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Inhibitory Effect of Hydroethanolic Extracts of *Annona muricata* on Human Platelet Aggregation and Hemolysis *In Vitro*



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ABSTRACT

Annona muricata (Annonaceae), a plant used in ayurvedic medicine is a small tree that bears edible fruits called soursop. Preliminary phytochemical screening of 50 % ethanolic extracts revealed the presence of phytochemical constituents (flavonoids, alkaloids, phenols etc.). Hence, an attempt has been made to evaluate the inhibitory effect of hydroethanolic extract on in vitro platelet aggregation and hemolysis. The platelet aggregation assay was carried out using platelet-rich plasma with different concentrations of plant extract (100-500 µg/ml). *A. muricata* extract showed dose-dependent effective antiplatelet activity with maximum activity of 88 % at 500 µg/ml concentration which is comparable with aspirin standard. Anti-hemolytic activity was found to be maximum (85.7 %) at 500 µg/ml concentration. The results obtained revealed that *A. muricata* has exhibited considerable antiplatelet and anti-hemolytic activity.



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INTRODUCTION

Herbal remedies used in traditional medicine provide an interesting and largely unexplored source for the development of new drugs. Platelets play a prominent role in homeostasis and thrombosis [1]. Platelets can adhere to the walls of the blood vessels, release bioreactive compounds and aggregate to each other. These properties increase to a well established level in conditions of arterial thrombosis and atherogenesis [2/Emanuele et al., 2011]. The evaluation of platelet function is a crucial datum in establishing necessary therapeutic measures associated with each clinical case. Platelets are important players in the processes responsible for the control of bleeding (hemostasis) and the formation of clots in injured blood vessels (thrombosis). For this reason, the pharmacological basis for therapy and prevention of cardiovascular disturbances is based on platelet structure and specific function [3/Giovanna et al., 2009]. Aspirin is a common antiplatelet drug that has been reported to have adverse side effects. One potential explanation for aspirin failure is variable response of individual patients to aspirin with consequent inadequate platelet inhibition (4/Enma *et al.*, 2012). Hence, it necessitates exploring medicinal plants and their natural constituents with minimal side effects.

Destruction of erythrocytes causes hemolysis by which hemoglobin and other internal cell components are released into the surrounding fluids. Due to the preponderance of polyunsaturated fatty acids in erythrocyte membranes, they are highly susceptible to oxidative damage whose consequence is hemolytic process (5/Nagamani *et al.*, 2012.). In addition, oxidative and inflammation are evidently noticed even in malaria associated hemolysis (6/Suthin et al., 2014).

In recent years, many drugs have been isolated from plants and investigated for their potent antiplatelet aggregating and antihemolytic activities (7/Dong *et al.*, 1998). Important classes of natural antiaggregant compounds are flavonoids such as quercetin and myricetin (8/Hommam *et al.*, 2000). *Annona muricata L.* is an undersized, deciduous commonly known as Graviola and Soursop belongs to custard apple family and roundish canopy-like tree. Height of this fruit bearing tree is measured to be in the range between 5 and 8 m [9/Adewole *et al.*, 2006]. Fruits were consumed for reducing fever and improving mother's milk secretion. The seed extracts are used to kill external parasites, head lice, and worms [10/Taylor]. The phytoconstituents that are

naturally present in the plant exhibit disease preventive properties, though they are not essential nutrients to human health. Annonaceous acetogenins, lactones and isoquinoline, alkaloids, tannins, and coumarins are some of bioactive compounds present in the *Annona muricata* leaves. It is in this context, the present study is carried out.

MATERIALS AND METHODS

Collection and extraction of plant materials

The fully matured fresh leaves of *Annona muricata* were collected from nearby garden in Coimbatore district. The leaves were washed with tap water followed by distilled water and shade dried for few days and then powdered with blender.

Plant Extraction

Some amount of powdered plant material were taken in 4 different conical flasks and soaked with 1 litre of each solvent (hydroethanolic, chloroform, ethyl acetate and petroleum ether) and fed in Soxhlet apparatus. The extracts were filtered through Buchner funnel using Whatman filter paper no.1. The filtrate was evaporated to dryness under reduced pressure and crude extracts were obtained.

Phytochemical analysis

The prepared plant extracts were analysed for the presence of alkaloids, carbohydrates, saponins, tannins, flavanoids, phenols, vitamin A, C, E and proteins [11/Ammar et al., 2014].

Antiplatelet activity

Platelet rich plasma 0.13×10^{-7} for each assay was resuspended in tyrode buffer (pH adjusted to 7.4 with 0.25 M HCl). Aggregation of platelets was induced by CaCl_2 at a final concentration of 2 μM . Platelet aggregation was recorded by increasing transmittance value of spectrophotometric measurements. To determine the *in vitro* antiplatelet aggregation property, different concentration (100, 200, 300, 400 and 500 μg) of plant extract was added to the platelet suspension for 1 minute, exposed at 37°C before treatment with platelet aggregating agents. Aspirin at 500 $\mu\text{g}/\text{ml}$ was used as standard [12/Iman et al., 2006].

Antihemolytic assay

Blood was collected from healthy adult human volunteers and collected in sterile Alsevier's solutions and used within 5 hours of collection. The preparation of cell suspension was carried out as described above. In a series of test tubes, take 800 µl of 1 % w/v Triton X-100 and make it upto a volume of 3 ml with phosphate buffer. Similarly 3 ml of distilled water alone served as positive control. Different concentrations of the plant extracts (100-500 µg) were added in a series of tubes in which Triton X-100 was previously incubated. 500 µl of RBC suspension was added to all the tubes mixed gently. Tubes were incubated in a water bath at 37°C for 1 hour and centrifuged at maximum speed for 5 minutes. The supernatant was collected and the absorbance was read at 541 nm against phosphate buffer as blank for calculating the percentage of hemolysis [13/Oguiura N Et Al., 2011].

RESULTS AND DISCUSSION

Antiplatelet activity of hydroethanolic extract of *Annona muricata* leaves:

Hydroethanolic extract of *Annona muricata* leaves was found to exert 88 % inhibitory action at 500 µg/ml concentration against calcium chloride induced aggregation (Figure 1). The antiplatelet activity is concentration dependent but it was found to be slightly lower when compared to standard Aspirin which showed 94 % antiplatelet aggregating activity. Activation of platelets plays a key role in hemostasis and circulation. Platelet aggregation is due to result of complex signal transduction cascade reactions brought about by stimulants (14/OGAWA et al., 1998). After activation, platelets provide a catalytic membrane surface for thrombin generation, which accelerates the formation of fibrin, necessary to stabilize thrombin. Platelet dysfunction contributes to the development and progression of many cardiovascular diseases like arterial hypertension, atherosclerosis and thrombosis. Indeed, it has been reported that patients with hypertension or coronary heart disease tend to have increased platelet reactivity (15/ VIVIANA et al.). Therefore, the compounds that inhibit platelet function, isolated from medicinal plants are of great importance. Hence, many investigations were carried out towards the prevention of abnormal hyperactivity of platelets reported in cardiovascular disorders employing different therapies, including use of medicinal plants (16/Massberg et al., 2005). The results reveal that herbal based compounds might lead to development of promising drugs to inhibit platelet aggregation (17/ Koleckar et al., 2008).

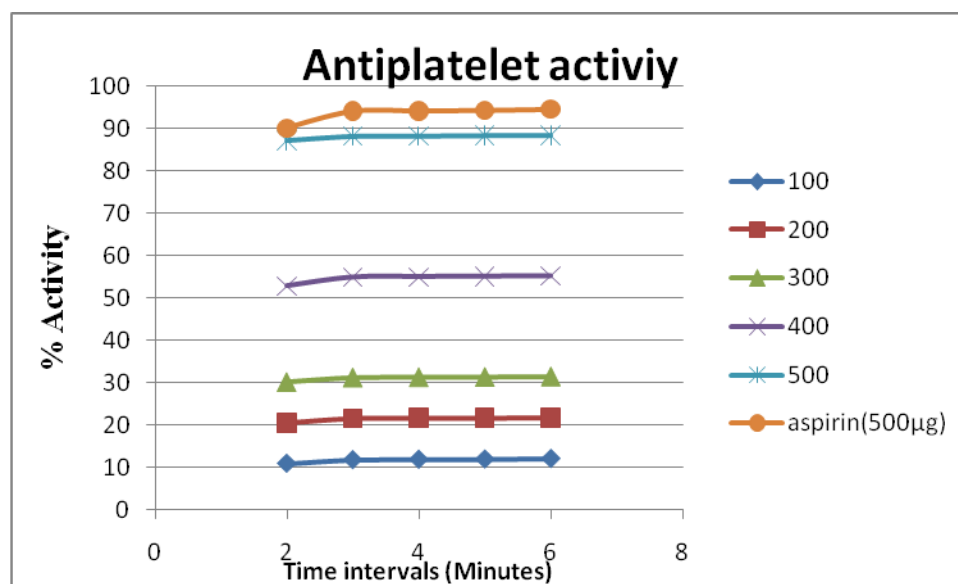


Figure 1. Effective anti-platelet activity of hydroethanolic leaf extracts (100-500 µg/ml)

Antihemolytic activity of hydroethanolic extract

Triton-X-100 is a chemical which causes human RBC (HRBC) lysis. It is a potent hemolytic agent. Injury to RBC membrane in turn will render the cell more susceptible to secondary damage through free radicals (18/DEVJANI et al.,). This study demonstrated the capability of the plant extract to stabilize RBC membrane, which is an indication of the ability of plant extract to prevent hemolysis. The maximum inhibition of 85.7 % was observed at 500 µg/ml concentration of hydroethanolic extract in concentration dependant manner (Figure 2). Thus, the hydroethanolic extract was found to substantially inhibit hemolysis caused by Triton-X-100. The erythrocyte model is widely used by researchers since it reveals the direct indication of toxicity of any injectable formulation as well as general indication of membrane toxicity. Another advantage of erythrocyte model is that blood is readily available and cells are easy to isolate from the blood. Moreover, RBC membrane has similarities with other cell membranes [19/Brindha et al.,]. Hence, erythrocytes have been used as a common *in vitro* model for the study of interaction of drugs with membranes (20/Mohammedi et al., 2014). From the results obtained, it proves that *Annona muricata* extract exhibits antihemolytic property that can be implicated in a wide range of applications for several disease states.

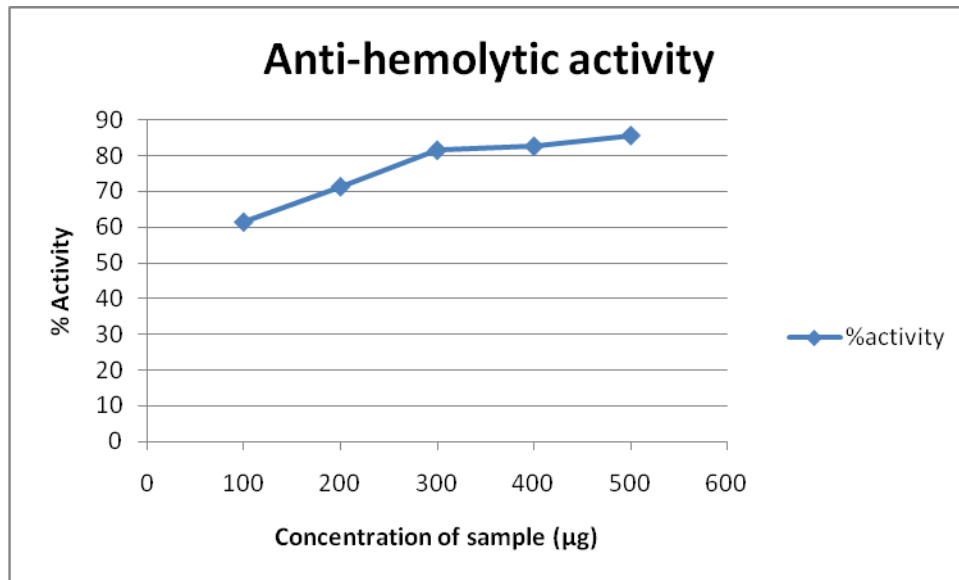


Figure 2. Anti-hemolytic activity of hydroethanolic leaf extract.

CONCLUSION

From the above findings it could be suggested that hydroethanolic extracts of *Annona muricata* leaf possess potent antiplatelet and antihemolytic activities limited to primary haemostasis in human blood and anti-hemolytic potential. Hence, platelet inhibition represents a promising approach for preventing thrombosis. However, further investigations are needed to confirm the mode of action and efficacy of *A. muricata* leaf extract in platelet aggregation and hemolysis in order to explore in therapeutic efficacy.

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