clot in a brain artery
- Heart attack: A clot in a coronary artery
- Pulmonary embolism: A clot that travels to the lungs

Like many other developing nations, thromboembolic disorders are a leading cause of both illness and death in India. These conditions present a significant and complex healthcare burden, emphasizing the urgent requirement for better medical strategies, well-maintained national data systems, and awareness programs to counter this escalating health concern. Confronting these issues is vital for improving patient health outcomes and minimizing the social and economic consequences of these disorders on the Indian population.

1.1. Clotting Mechanism

The human body relies on blood, and a dangerously high loss of this fluid can be fatal. Its major function is to carry oxygen to cells and tissues; it is produced during hematopoiesis. When blood loss becomes too great, the body's clotting system kicks in. In this process, clots are formed when platelets, clotting factors, prostaglandins, enzymes, and proteins work together with vascular responses. A temporary seal is formed at the site of damage by these components working together through vasoconstriction, platelet adhesion, activation, and aggregation. Then, fibrin, the active component of fibrinogen, strengthens this transient platelet plug to guarantee a steady clot [6].

The clotting process is divided into two main phases

- **Primary Hemostasis:** Development of an initial, fragile platelet plug
- **Secondary Hemostasis:** Strengthening of the platelet plug through formation of a fibrin mesh network

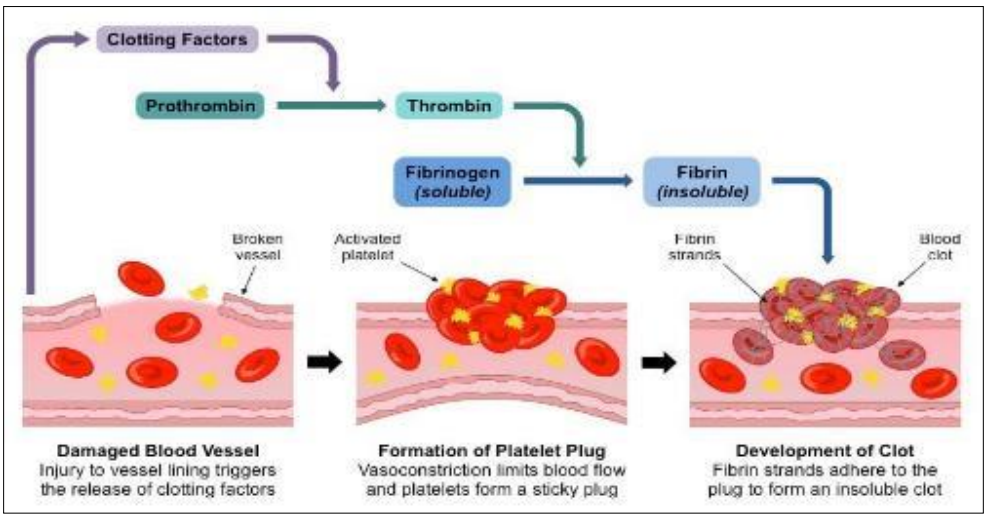

Figure 1 Stages of Clotting mechanism

1.2. Mechanism of Thrombolytic agents

1.2.1. Streptokinase

The first fibrinolytic to find practical usage in therapeutic settings, it is a protein formed by definite strains of hemolytic group C streptococcus. Streptokinase is not an enzyme like other plasminogen activators; hence it cannot hydrolyze plasminogen molecules on its own. A streptokinase-plasminogen complex is formed when it and plasminogen combine to produce an equimolecular molecule. The complex undergoes a metamorphosis into a streptokinase-plasmin complex, or free plasmin that decomposes fibrin, as a result of certain conformational changes in the plasminogenic area. These modifications cause a rupture in a few peptide links. Patients with severe, major pulmonary embolisms, vein thrombosis, and myocardial infarctions are treated intravenously with this medication, which has a plasma half-life of 15-30 minutes [7].

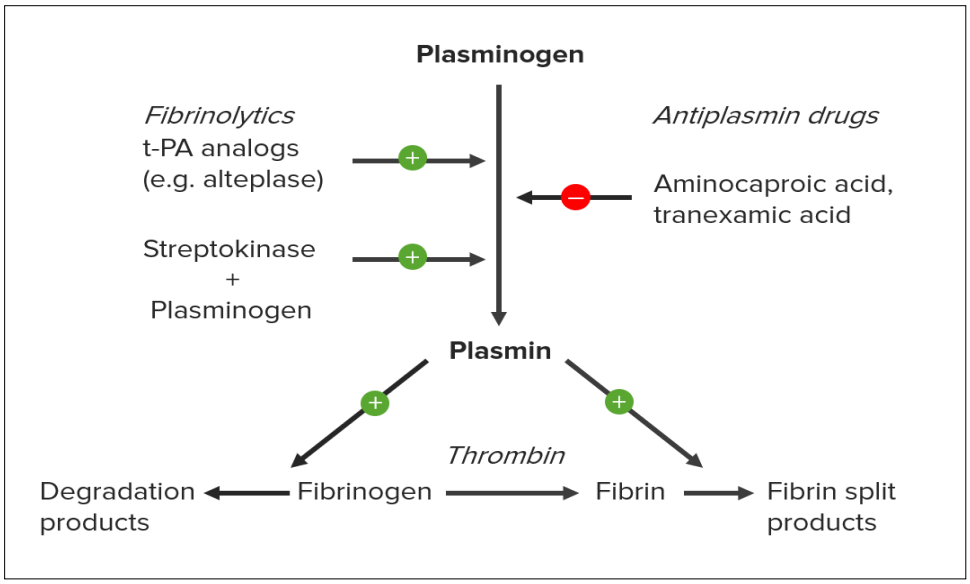

Figure 2 Mechanism of action of Thrombolytic agents

1.2.2. Urokinase

Purified from human urine or kidney cells, urokinase is an enzyme that converts plasminogen into plasmin by directly cleaving certain peptide links, most notably the Arg-560-Val-561 connection. Its indications of usage are identical to those of streptokinase. This medication has other synonyms, such as abbokinase [8].

1.2.3. Alteplase

The medicine alteplase stimulates t-plasminogen, which is found in human tissues. Molecular weight 68,000 and produced by vascular endothelial cells, it is a glycoprotein. Originally derived from human melanoma cells grown in a lab, t-PA cells are now designed to be a variant of rt-PA with a different genetic makeup. Systemic fibrinolysis is less likely to occur as a result of its treatment compared to streptokinase and urokinase since its activity is confined in thrombotic areas. Hence, infusion techniques are the exclusive means of administering them. Patients incompatible with streptokinase, such as those who have just recovered from a streptococcus infection, can be given these instead. Activase and other similar drugs are its synonyms [9].

Herbal remedies have been utilized for management of many ailments since dawn of time. The fact that herbal remedies are "natural" gives the impression that they are risk-free. Evidence from epidemiologic research suggests that foods having anti-thrombotic effects that have been demonstrated in experiments may help lower the incidence of thrombosis. Research on herbs with thrombolytic properties has yielded some noteworthy findings [3]. Herbal remedies have recently seen a renaissance in popularity, thanks to developments in phytochemistry and the discovery of plant chemicals with medicinal properties [4].

The ideal thrombolytic treatment would be defined by maximally stable coronary arterial thrombolysis with little bleeding, and more research in this area will shed light on this and get the field closer to this goal. [5]

2. Experimental work

2.1. Collection and Authentication of Plant

In this study selected two medicinal plants *Curcuma caesia* and *Coccinia grandis* previously reported for their pharmacological properties. . Both plants were collected during the June–July monsoon season from Village-Shigaon, Tarf Walwa, Sangli, State-Maharashtra, India.

A taxonomist from the Department of Botany at Kanya Mahavidyalaya in Islampur, State-Maharashtra, India, verified the authenticity of the plant materials after they were rinsed with filtered water and shade-dried at room temperature (~25 °C) to preserve their phytochemical composition. The same is produced and preserved voucher specimens for future reference.

2.1.1. Curcuma caesia

One of the oldest plants used as a spice, turmeric also has pharmacologic effects demonstrated in both laboratory and animal investigations [10]. Cases of several ailments have been on the rise due to the fact that urbanisation altered people's lifestyles. Soil rich in moisture and clay is ideal for the growth of *C. caesia*, another name for black turmeric (English), kali haldi (Hindi), and kunyit hitam (Malaysia) [11]. Its birthplace was in the Himalayan area, which includes countries in South and Southeast Asia and India [12].

A rhizomatous perennial, *C. caesia* can reach a height of 0.5 to 1 meter when grown upright. The plant is characterized by its enormous system of tuberous roots, broad, vertical oblong leaves, and pale yellow flower with a reddish border. The inside of the rhizome can be either buff or bluish-black in hue. Despite the lack of any credible information on the plant's usage as a food source [13]. As shown in Table No.1, its rhizome has a large number of known pharmacological uses.

2.1.2. Coccinia grandis

Commonly referred to as a gourd, the *Coccinia grandis* plant is really a member of the *Cucurbitaceae* family. The plant is known by a variety of names, including Telachucha, Tindora, Scarlet-fruited gourd, and Ivy-gourd. Central Africa, India, and Asia are the original habitats of this plant species [14].

The climbing perennial plant *Coccinia grandis* can spread by seed or by means of vegetative growth. For both food and industrial use, the oils and proteins found in this plant's seeds are invaluable. In rare cases, adventitious roots can develop at the plant's base from its herbaceous, thin climber stems. The lengthy, elastic tendrils have a springy, coil-like quality that makes them able to round the host and provide support [15].

Figure 3 Rhizomes of *C. caesia*

Figure 4 Leaves of *C. grandis*

Table 1 Biological and pharmacological properties shown by *Curcuma caesia* & *Coccinia grandis*

Curcuma caesia	***Coccinia grandis***
Antioxidant [18].	Antidiabetes [23].
Anti-inflammatory [18].	Anti-inflammatory [23].
Antimicrobial [18].	Digestive Aid [27].
Anticonvulsant [18]	Antimicrobial [24].
Analgesic [19].	Antieczema [25].
Anti-asthmatic [20].	Analgesic [25].
Smooth Muscle Relaxation [21].	Antipyretics [25].
Anticancer [22].	Antioxident [24].
Menstrual Disorders [22].	To treat Leprosy [26].

2.1.3. Extraction

After collecting, cleaning, and chopping the *Curcuma caesia* rhizomes and *Coccinia grandis* leaves into smaller pieces, they were shade dried for a week. After drying, materials were coarsely powdered. The powdered plant materials were separately extracted using the Soxhlet extraction method with ethanol as the solvent until the extract in the thimble turned colorless. The resulting extracts were combined and concentrated using a rotary evaporator [28] [29].

2.1.4. Preparation of Standard

A vial containing lyophilized streptokinase (15,000,000 IU) that is commercially available was mixed well with five milliliters of phosphate-buffered saline. Since streptokinase is a commonly used thrombolytic drug, its concentration in the solution was adjusted to 30,000 IU to serve as a reference standard for thrombolytic activity evaluation [28].

2.1.5. Blood Withdrawal

Ten healthy adults (22–24 years old) who had not recently used anticoagulant or oral contraceptive medication had their venous blood drawn. In order to facilitate clot formation, around 500 µl of blood was added to each pre-weighed blood collection tube, and each tube was labelled with a distinct identification number [28].

3. Methodology

Individual sterile blood collection tubes, with a volume of 500 µl per tube, were quickly filled with venous blood samples taken from 10 healthy subjects. To induce coagulation, 200 µl of a 2% calcium chloride solution was added to each tube, mixed well, and then incubated at 37°C for 45 minutes. After clots had developed, the serum was delicately extracted so as not to disrupt the clot. The clot weight was determined by weighing the tubes again, this time with the clot removed.

For the evaluation of *in-vitro* thrombolytic activity, ethanolic extracts of *Curcuma caesia* (rhizomes) and *Coccinia grandis* (leaves) were used. The LD_{50} values of both plants were conserved for dose selection and different doses and its combination were made as follows. [20] [32] To evaluate the dose-dependent thrombolytic effect, three different concentrations of each plant extract were tested as follows:

Doses for *Curcuma caesia* extract: Dose 1 200mg, Dose 2 100mg, Dose 3 400mg. and for *Coccinia grandis* extract: Dose 1 400mg, Dose 2 200mg, Dose 3 600mg.

Further the combination of both plants were made with different proportions to check its effect and the combination were made as dose 1 25% *C. caesia* + 75% *C. grandis*, dose 2 50% C. caesia + 50% *C. grandis*, dose 3 75% *C. caesia* + 25% *C. grandis*. For the combination 200mg of *Curcuma caesia* is considered as 100% and 400mg of *Coccinia grandis* is considered as 100%. Each dose was prepared in appropriate volume using a suitable solvent (e.g, distilled water).

Further normal saline (as a negative control), and the 30,000 IU streptokinase reference standard was added to each clot-containing tube. In same way control is prepared except extract and stdandard. Then incubated all of the samples for 90 minutes at 37°C. After carefully aspirating the residual fluid, the tubes were weighed again. The differential between the pre- and post-clot weights of the tubes was used to determine the clot lysis percentage [30].

Statistical Analysis: The proportion of clot lysis was shown as the mean plus or minus the standard deviation. To assess statistical significance, a paired Student's t-test was used. A paired t-test and one-way analysis of variance (ANOVA) were used to determine the overall significance of clot lysis percentages. Statistics were deemed significant when the p-value was less than 0.05 [28] [31].

% clot lysis = (weight of clot after lysis by sample and removal of serum / weight of clot before lysis by sample) × 100.

4. Result

4.1. Phytochemicals present in rhizomes of *C. caesia*

Table 2 Phytochemicals of rhizomes of *C. caesia*

Sr. No.	Test	Observation	Inference
1.	For Alkaloids: Mayer's test Dragendroff's test	 Creamy white Orange red	 +
2.	For Glycosides	Yellow colour formed	+
3.	For Steroids	Red colour formed	+
4.	For Tannins	Greenish black ppt. formed	+
5.	For Flavonoids	Immidiate formation of red colour	+
6.	For Saponins	Foam formed	+
7.	For Terpenoids	Formation of reddish brown	+
8.	For Carbohydrates (benedict test)	Brownish red colour formed	+

4.2. Phytochemicals present in leaves of *C. grandis*

Table 3 Phytochemical constituents present in leaves of *C. grandis*

Sr. No.	Test	Observation	Inference
1.	For Alkaloids: Hager's test Dragendroff's test	 Yellow colour Orange red	 +
2.	For Glycosides	Yellow colour formed	+
3.	For Steroids	Red colour formed	+
4.	For Tannins	Greenish black ppt. formed	+
5.	For Flavonoids	Immidiate formation of red colour	+
6.	For Saponins	Foam formed	+
7.	For Terpenoids	Formation of reddish brown	+

8.	For Carbohydrates (Benedict test)	Brownish red colour formed	+

Table 4 % clot lysis with different treatment (by method as described by *Prasad et al*.) [30]

Treatment	**Negative control (Normal saline)**	**Positive Control (Streptokinase 30,000IU)**	***C. caesia* rhizome extract**	***C. grandis* leaves extract**	**Combined (*C. caesia* rhizome extract + *C. grandis* leaves extract)**		
					Groups (proportion of extract of both plants for combined effect)		
					CG1=25%C + 75%G	**CG2=50%C + 50%G**	**CG3=75%C + 25%G**
%clot lysis (mean± Std. deviation)	7.5% ± 0.45	64.6% ± 4.11	44% ± 6.9	46.03% ± 6.07	50.4% ± 1.53	56.5% ± 1.60	61.4% ± 1.8

Figure 5 % clot lysis occurs with different treatment

Table 5 Result obtained by Prothrombin time test

Treatment	**Prothrombin Time (seconds)**
Normal saline solution	12.8
Streptokinase (30,000 IU)	14.1
C. caesia rhizome extract	13.0
C. grandis leaves extract	13.4
Combination (75% *Curcuma caesia* rhizome extract + 25% *Coccinia grandis* leaves extract*)*	13.9

5. Discussion

Thrombotic conditions as myocardial infarction, stroke, and deep vein thrombosis are major contributors to global mortality. While agents like streptokinase are effective in thrombolysis, they pose risks such as bleeding, allergic reactions, and high cost. This has driven the exploration of plant-based alternatives that are safer and more affordable.

Individually, *Curcuma caesia* and *Coccinia grandis* exhibited moderate clot lysis (44% and 46%, respectively). Flavonoids, alkaloids, tannins, and phenolic compounds are bioactive phytochemicals that may explain this mild action. (32) These constituents are known to exhibit antioxidant, anti-inflammatory, and fibrinolytic activities. Specifically, the antioxidant properties may reduce oxidative stress at the site of the clot, while anti-inflammatory compounds could reduce endothelial damage and support vascular health, indirectly contributing to thrombolytic activity.

Remarkably, the combination of 75% *Curcuma caesia* and 25% *Coccinia grandis* demonstrated a significantly higher thrombolytic activity (61.4%), which nearly approached the efficacy of streptokinase. This observation strongly suggests a synergistic interaction between the phytoconstituents of both extracts. It is plausible that the combined effect of curcuminoids from *Curcuma caesia* and polyphenols from *Coccinia grandis* may enhances fibrin degradation or facilitates plasmin activation, thereby accelerating clot lysis. The formulation ratio might play a critical role in maximizing therapeutic outcomes and should be further optimized through dose-dependent studies.

To further validate these observations, Prothrombin Time (PT) analysis was conducted. The results showed increased PT values in all test samples compared to normal saline (12.8 seconds), with the highest observed in the streptokinase group (14.1 seconds). The individual extracts of *C. caesia* and *C. grandis* recorded PTs of 13.0 and 13.4 seconds, respectively, while the combination yielded 13.9 seconds—again, closely aligning with streptokinase. The elevation in PT indicates delayed clot formation, reinforcing the thrombolytic activity observed in clot lysis assays.

6. Conclusion

The study successfully demonstrated the thrombolytic potential of plant-based extracts in comparison to a standard pharmaceutical agent. Streptokinase, used as the positive control, exhibited the highest clot lysis activity at 64.6%, while normal saline, serving as the negative control, showed minimal activity at 7.5%. Among the individual plant extracts tested, *Curcuma caesia and Coccinia grandis* showed moderate clot lysis effects of 44% and 46%, respectively. Their antioxidant and membrane-stabilizing properties further enhance their therapeutic potential. Notably, the combination of 75% *Curcuma caesia* rhizome extract and 25% *Coccinia grandis* leaves extract resulted in a clot lysis rate of 61.4%, closely approaching the efficacy of streptokinase.

To support this finding, prothrombin period (PT) analysis (PT) was performed to assess the effect of the extract on blood clotting. This shows a significant thrombolytic effect. This increase in the PT period reflects delayed coagulation and increases the thrombolytic effect observed in the coagulation solubility assay.

These findings suggest that the synergistic effect of the combined plant extracts holds significant promise as a natural alternative for thrombolytic therapy. Future studies shall focus on in vivo evaluations and mechanistic insights to establish their efficacy and safety for clinical applications.

Compliance with ethical standards

Disclosure of conflict of interest

The authors have no conflicts of interest to declare. All co-authors have seen and agree with the contents of the manuscript and there is no financial interest to report. We certify that the submission is original work and is not under review at any other publication.

Statement of informed consent

Informed consent was obtained from all individual participants included in the study.

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