



## RESEARCH ARTICLE

### BLOOD COAGULATION PROPERTIES OF KHANDU CHAKKA (*EHRETIA LAEVIS*) PLANT LEAVES

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#### ABSTRACT

This study aims to investigate the potential of *Ehretia laevis* as coagulant. To do this the dried powder of this plant was extracted with acetone and isopropanol using soxhlet assembly. These solvents were evaporated to make aqueous extract which was subjected to coagulation (prothrombin test) using plasma and blood sample of healthy individual. The blood coagulation time of prothrombin test of both the plasma and blood samples was reduced as compared to the control.

**Key words:** *Ehretia laevis*, coagulation time, Prothrombin time, extract.

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#### INTRODUCTION

Blood is a fluid in our body that helps to survive; transport all essential nutrients to body parts. Blood circulates through our body and delivers essential substances like oxygen and nutrients to the body's cells. There are four basic components that comprise human blood: plasma, red blood cells, white blood cells and platelets. It contains some factors that help to coagulate the blood when it flows outside the body. Coagulation is blood property that is evaluated in medical laboratories. Coagulation is an important mechanism of the blood to stop blood loss when damage occurs in the body (Blood coagulants and anti-coagulants, 2008; Segen, 2004). There has been a number of literatures that studied coagulations (Maschirow *et al.*, 2015; Luz *et al.*, 2013; Vivas-Ruiz *et al.*, 2013; Su *et al.*, 2015; Menkiti *et al.*, 2015; Merheb-Dini *et al.*, 2012; Farhat *et al.*, 2015). Romeo C. and Ongpoy J.R. (2016) studied coagulation and hemagglutination properties of the crude extract derived from the leaves of *Euphorbia hirta* L., *Tridax procumbens* L., and *Vernonia cinerea* (L) Less (Romeo and Ongpoy, 2016). Essien *et al.* (1985) reported coagulant properties of root bark and root wood extracts of *Fagara xanthoxyloides* lam plant (Essien *et al.*, 1985). Okuo and Ejimadu (2005) determined clotting time of blood samples drawn from eight patients using three variants of plant extracts (crude, aqueous and chloroform) of the leaf of *Bryophyllum pinnatum*. These extracts clotted the blood samples faster than the untreated blood samples, which ranged between 0.34-3.27 min (Okuo and Ejimadu, 2005). Edemeka and Oqwu (2000) effect of aqueous and methanol extracts of *Ocimum gratissimum* on the prothrombin time (PT)

and activated partial thromboplastin time (APTT) of plasma and factor VIII-deficient plasma. The biological activity suggests the extract contains unidentified constituents that promote blood coagulation (Edemeka and Oqwu (2000). In diabetic and high blood pressure patients' process of blood coagulation in wounds get delayed and blood flows continuously. This study was carried on blood coagulation property of *Ehretia laevis* (Khandu chakka). *Ehretia laevis* is a medicinal plant mainly used for wound healing, joint pain and minor fractures by local people with promising result. Thakre Rushikesh *et al* (2016) studied on ethano botanical properties of unexplored plant khandu chakka (*Ehretia laevis roxb.*), used on wound healing. Shailesh Dhenge and Kiran Khandare (2016) did a case study on the efficacy of local application of khandu chakka (*Ehretia laevis roxb*) ghrita in dushtavrana. The purpose of this study is to evaluate the coagulation properties of the *Ehretia laevis*.

#### MATERIALS AND METHODS

The plant material was collected from nearby jungle area of Nagpur. The samples were washed with distilled water and dried in sunlight to make powder form. The dried powdered material was extracted with soxhlet apparatus for 5-6 hours using acetone and isopropanol respectively. The extracts were collected and tested for coagulation activity (prothrombin test).

#### Collection of Blood sample for coagulation test

Blood sample from healthy individual having no disease and disorder was withdrawn from vein with the help of sterile syringe. It was placed in two separate vials containing trisodium citrate to prevent clotting of blood.

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**Table 1. Plasma coagulation time using aq. Extract**

Different TT for test	Control (Saline water)	Control-1 (Acetone)	Control-2 (Isopropanol)	TT -1 Aq. Extract after evaporation of Acetone	TT-2 Aq. Extract after evaporation of Isopropanol
Coagulation time in min.	3.16	No coagulation	No coagulation	2.27	2.12

**Table 2. Blood coagulation time using aq. Extract**

Different TT for test	Control (Saline water)	Control -1 (Acetone)	Control -2 (Isopropanol)	TT -1 Aq.extract after evaporation of acetone	TT -2 Aq.extract after evaporation of Isopropanol
Coagulation time in min	2.35	No coagulation	No coagulation	1.40	1.32

One tube was centrifuged at 3000 rpm for 15 minutes to separate plasma from blood cell in order to obtain pure platelet plasma for prothrombin time test. The obtained blood plasma separated in a container with the help of micropipette and for further use.

#### **Determination of coagulation time (plasma) using aq. Extract**

As per the procedure 0.2 ml plasma, 0.1 ml of crude extract (400 µg/ml), and 0.3 ml of CaCl<sub>2</sub> (25 mM) were added together in a clean fusion tube. The mixture was incubated at 37°C in water bath. For control experimental extract solution was replaced by same volume of 0.9 % saline water. For control 1 and 2 extract solution was replaced by acetone and isopropanol respectively. Control 1 & 2 was taken to check interference of acetone and isopropanol with the coagulation time. The clotting time was recorded with stopwatch by tilting the test tubes for every 5 seconds.

Similar test was performed for blood also.

## **RESULTS**

### **DISCUSSION**

By the determination of coagulation time test it was found that coagulation time was decreased as compared to normal coagulation time i.e. control. Normal coagulation time (PT) was 3.16 minutes (control), and in experimental test tube 1 (aqueous extract from acetone extract), and 2 (aqueous extract from isopropanol extract) was 2.27 minutes, and 2.12 minutes respectively (Table 1). Whereas normal blood coagulation time (PT) was 2.35 minutes (control), and coagulation time in experimental test tube 1 (aqueous extract from acetone extract) and 2 (aqueous extract from isopropanol extract) was 1.40 minutes and 1.32 minutes respectively for aqueous extract from isopropanol extract (Table 2). The reduction in coagulation time of plasma and blood may be due presence of some blood coagulating compounds in these extracts. Chung KT et al (1998) reported that presence of tannic acid in khandu chakka accelerates blood clotting.

#### **Conclusion**

From all the observations we concluded that both the aqueous extracts have shown plasma and blood coagulant activity, and can be very useful medicine for treatment of blood coagulation.

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