



PHYTOCHEMICAL AND ANTI COAGULANT STUDIES OF *PSIDIUM GUAJAV* LEAF METHANOLIC EXTRACT ON WISTAR RATS

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ABSTRACT

Psidium guajava L, belonging to the Myrtaceae family, has been reported to have anti-diarrheal, hepatoprotective, hypoglycemic, lipid lowering, antibacterial and antioxidant activities. It contains important phytoconstituents such as tannins, triterpenes, flavonoid: quercetin, pentacyclic triterpenoid: guajanoic acid, saponins, carotenoids, lectins, leucocyanidin, ellagic acid, mariticide, beta-sitosterol, uviol, oleanolic acid and ursolic acid. In view of the immense medicinal importance of the plant, this review is an effort to compile all the information reported on its phytochemical and pharmacological activities. The present is an attempt to study the effect of methanolic extract of *Psidium guajava* L. on blood coagulant activity in rats.^[15]

KEYWORDS: Preliminary phytochemical analysis, Antithrombotic tests.

INTRODUCTION

Our understanding of blood clotting is intimately tied to the history of civilization. With the advent of writing 5000 years ago, it could be argued that the first symbols used for blood, bleeding, or clotting represented the first published coagulation pathway. The ancient peoples of the world always held blood in utmost mystical esteem. Through the ages, this esteem has been transmitted to modern times in the many expressions that use "blood," such as "blood is thicker than water," "blood of our fathers," and others.

Mysticism aside, the study of blood clotting and the development of laboratory tests for blood clotting abnormalities are historically inseparable. The workhorse tests of the modern coagulation laboratory, the prothrombin time (PT) and the activated partial thromboplastin time (aPTT)^[17], are the basis for the published extrinsic and intrinsic coagulation pathways, even though it is now known that these pathways do not accurately reflect the function of blood clotting in a living organism. In this chapter, and ultimately this textbook, the many authors hope to present a clear explanation of coagulation testing and its important place in the medical armamentarium for diagnosing and treating disease.^[2,3,4]

MATERIALS AND METHODS

Plant Material

The dried leaf of *Psidium guajava* Linn in the present study were collected from the natural habitat in and around Guntur and Rajamahendravaram.

Plant Extraction Procedure

The leaves were dried in hot air oven and coarsely powdered. The powdered material was extracted with methanol using Soxhlet apparatus for about 24hrs. The extract was filtered and concentrated by using water bath, after evaporating the extract it was dried and stored in the desiccator. The small amount of powdered extract was dissolved in 10ml of distilled water and filtered the extracted solution. The *Psidium guajava* extract was given 50mg/kg orally.^[5,6,7]

Preliminary Phytochemical Analysis

The methanolic extract of the leaf of *Psidium guajava* was subjected to preliminary phytochemical screening.^[8,9]

1. Test for Alkaloids

- **Mayer's Test:** Sample was treated with Mayer's reagent; appearance of cream color indicates the presence of alkaloids.
- **Dragendorff's Test:** Sample was treated with Dragendorff's reagent; appearance of reddish brown precipitate indicates the presence of alkaloids.
- **Hager's Test:** Sample was treated with Hager's reagent; appearance of yellow color indicates the presence of alkaloids.
- **Wager's Test:** Sample was treated with Wager's reagent; appearance of brown precipitate indicates the presence of alkaloids.

2. Test for Carbohydrates

- **Molisch Test:** The extract was treated with 3ml of alpha naphthol in alcohol and Conc. Sulfuric acid was carefully added to side of the test tubes. Formation of violet ring at the junction of the two liquids indicates the presence of carbohydrates.
- **Fehling's Test:** To the sample Fehling's solution A and B was added and heated for 2mins. Appearance of reddish brown color indicates the presence of reducing sugars.
- **Benedict's Test:** To the sample Benedict's solution was added and heated, appearance of reddish orange precipitate indicates the presence of reducing sugars.
- **Barfoed's Test:** The sample was treated with Barfoed's reagent and heated, appearance of reddish orange precipitate indicates the presence of reducing sugars.

3. Test for proteins

- **Biuret's Test:** To the extract copper sulphate solution followed by sodium hydroxide solution was added, a violet color precipitate indicates presence of proteins.
- **Xanthoproteic Test:** To the 5 ml of test solution add 1ml of Conc.HNO₃ and boil yellow precipitate is formed. After cooling it add 40% NAOH solution, orange color is formed.
- **Seliwanoff's Test:** To the test solution add crystals of resorcinol and eqvolurage of Conc. HCL and heat on water bath, rose color is produced.

- **Millon's Test:** To the extract million's reagent was added, appearance of pink color indicates the presence of proteins.

Animals and Experimental Design

The study was carried out on twenty one locally bred male rats weighing between (180- 220 gms). Animals were procured and were acclimatized to the laboratory conditions of animal house. The animals were maintained under standard laboratory conditions i.e 12 hours dark and 12 hours light cycle. All animals were divided into three groups, control (Vehicle), standard (Warfarin) and test group (*Psidium guajava* extract) each comprising of 7 rats. Animals were kept in plastic cages under controlled conditions of temperature 23±2⁰ C and were given free access to food and water. The animals were transferred to the laboratory at least one hour before the start of the experiment. All experiments were performed during day time.^[10,11]

Study schedule

Drugs were given orally to overnight fasted animals as single dose every day in the morning for fourteen consecutive days and food was provided after one hour of drug administration so that food could not interfere with drug absorption. Antithrombotic tests were done after fourteen doses and on the day of experiment i.e. on fourteenth day, the drugs were given forty minutes before the start of experiment.^[12]

Experimental details

S.No	No. of Animals & sex	Group details	Duration of Exposure	Study duration #
1	7M	Group I (Control) Phosphate buffer saline	Single exposure daily	14 days
2	7M	Group II Group II (Test I) 150 mg/mL DEBRIDACE	Single exposure daily	14 days
3	7M	Group II Group III (Test II) (Test II)	Single exposure daily	14 days

Chemicals Required

Drugs

Warfarin sodium 5mg tablets were crushed, diluted in distilled water and administered to animals in a dose of 0.54mg/kg orally.^[13]

Gum Tragacanth powder was used as suspending agent to prepare suspension of the test drug (*Psidium guajava*) and was administered to control animals as placebo in the dose of 150mg/kg orally - 100ml of warm distilled water was added in 2 gms Gum Tragacanth powder to form 2% suspension. Suspensions were prepared freshly at the time of administration.^[14]

Measurement for coagulation parameters

Blood samples were collected in coagulation tubes; plasma was suspended by centrifugation at 1500 rpm for 15 minutes in 14k Hum ax centrifuge. PT and aPTT were

measured by Huma clot duo, using standard reagent kits of Merck.^[15,16,17]

Statistical analysis

The data were subjected to analysis by taking mean and standard error to the mean using student T-test, P -values of < 0.01 were considered as significant and P < 0.001 as highly significant. All statistical methods were performed using SPSS software version 16.5.

RESULTS

Phytochemical screening

Table 1.1: Preliminary phytochemical analysis of methanol extract of *Psidium guajava*.

PLANT CONSTITUENTS	Methanolic Extract
ALKALOIDS	Absent
CARBOHYDRATES & GLYCOSIDES	Present
SAPONINS	Present
FLAVONOIDS	Present
PROTEINS & AMINOACIDS	Absent
TANNINS	Absent
VOLATILE OILS	Present
FIXED OILS & FATS	Present

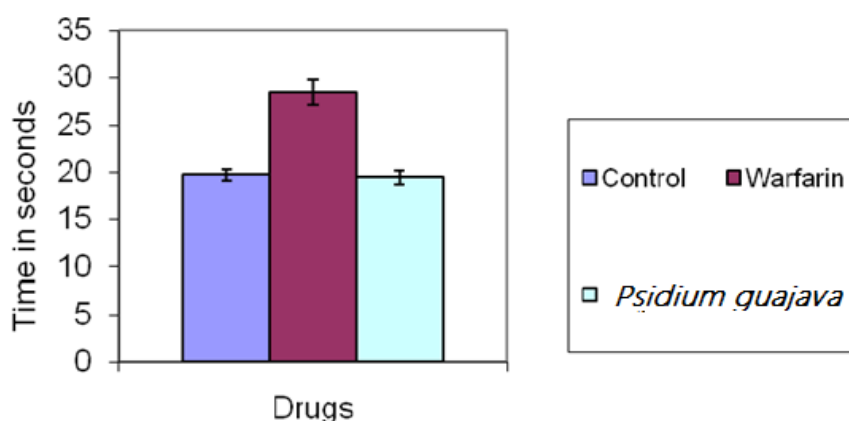
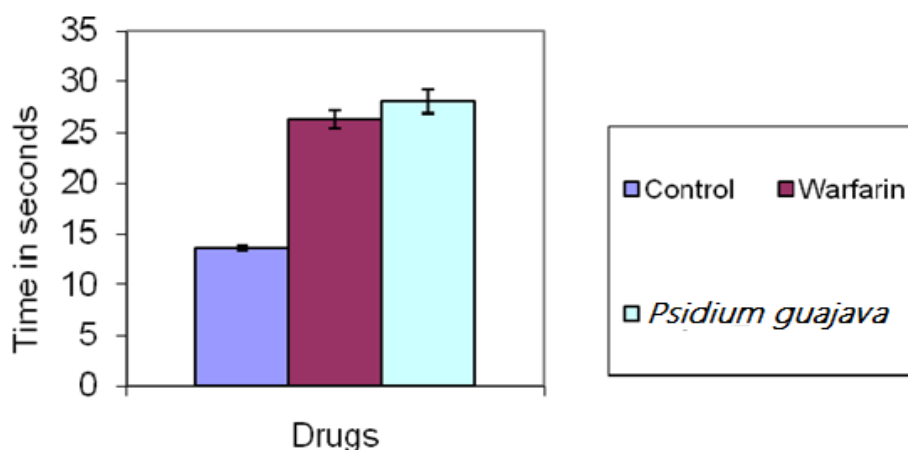
Table 1.2 Figure I and II elaborate the comparative effect of *Sodium guajava* extract (50mg/kg) and warfarin (0.54mg/kg) on coagulation parameters after 14 days continuous administration of drugs to rats. The antithrombotic effect was assessed by determining aPTT and PT. *Psidium guajava* extract did not show any

significant effect on aPTT as compared to control; however there was highly significant increase in PT i.e. 28.00 ± 1.2 seconds as compared to control i.e. 13.57 ± 0.30 seconds, whereas Warfarin altered both parameters i.e. aPTT and PT highly significantly as compared to controls.

Table 1.2: Effect of Multiple Dosing of *Psidium Guajava* and Warfarin on APTT and PT.

Parameters	Control (n=7)	Warfarin (n=7)	<i>Psidium guajava</i> (n=7)
aPTT	19.71 ± 0.57	$28.43 \pm 1.3^{**}$	19.43 ± 0.78
PT	13.57 ± 0.30	$26.29 \pm 0.87^{**}$	$28.00 \pm 1.2^{**}$

Mean $\pm$ S.E.M $^{**}p < 0.001$, highly significant as compared to control.

Figure I: Comparative Effect of Multiple Dosing of *Psidium Guajava* and Warfarin On APTT.Figure II: Comparative Effect of Multiple Dosing of *Psidium Guajava* and Warfarin On PT.

DISCUSSION

The process of blood coagulation has a vital role in an organism's response to vascular injury on one hand and in thrombosis and cardiovascular diseases (CVD) on the other hand. Oral anticoagulant therapy must be monitored to ensure that the dose is providing the required response. Activated partial thromboplastin time (aPTT) and Prothrombin time (PT) are generally used to determine variations in coagulation factors. PT is an effective method of monitoring oral anticoagulant therapy and it reflects the overall efficiency of extrinsic clotting pathway in clinical test of blood coagulation. A prolonged Prothrombin time indicates a deficiency in clotting factors V, VII and X. Whereas activated aPTT is a test of intrinsic clotting activity. A prolonged activated partial thromboplastin time usually represents a deficiency in factors VII, IX, XII, XIII and von Willebrand's factor. Present study revealed significant increase in PT with *Psidium guajava* similar to that of Warfarin suggesting its possible effects on the extrinsic pathway, while aPTT was not altered suggesting that it might have no effects on the intrinsic pathway but further studies are required on different species and large number of animal to investigate the exact mechanism of action.

CONCLUSION

It is concluded that Methanol extract of *Psidium guajava* Linn has mild antithrombotic effect which may be of value in thrombotic states and cardiovascular diseases, however, nothing can be said definitely; hence further studies are needed to reach at final conclusion.

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