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Evaluation of *In-Vitro* thrombolytic activity of *Manilkara zapota* leaf extract

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Abstract
The present study was aimed to evaluate the *in vitro* thrombolytic activity of *Manilkara zapota* for the potential against thrombosis by dissolving the clot. The fresh leaves of *Manilkara zapota* were collected, grinded to obtain coarse powder then subjected to hydro ethanolic extraction by Soxhlet apparatus. Finally the extracts were air dried and then evaluated for thrombolytic activity according to Prasad S *et al* described method. The results of study were tabulated and analyzed for the thrombolytic activity and it is found that the hydro ethanolic extract of the plant possess moderate thrombolytic activity which may be due to the presence of several phytoconstituents in the plant extract.

Keywords: Herbal medicines, *Manilkara zapota*, thrombolytic activity, extract.

Introduction
Herbal medicines are defined as a plant or plant part or an extract used for the treatment of several diseases [1]. In the recent trends the demand of herbal medicines is increasing exponentially. Because of public as well as medical establishments, studies leading to the scientific explanation of plant therapeutic capabilities are allowing this practice to gain increasing credibility and acceptance within the medical community [2].
Rapid changes in lifestyles of people on the globe enhance the occurrence of chronic non-communicable diseases including obesity, diabetes mellitus, cardiovascular disease (CVD), hypertension, stroke and some types of cancer which are significant causes of disability and pre mature death in developing and newly developing countries, placing additional burdens on already overtaxed national health budget [3].
The importance of dietary supplements derived from plants has accelerated in recent years because plants have remedy for various diseases. Pharmacologists, microbiologists, botanists, and natural-products chemists are engaged to develop a good herbal drug for treatment of various diseases. In fact, according to the World Health Organization, approximately 25% of modern drugs used in the United States have been derived from plants [4].
Many of the pharmaceuticals currently available to physicians have a long history of use as herbal remedies, including opium, aspirin, digitalis, and quinine. At least 7,000 medical compounds in the modern pharmacopoeia are derived from plants [8]. All plants produce chemical compounds as part of their normal metabolic activities, these phytochemicals are mainly responsible for the healing of the disease [5].
Manilkara zapota, commonly known as the sapodilla whose compounds extracted from the leaves showed anti-diabetic, antioxidant and hypocholesterolemic effects in rats [6]. Keeping this in view, the present study is aimed to evaluate the *in vitro* thrombolytic activity of *Manilkara zapota* for the potential against thrombosis by dissolving the clot.

Materials and Methods
Collection of plant part
The fresh leaves of *Manilkara zapota* were collected from the botanical garden of Anurag Pharmacy College, Kodad.

Drying, extraction and fractionation
The fresh leaves of *Manilkara zapota* were cleaned and then it was grinded to obtain coarse powder of standard size suitable for extraction.

The powdered peel powder was subjected to hydro ethanolic (Water: Ethanol= 60: 40) extraction by Soxhlet apparatus. After that the hydro ethanolic extract was defatted with petroleum ether to remove the chlorophylls. Finally the extracts were air dried and then evaluated for thrombolytic activity.

Method for evaluation of *in-vitro* thrombolytic study

Preparation of Extract solution

100 mg hydroethanolic extract of *Manilkara zapota* was suspended in 100ml distilled water and the mixture was shaken vigorously to make it soluble and a concentration of 1mg/mL was obtained. This solution was further diluted to obtain a concentration of 100µg/ml for performing the thrombolytic study.

Preparation of Standard solution

To the commercially available lyophilized Streptokinase (SK) vial of 15,00,000 I.U., 5 ml sterilize distilled water was added and was properly mixed. This suspension was used as a stock. From this stock solution 100µl (30,000 I.U) was used for performing the *in vitro* thrombolysis.

Sampling of blood

Five human volunteers were selected for this study without having recent history of oral contraception or anticoagulant therapy for last 2 weeks days. Sterile microcentrifuge tubes were weighed and labelled properly. Under aseptic condition, 2.5 ml of fresh blood was drawn from each human volunteer and 500µl freshly collected blood was then transferred to separate previously weighed microcentrifuge tubes. They were then kept to form clot.

Assay [7-9]

Each properly labelled blood filled microcentrifuge tube was then incubated at 37^0C for 45 minutes. After clot formation, serum was withdrawn completely, without disturbing the clot. After that each tube was weighed again to get the weight of clot.

Clot weight = Weight of clot filled tube – Weight of empty tube

To each microcentrifuge tube containing clot, 100µl of hydroethanolic extract of *Manilkara zapota* (Concentration 100 µg/ml) was added.

In the negative non-thrombolytic control, 100µl of distilled water was added and in the positive control 100µl of Streptokinase was added.

All the tubes were then incubated at 37^0C for 90 minutes and observed for clot lysis. After incubation, supernatant fluid released was removed and tubes were again weighed to observe the difference in weight after clot disruption. Difference between previous weight and at now was plot as ratio to obtain the percent of clot lysis.

% of clot lysis= (Weight of lysis/weight of clot before lysis) ×100

Results and Discussion

The observation and results of thrombolytic study of the hydroethanolic leaf extract of *Manilkara zapota* are given below.

Table 1: Determination of the percentage clot lysis in control, test [100 (µg/ml)], standard [Steptokinase 100µl (30,000 I.U)]

Treatment	Percentage clot lysis
Control	4.01%
Test [100 (µg/ml)]	23.94%
Standard [Steptokinase 100µl]	60.98%

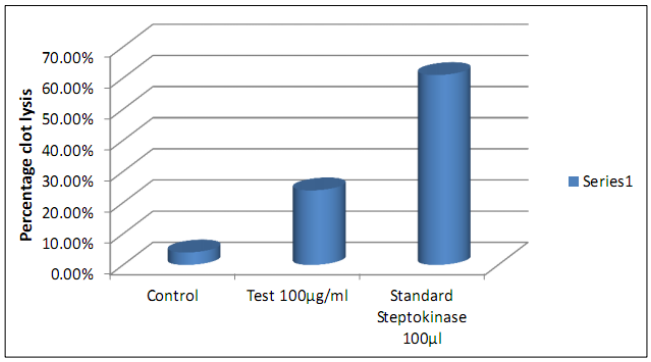

Fig 1: Clot lysis by standard drug, test and control

Thrombosis is the formation of blood clot inside a blood vessel that can block the blood flow in the circulatory system. When a blood vessel is damaged or roughened, the body's defense mechanism uses thrombocytes (platelets) and fibrin to form a blood clot for preventing blood loss. The clotting process starts when the activated platelets form bonds with other platelets in order to form platelet plug and also form bonds with the white blood cells, and form a complex process of clot formation and growth [10].

There are many causes which can lead to coagulation such as hypercoagulability, endothelial cell injury, disturbed blood flow etc. [11].

Human body has a naturally existing, fibrinolytic agent called "plasmin", which breaks the clot by disrupting the fibrinogen and fibrin present in a clot. Fibrinolysis can also be started by the plasminogen present at the cell surface after its conversion to the active form, plasmin [12].

The herbal products are popular now-a-days because it has less adverse effects. Several herbal plants have clot lysis activity. Interestingly, some of them are also used as food supplements. Different plant source, particularly several fruits, vegetables have been considered for their supplements having fibrinolytic, antiplatelet and anticoagulant activity, and there is indication that consuming such food leads to prevention of coronary events and stroke [13].

A number of thrombolytic drugs (Alteplase, Reteplase, Tenecteplase etc.) from different sources have been discovered by the scientists and some of them have been modified with recombinant technology in order to make those thrombolytic drugs more site specific and effective.

Thrombolytic agents work by activating the enzyme plasminogen, which digest the fibrin threads, which makes the clot soluble and subject to further proteolysis by other enzymes and clears the occluded blood vessels. Thus thrombolytic agents are useful for the treatment of myocardial infarction, thromboembolic strokes, deep vein thrombosis. Unluckily, these thrombolytic agents possess several adverse effects related to bleeding and embolism, which can lead to further health related complications [14].

In this thrombolytic study, when we compare the clot lysis percentage obtained through Streptokinase and water, a tremendously significant thrombolytic activity was detected

after treating the clots with *M. zapota* extract.

This particular hydroethanolic extract of *Manilkara zapota* exerted 23.94% clot lysis from clotted blood in thrombolytic activity test while for standard (streptokinase) and control are 60.98% and 4.01% respectively which are mentioned in table 1 as well as in the figure 1. So, this draws out that the hydroethanolic extract of *Manilkara zapota* possess slight thrombolytic activity.

The comparison of standard with control clearly demonstrated that clot dissolution does not occur when water was added to the clot. On the basis of the result obtained in this present study we can say that the extract has moderate thrombolytic activity compared to control (water).

The plant contains several phytochemical constituents belonging to categories such as alkaloids, carbohydrates, glycosides, tannins, triterpenes and flavonoids etc. The moderate thrombolytic activity of the extract of *Manilkara zapota* may be due the presence of such phytoconstituents which have ability to digest blood clot.

Conclusion

From this study, it can be concluded that hydroethanolic extract of *Manilkara zapota* leaves has moderate thrombolytic activity and can become a candidate for future thrombolytic agent. This is only a preliminary study and to make final comment the extract should be thoroughly investigated phytochemically and pharmacologically to exploit their medicinal and pharmaceutical potentialities.

References

1. Atanasov AG, Waltenberger B, Pferschy-Wenzig EM, Linder T, Wawrosch C, Uhrin P *et al*. Stuppner H; Discovery and resupply of pharmacologically active plant-derived natural products: A review. Biotechnol Adv. 2015; 33:1582-614.
2. Tapsell LC, Hemphill I, Cobiac L. Health benefits of herbs and spices: the past, the present, the future. Med. J Aust. 2006; 185(4 Suppl):S4-24.
3. Diet, nutrition, and the prevention of chronic diseases; Report of a WHO Study Group Geneva, World Health Organization, 1990 (WHO Technical Report Series, No.797 - TRS 797).
4. Interactive European Network for Industrial Crops and their Applications (2000–2005). Summary Report for the European Unio. QLK5-CT-2000-00111. Archived from the original on, 2013.
5. Karen S, Toni MK; Introduction to the different classes of natural products. In Lanzotti, Virginia. Plant-Derived Natural Products: Synthesis, Function, and Application. Springer, 2009, 3.
6. Fayek NM, Monem AR, Mossa MY, Meselhy MR, Shazly AH; Chemical and biological study of *Manilkara zapota* (L.) Van Royen leaves (Sapotaceae) cultivated in Egypt. Pharmacognosy Res. 2012; 4(2):85-91.
7. Prasad S, Kashyap, RP, Deopujari, JY, Purohit, HJ, Taori, GM and Daginawala, HF; Effect of Fagonia Arabica (Dhamasa) on *in vitro* thrombolysis; BMC, Complementary and Alternative Medicine, 2007, 7:36.
8. Prasad S, Kashyap, RS, Deopujari, JY, Purohit HJ, Taori GM, Daginawala HF; Development of an *In vitro* model to study clot lysis activity of thrombolytic drugs. Thromb J, 2006, 4:14.
9. Jainul AM, Azam S, Chowdhury A, Mubarak RU, Faruq KO; *In-vitro* thrombolytic and cytotoxic activity of methanolic extract of flemingia macrophylla leaves. Asian J Pharm Clin Res. 2013; 6(4):86-88.
10. Furie B, Furie BC; Mechanisms of thrombus formation". New England Journal of Medicine. 2008; 359(9):938-949.
11. Borissoff JI, ten Cate H; From neutrophil extracellular traps release to thrombosis: an overshooting host-defense mechanism? Journal of thrombosis and haemostasis: JTH. 2011; 9(9):1791-4.
12. Pantzar M, Ljungh A, Wadström T, Infect Immun. 1998; 66(10):4976-4980.
13. Shahik SM, Faruk Sikder MO, Amin Patwary NI, Sohel M, Islam MS, Faizah T; *In vitro* thrombolytic and cytotoxic evaluation of Mentha arvensis L., Mentha spicata L. and Mentha viridis L. IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS). 2014; 9(5):97-102.
14. Das A, Dewan SMR, Ali MR, Debnath PC, Billah MM; Investigation of *in vitro* thrombolytic potential of ethanolic extract of *Momordica charantia* fruits: An anti-diabetic medicinal plant. Der Pharmacia Sinica. 2013; 4(2):104-108.