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EVALUATION OF *IN-VITRO* ANTI-COAGULANT ACTIVITY OF FRUIT EXTRACT OF *MANILKARA ZAPOTA*

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ABSTRACT

Coagulation refers to hemostatis mechanism where the blood changes from liquid state to gel, forming a clot to cease a bleeding. In some cases it forms a abnormal clot & coagulate excessively, that can lead to serious conditions like stroke, heart attack and deep vein thrombosis. The level of study is planned to evaluate the in-vitro anti- coagulant activity of a fruit *Manilkara zapota*. The fresh fruit pulp of *Manilkara zapota* is collected, dried and extracted by maceration extraction technique followed by phytochemical screening. The Phytochemical scrrening of fruit extract shows the presence of flavonoids, terpenoids, tannins, glycosides, quionenes and saponins. The anti-coagulant activity of sapodilla extract is evaluated by using blood plasma of a healthy poultry bird. The clotting time is assessed by the Prothrombin time assay method. Increasing concentrations of the plant extract resulted in a dose-dependent inhibition of clot formation, with concentrations like 50, 100, 150 µl which significantly ($p<0.05^{*}$, $p<0.01^{**}$, $p<0.001^{***}$) prolonging Prothrombin Time respectively. The study concluded that sapodilla extract shows better efficacy for anti-coagulant activity in a dose dependent manner.

KEYWRODS: Phytopharmacology, *Manilkara zapota*, Anti-coagulant activity, Prothrombin time.

INTRODUCTION

Haemostasis is an interaction process between coagulation and anticoagulants that retains the blood within the injured vascular system during periods of injury. Haemostasis comprises a complex mechanism that contains three major steps: (1) Vasoconstriction, (2) temporary blockage of a break by a platelet plug, and (3) blood coagulation, or formation of a fibrin clot. Anticoagulant drugs are needed for the short-term treatment of arterial and venous thrombotic disorders and for the long-term prevention of recurrences. Although heparin has been the mainstay of 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. Medicinal plants have historically been the first source of anticoagulant and antithrombotic molecules.

Manilkara zapota: Sapodilla (Spanish: sapote, chicozapote, chicoo, chicle, naseberry, nispero, or soapapple, among other names, is an evergreen tree native to southern Mexico and Central America The sapota or sapodilla is a tropical evergreen tree that is indigenous to Central America and Southern Mexico, but it has since spread to many other nations with tropical and subtropical climates. Thailand, Pakistan, Sri Lanka, Vietnam, Bangladesh, Maldives, Belize, Indonesia, Malaysia, and Caribbean are other countries where sapota is cultivated.

Pharmacological Profile of Sapodilla

Manilkara zapota, commonly known as sapodilla, has been traditionally used for various medicinal purposes in different cultures. While scientific research on its pharmacological activities is ongoing, several potential health benefits have been reported. Some of the pharmacological activities associated with Manilkara zapota include; Antioxidant Activity: Sapodilla contains phenolic compounds such as flavonoids and tannins, which exhibit antioxidant properties. Antioxidants help neutralize free radicals in the body, thereby protecting cells from oxidative damage and reducing the risk of chronic diseases like cancer and cardiovascular diseases.

Antimicrobial Properties: Extracts from different parts of the sapodilla tree, including the leaves, bark, and seeds, have shown antimicrobial activity against various pathogens,

including bacteria and fungi. These antimicrobial properties suggest potential applications in treating infections.

Anti-inflammatory Effects: Some studies have indicated that extracts from Manilkara zapota possess anti-inflammatory properties. These effects may help reduce inflammation and alleviate symptoms associated with inflammatory conditions such as arthritis and inflammatory bowel disease.

Antidiabetic Potential: Research suggests that sapodilla may have hypoglycemic effects, meaning it could help lower blood sugar levels. This potential antidiabetic activity may be attributed to certain compounds found in the fruit, which could be beneficial for individuals with diabetes or those at risk of developing the condition.

Wound Healing: Sapodilla extracts have been investigated for their wound healing properties. Some studies suggest that they may promote the proliferation of fibroblasts and enhance collagen synthesis, which are essential processes in wound healing. Antiulcer Activity: Certain compounds present in sapodilla have been studied for their potential gastroprotective effects against ulcers. These compounds may help protect the stomach lining and reduce the risk of ulcer formation.

Anticancer Potential: Preliminary studies have explored the potential anticancer properties of sapodilla extracts. Some research suggests that certain compounds in sapodilla may inhibit the growth of cancer cells or induce apoptosis (programmed cell death) in cancer cells.

MATERIALS AND METHODS

Collection and authentication of plant material

The fruits of *Manilkara zapota* were collected from hill farm Agrico, Kerala in the region of Payyambalam, Kannur in the month of April. Dr. K.P Prasanth Assistant Professor and Head of Department of Botany Reasearch centre Sree Narayana College, Kannur, recognised the authenticity of the specimen.

Sample Preparation

Choosing sapodilla fruit of size is not too large, if the outer part of sapodilla fruit is rubbed which will look green on the inner skin which indicate the sapodilla fruit is still young there is still much sap. Then wash with water until clean, then dry (at room temperature) Next, cut into small sapodilla fruit with a knife and dioven at a temperature of 45 degree celcius, dry

and mashed with a blender. Extractions are prepared of in this study by using maceration technique. Young sapodilla fruit powder weighed 50g, put in to maceration container, then added 70% methanol 150ml until a samples were submerged by solvent, then shake for 2 hours by using a mechanical shaker then covered with aluminum foil, left for 12hours. The maceration obtained is filterd with Whatman no 1 paper obtained by filteration and residue. Once the maceration is repeated. The obtained filteration is collected.



Fig. 1: Maceration Extraction of *Manilkara Zapota.*

PHYTOCHEMICAL SCREENING

Qualitative chemical screening was used to identify different classes of active chemical compounds such as saponins, flavonoids, tannins, quinones, terpenoids, and glycosides.

DETECTION OF SAPONIN

To detect saponins, 1 ml of extract was added to 1 ml of distilled water and shaken vigorously. The formation of foam confirms the presence of saponin.

DETECTION OF FLAVANOIDS

To detect saponins, 1 ml of extract was added to few drops of dilute sodium hydroxide and intense yellow colour is formed, if solution is colourless by adding Hcl, this is a sign of the presence of flavonoids.

DETECTION OF TANNINS

To detect tannin, 1 ml of extract was mixed with 5% FeCl3. The color of the black-green sediment confirms the presence of tannins.

DETECTION OF GLYCOSIDES

To reveal the presence of glycoside, 1 ml of the extract was added to sulfuric acid (H2SO4) and glacial acetic acid (2 ml) containing a few drops of iron chloride. A purple ring may form below the brown ring, indicating the presence of a glycoside.

DETECTION OF QUINONES

To test quinones, 0.5 ml of concentrated hydrochloride was added to 1 ml of extract. The presence of quinones is indicated by the production of a yellow precipitate.

DETECTION OF TERPENOIDS

To identify terpenoids, 1 ml of extract was mixed with 5 ml of concentrated H2SO4 and 2 ml of chloroform in a mixture. Reddish-brown color indicates the presence of terpenoids.

DETECTION OF PHENOLS

The total phenols in different solvent extract was determined with Folin Ciocalteu reagent(FCR) using different concentration of 1ml of extract mixed with 0.4ml FCR After 5 minutes 4ml of 7% sodium carbonate solution was added. The final volume of tubes were made up to 10ml distilled water and allowed to stand for 90 minute at room temperature. Absorbance of the sample was measured against blank at 750 nm using spectrophotometer.

DETERMINATION OF PROTHROMBIN TIME

Collection of blood and separation of plasma

About 5 ml of blood was drawn from healthy poultry bird (having no medicine consumption history) Trisodium citrate solution (3.8 % w/v) was used added to the blood in 1:9 ratio to avoid natural coagulation. Centrifugation was carried out for 15 min at 3000rpm for separation of plasma. This was used for testing prothrombin time (PT) analysis. Calcium chloride (0.025M) was used for initiating coagulation. Plant extract was used at a concentration of 100mg/ml. Tranexamic acid at 2% w/v concentration was used as stock for standard. For this assay plasma sample was divided into five groups.

Group I: 0.2 ml plasma + 10 µl 0.9 % saline + 30 µl calcium chloride

Group II: 0.2 ml plasma + 50 µl plant extract + 30 µl calcium chloride

Group III: 0.2ml plasma + 100 µl plant extract + 30 µl calcium chloride

Group IV: 0.2 ml plasma + 150 µl plant extract + 30 µl calcium chloride

Group V: 0.2ml plasma + 100 µl Tranexamic acid solution + 150µl plant extract + 30 µl calcium chloride.

All the tubes are tilted at an angle of 45° for every 10 seconds to measure the clotting time. Stop watch was used for measuring the clot formation. The total experiment was conducted for 600 seconds. This time is called as PT.

RESULTS

Table 1: Phytochemical Screening of Manilkara Zapota.

COMPONENTS	RESULTS
SAPONIN	++
TANNIN	++
FLAVANOIDS	+++
GLYCOSIDES	+++
QUINONES	++
TERPENOIDS	++
PHENOLS	+++

Table 2: Values showing Clotting time.

Group	sample	Result (Time in seconds)
Group 1:-Control group	0.2 ml plasma + 10 µl 0.9 % saline + 30 µl calcium chloride	30±0.12
Group 2: -1 st Test group	0.2 ml plasma + 50 µl plant extract + 30 µl calcium chloride	50±0.213*
Group 3: -2 nd Test group	0.2ml plasma + 100 µl plant extract + 30 µl calcium chloride	70± 0.02*
Group 4: -3 rd Test group	0.2 ml plasma + 150 µl plant extract + 30 µl calcium chloride	120 ± 0.24**
Group 5: Standard group	0.2ml plasma + 100µlTranexamic acid solution + 150µl plant extract + 30 µl calcium chloride	540 ± 0.54***

Value are expressed as mean (±), SEM p< 0.05*,p<0.01 **,p<0.001*** as compared with control group.

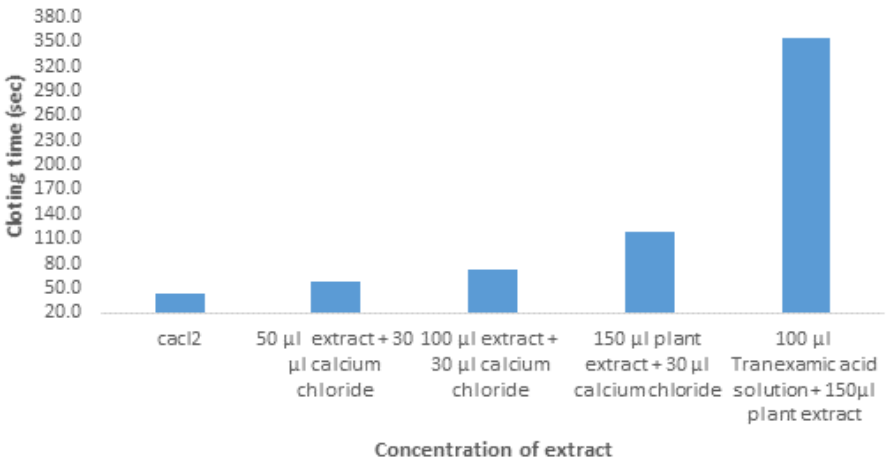


Fig 2: Graph representing Concentration of extract Vs Clotting time.

DISCUSSION

As the determination of prothrombin test, the effect of plant extract was compared with control group and which shown inhibition by prolonging Prothrombin time. From the Table 2 it is clearly understood that the fruit extract of *Manilkara zapota* shows significant anti-coagulant activity by increasing the concentration as 50µl, 100µl, 150µl of fruit extract.

CONCLUSION

The study evaluated the anti-coagulant activity of a plant extract using the prothrombin time (PT) assay compared to a control group and a standard (Tranexamic acid). Increasing concentrations of the plant extract resulted in a dose-dependent inhibition of clot formation, with higher concentrations significantly ($p<0.001^{***}$) prolonging Prothrombin Time. However, the extract's efficacy was better while increasing the concentration of extract respectively. Further research may explore for optimizing its concentration to enhance its anti- coagulant activity.

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