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Research Paper

Invitro Anticoagulant Activity of Nux Vomica, Sapota and Soapnut Seed Extracts

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ABSTRACT

Plant derived extracts demonstrate different levels of anticoagulant activity. Some significantly prolong blood clotting time, whereas others show little or no effect. Several phytochemicals including Alkaloids, Flavonoids, Coumarins, Saponins have been reported to contribute to this anticoagulant property. Homeostasis is the physiological process that regulates blood clot formation at the sites of vascular injury, preventing excessive bleeding while maintaining normal blood circulation. In the present study anticoagulant potential of aqueous extracts obtained from strychnos nux vomica seed, Sapota seed (manilkara zapota), soap nut (sapindus spp) was investigated. The anticoagulant activity of these extracts was evaluated invitro using the prothrombin time (PT) assay. Plasma obtained from blood samples of healthy individuals was treated with the plant extracts, and changes in clotting time were measured. Ethylenediamine tetra acetic acid (EDTA) and normal saline served as a negative and positive control, respectively. The clotting times of extract-treated plasma were compared with those of the control groups. The prolonged prothrombin time observed in the treated samples suggest that the crude extracts contain bioactive phytochemicals capable of interfering with the blood coagulation process. Further purification and characterization of these bioactive compounds may facilitate their development for therapeutic applications these findings indicate that the investigated plant extracts represent promising natural sources of anticoagulant agents.

INTRODUCTION

Coagulation, commonly referred as blood clotting, is a physiological process in which blood changes from a fluid into semisolid gel, leading to the

formation of a blood clot. This process helps achieve hemostasis, preventing excessive blood loss from an injured blood vessel and supporting tissue repair. Blood coagulation involves platelet

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activation, adhesion and aggregation followed by the formation and stabilization of fibrin network

The coagulation cascade is initiated through two primary pathways ,the intrinsic and extrinsic pathways . The extrinsic pathway is triggered when circulating blood comes into contact with tissue factor released from damaged the extravascular cells, in contrast , the intrinsic pathway is activated by factors present within the injured blood vessels. Platelet activation occurs in response to various stimuli, including collagen and arachidonic acid , which promote the synthesis of Thromboxane B₂ via the Cyclooxygenase 1 and Thromboxane A₂ synthase enzymatic pathway (TXAS). TXB₂ plays a significant role in enhancing platelet aggregation, and facilitating platelet to platelet interactions there by contributing to thrombus formation and increasing the risk of thrombotic disorders.

Numerous medicinal plants have been reported to exhibit anticoagulant properties by reducing platelet aggregation, extending blood clotting time ,and influencing various coagulation pathways.Among these soapnut seeds ,sapota seeds and nux vomica seeds have shown notable anticoagulant effects. Nux vomica seeds are rich in alkaloids and chlorogenic acid ,soap nut seeds were rich in saponins, ,flavonoids and phenols where as sapota seeds were rich in flavonoids ,tannins and phenolic compounds which may inhibit clotting enzymes and increase coagulation parameters such as Prothrombin time and activated partial prothromboplastin time.

MATERIALS AND METHODS:

Plant collection and extract preparation:

Three plant species of *Strychnos nux vomica* (Nux vomica seeds) ,*Sapindus mukkorossi* (soap nut seeds) and *Manilkara zapota*(sapota seeds) were purchased from local Sangareddy market.

Extract preparation of *Strychnos Nux vomica* seed:

Nux vomica seeds were washed cleanly , dried and crushed in a homogenizer and sieved and further stored in a dry place at room temperature until the day of extraction. Seed powder of about 50gm was taken and extracted with ethanol(50%)o by using soxhlet extractor .The extract thus obtained is filtered by using whatmann filter paper and filtrate is evaporated to dryness.It is further diluted and various concentrations were prepared and used for anticoagulant studies.

Extract preparation of *Sapindus mukkorossi* (Soap nut seeds):

Soap nut seeds were collected and washed cleanly , dried and outer shell is removed and kernels were collected and crushed in a homogenizer and sieved and further stored in a dry place at room temperature until the day of extraction. Seed powder of about 50gm was taken and extracted with ethanol(50%)o by using soxhlet extractor .The extract thus obtained is filtered by using whatmann filter paper and filtrate is evaporated to dryness.It is further diluted and various concentrations were prepared and used for anticoagulant studies.

Extract preparation of *Manilkara zapota*(S

All the seed extracts were subjected to phytochemical tests to determine the presence of Alkaloids, Flavonoids,Saponins and Coumarins.

Blood collection and platelet plasma preparation:

A healthy volunteer of about 18-35 years old were selected and blood samples were withdrawn from the selected donor via vein puncture and collected blood is transferred into separate tubes containing EDTA to prevent clotting.Centrifuge the blood samples for 15 minutes at a rate of 3000 rpm to separate the blood cells from plasma in order to



obtain pure platelet plasma (ppp) for performing Prothrombin time test.

Prothrombin Time Test:

For the plasma recalcification assay, 0.2ml of plasma was mixed with 0.1ml of ethanoic plant seed extracts at various concentrations along with different volumes of 25mm calcium chloride (CaCl_2) in a clean test tube. The mixture was incubated in a water bath maintained at 37°C . in the control group, the extract was substituted with an equal volume of 0.9% normal saline. Clot formation was monitored by gently tilting the test tube every 5 seconds and the time required for clot

formation was measured using a stop watch. The recorded clotting time was considered as prothrombine time.

RESULTS

This study was conducted to investigate the anticoagulant potential of Nux vomica seeds, Soap nut seeds and Sapota seeds using blood samples collected from healthy individuals. The anticoagulant activity of these plant extracts was assessed by determining their influence on blood coagulation time

Phytochemical screening:

Table no:1

Phytochemical screening	Ethanoilc Nux vomica seed extract	Ethanolic Soap nut seed extract	Ethanolic Sapota seed extract
Test for Alkaloids	+	+	-
Test for Coumarins	+	+	+
Test for flavanoids	+	-	-
Test for Saponins	+	+	+

+ Presence, - Absence

From the phytochemical screening we found that Nuxvomica seed extracts have shown the presence of Alkaloids, Coumarins, Flavonoids and Saponins. Sopa nut seeds have showed the presence of alkaloids, coumarins and saponins. Sapota seeds have revealed the presence of coumarins and saponins.

Anticoagulant activity

Ethanoilc extracts of soapnut seeds have exhibited coagulation time of about 7min and Nux vomica seed extract coagulation time was about 4min 30sec and Sapota seed extract coagulation time was 5min 24sec. Ethanolc extract of Nux vomica seed have exhibited more clotting time when compared to soapnut and sapota seed extracts. Its activity may be due to the presence of Phytoconstituents like Alkaloids, saponins, flavonoids and coumarins in the sample.

Anticoagulant Assay (Prothrombin Time Assay)

Table no:2

Group	Name of the plant extract	Amount of plasma (ml)	Amount of extract (ml)	CaCl_2 solution (ml)	Time of coagulation (min)
Group 1	Ethanolc extract of Nuxvomica	0.2	0.1	0.3	07.00min



	Seeds				
Group 2	Ethanollic extract of Soapnut seeds	0.2	0.1	0.3	4.30sec
Group 3	Ethanollic extract of Sapota seeds	0.2	0.1	0.3	5.24sec
Group 4	Control	0.2	0.1	0.3	2.38sec

CONCLUSION

In this summary ,present study is the first investigation to evaluate the anticoagulant potential of differenr plant seed extracts of Nux vomica,Soapnut and Sapota seed extract .findings suggest that Nux vomica seed extract exhibits promising anticoagulant activity and may have potential as a natural therapeutic option for management of thromboembolic disorders.

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