WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.084
Volume 12, Issue 19, 752-759. Research Article ISSN 2277–7105

STUDY OF IN-VITRO ANTICOAGULANT ACTIVITY ON *KAEMPFERIA ROTUNDA*

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Article Received on
05 September 2023,
Revised on 26 Sept. 2023,
Accepted on 16 Oct. 2023
DOI: 10.20959/wjpr202319-30016

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ABSTRACT

Hemostasis is a process resulting from the interaction between coagulation and anticoagulants that keeps blood within the injured vascular system during an injury. Three main steps make up the delicate mechanism of hemostasis involves vasoconstriction, platelet plug temporarily blockage and blood clotting or fibrin clot formation. Anticoagulant medications are required for the long-term prevention of recurrences as well as the short-term therapy of arterial and venous thrombotic diseases. Folklore practitioners have claimed that *Kaempferia rotunda* has anticoagulant properties. The purpose of this study is to highlight the potential of *Kaempferia rotunda*, a member of Zingiberaceae family, as an anticoagulant agent. A suitable solvent, such as methanol, was used to prepare the rhizome extract using the Soxhlet extraction method, and platelet-poor plasma samples were used to measure the extract's anticoagulant activity using the APTT and PT assays. The outcomes were contrasted with those attained with conventional heparin. The maximum anticoagulant activity was shown by the platelet-poor plasma sample treated with the concentration 100 µg/ml (83.6 sec: APTT; 18.68 sec: PT).

KEYWORDS: *Kaempferia rotunda*, Anticoagulant, Platelet-poor plasma, APTT, PT, Assays.

INTRODUCTION

The World Health Organization (WHO) defines herbal medicine as a practice which includes herbs, herbal materials, herbal preparations and finished herbal products that contain as active ingredients parts of plants, or other plant materials, or combinations. *Kaempferia rotunda* popularly known as "chengazhuneerkizhangu" among the traditional practitioners of Kerala. It is a small herbaceous plant that develops from an underground, horizontal stem known as a rhizome. It is native to China, Taiwan, India, Indonesia, Malaysia, Myanmar, Sri Lanka, and Thailand where it is found in open grasslands.[1, 2, 3]

The efficient functioning of the body depends on the blood's circulation. Blood thickens into a gel-like consistency during coagulation. Hemostasis is a bigger process that includes coagulation and is how the body controls when and how much bleeding occurs. Coagulation is a crucial phase in the healing process of a wound when it functions as it should. Coagulation aids in the formation of a clot, which is made of a material called fibrin, when a blood vessel breaks, as with a cut or other damage. Until the tissues can heal themselves, the clot closes the wound. Coagulation can generate a clot that plugs a blood vessel instead of healing it when it occurs where it should not. A blood clot or thrombus that restricts blood flow through the circulatory system, known as thrombosis, forms inside a blood vessel.[4, 5]

Anticoagulants are a family of medications that stop your blood from clotting too easily. They can break down existing clots or prevent clots from forming in the first place. These medications can help stop life-threatening conditions like strokes, heart attacks and pulmonary embolisms, all of which can happen because of blood clots. Heparin has been used as for anticoagulant treatment for acute thrombotic disorders for decades, this drug presents some limitations related to its clinical application, such as inefficacy in antithrombin deficient patients, bleeding complications, potential for the development of heparin-induced thrombocytopenia, immunosuppression and osteoporotic effect with long-term application as side effects. So, the search for new substances with anticoagulant and antithrombotic activities is relevant.[6, 7]

MATERIALS AND METHODS

Collection of plant material

The entire fresh plant material of *Kaempferia rotunda* was collected from the region of Kizhissery, Malappuram district, Kerala, India on May 2023. It was then identified as *Kaempferia rotunda* species, belonging to *Zingiberaceae* family.

Extraction of plant material

The freshly collected rhizomes of *Kaempferia rotunda* were washed with water thoroughly to remove physical impurities and was made into small pieces and dried well under shade for a period of 3 weeks. It was then coarsely powdered and 100g of the powder was extracted with methanol using soxhelet apparatus. The extract was concentrated and evaporated to dryness to obtain the crude extract. To find the presence of several types of phytoconstituents, the produced extract is subjected to qualitative chemical assays.[8]

Fig. 1: Dried pieces of *K. rotunda* rhizomes.

Fig. 2: Soxhlet extraction.

EVALUATION OF ANTICOAGULANT ACTIVITY

a) Anticoagulant action measured using activated partial thromboplastin time (APTT)

Activated partial thromboplastin time was determined using the method of Anderson et al. (1976) using platelet-poor plasma. In these assays, platelet-poor plasma samples (0.1 ml) were mixed with different amounts of extracts (50, 75, 100 µg/ml) and pre-warmed APTT reagent (0.1 ml) was added into respective test cuvette and the mixture was allowed to incubate for 3 minutes at 37°C. Then pre-warmed 0.1 ml calcium chloride was then added into the test cuvette, and the APTT was recorded as the time (sec) for clot formation. Solution of heparin (2 IU/ml) was taken as standard. Also, recorded the time for clot formation in control.[9, 10]

b) Anticoagulant action measured using prothrombin time (PT)

Prothrombin time was determined according to the method of Quick (1940). The reaction mixture containing different sample amounts (50, 75, 100 µg/ml) was incubated with platelet-poor plasma samples (0.1 ml) for 3 minutes at 37°C. Then pre-warmed PT reagent was added and the time (sec) for clot formation was recorded. Solution of heparin (2 IU/ml) was taken as standard. Also, recorded the time for clot formation in control.[11]

Fig. 3: Single Channel Coagulation Analyser.

RESULTS AND DISCUSSION

Extraction of the rhizomes

Successive solvent extraction was performed by soxhelet extraction method using methanol as solvent.

Table 1: Results showing characteristics of extract.

Solvent	Methanol
Colour and consistency	Dark brown, semi-liquid
Weight in grams	7.76 gm
Percentage yield	7.76w/w

Phytochemical study

Table 2: Qualitative Phytochemical analysis of rhizome of *K. rotunda*.

S. No.	Chemical Tests	Inferences
1	**Test for Alkaloids**	
a.	Dragendroff's test	+
b.	Mayer's test	+
2	**Test for Carbohydrates**	
a.	Fehling's test	+
b.	Benedict's test	+
3	**Test for Flavonoids**	

a.	Shinoda test	+
b.	Lead acetate test	+
4	**Test for Phenols**	
a.	Ferric chloride test	+
b.	Acetic acid test	+
5	**Test for Saponins**	
a.	Froth test	+

Anticoagulant activity study

The anticoagulant study was assessed using APTT and PT assays in platelet-poor plasma samples. Three different concentrations of the crude drug extract (50, 75, 100 µg/ml) were used for the study. The time for clot formation at different concentrations of the crude drug extract showed that, there was an increase in the time for clot formation with increase in concentration of the crude drug extract.

Table 3: The time for clot formation at different concentrations of extract by using APTT assay.

Concentration of extract	Time for clot formation (Seconds)
Control	40.80
50 µg/ml	62.50
75 µg/ml	69.90
100 µg/ml	83.60
Standard	82.42

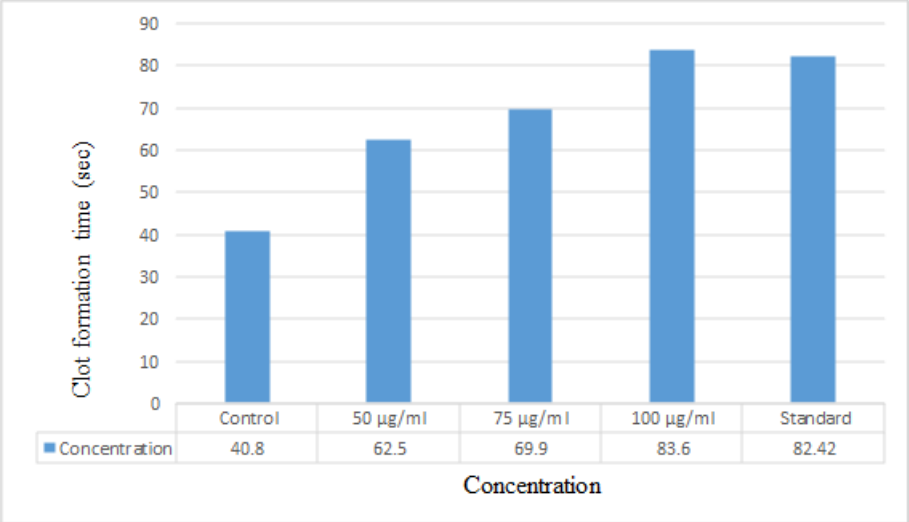

Fig. 4: Graphical representation showing the time for clot formation at different concentrations of extract by using APTT assay.

Table 4: The time for clot formation at different concentrations of extract by using PT assay.

Concentration of extract	Time for clot formation (Seconds)
Control	14.15
50 µg/ml	16.88
75 µg/ml	17.19
100 µg/ml	18.68
Standard	17.80

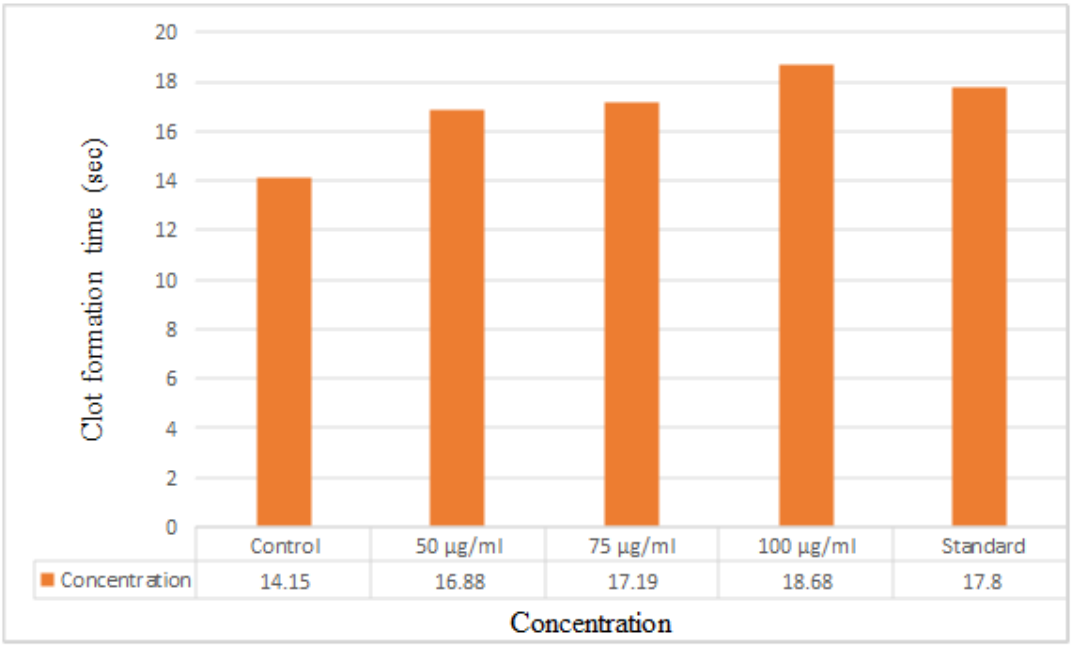

Fig. 5: Graphical representation showing the time for clot formation at different concentration of extract by using PT assay.

Fig. 6: Clot formation in control.

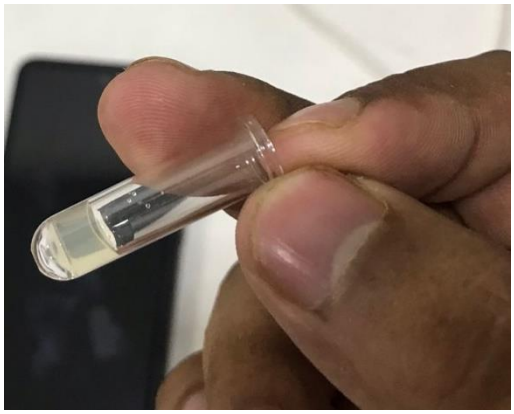

Fig. 7: Clot formation in test sample.

Fig. 8: Clot formation in standard.

CONCLUSION

The methanolic extract of rhizomes of *Kaempferia rotunda* were prepared by Soxhlet extraction method. Phytochemical analysis was carried out and showed the presence of alkaloids, carbohydrates, flavonoids, saponins and phenolic compounds on the methanolic extract of rhizomes of *K. rotunda*. In the pharmacological study, i.e., invitro anticoagulant activity was evaluated by APTT and PT assays using platelet-poor plasma samples. The results obtained showed that the clot formation time in platelet-poor plasma samples with the concentrations 50 µg/ml, 75 µg/ml and 100 µg/ml were statistically highly significant when compared with the control and the results obtained for the concentration 100 µg/ml was highly significant when compared with both control and standard heparin solutions. The platelet-poor plasma sample treated with the concentration 100 g/ml demonstrated the highest anticoagulant activity (83.6 sec for APTT; 18.68 sec for PT). It is clear from the findings of this study that *Kaempferia rotunda* rhizomes have highly significant anticoagulant activity.

ACKNOWLEDGEMENT

The authors thank Mrs. Safeera Mayyeri, Associate Professor, Department of Pharmacognosy, Jamia Salafiya Pharmacy College, Kerala University of Health Sciences, for her full cooperation, guidance and support.

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