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Invitro Anticoagulant Activity of *Tabebuia Pallida* Leaves on Healthy Human Plasma

Radhika Batchu, Sinduja Baraju, Sindhuja Chandragiri, Samatha Enugurthi, Venkat Ramana Mudari, Mohan Gugulothu
Vaageswari College of Pharmacy, Thimmapur, Karimnagar-505001

Abstract— *Hemostasis,a process that involves clot formation in the walls of ruptured or damaged blood vessels in order to prevent abnormal bleeding as well as to keep intravascular blood in fluid form. To evaluate the anticoagulant activity, the methanol extract of the plant obtained from processes maceration and soxhlation were used. This work ofinvitro anticoagulant activity of Tabebuia pallida determines its ability to prolong the clot formation time through Prothrombin Time (PT) test via various concentrations (0.125g/ml, 0.250g/ml, 0.500g/ml) of its methanolic extracts.*

I. INTRODUCTION

Anticoagulants play a vital role in people with antithrombin deficiency which in turn leads to deep vein thrombosis and pulmonary embolism. Anticoagulant that areseldomlyreferred as blood thinners even though they don't actually thin the blood.

The interaction process between the coagulation and anticoagulant that retains the blood within the injured blood vessels during the periods of injury known to be hemostasis. On the otherhand,haemorrhage is the exact opposite process.[1]Hemostasis is primary step in wound healing. It is a complex process includes majorly three steps:

1. Formation of a prothrombin activator by initiation of a cascade of chemical reactions by numerous blood coagulation factors.
2. Prothrombin activator transforms prothrombin into thrombin.
3. Thrombin converts fibrinogen to fibrin fibres.

The mechanism of blood coagulation involves the sequential activation of number of proenzymes that results in stepwise response amplification.[2]Disorders of cardiovascular system which include hypertension, coronary thrombosis, arteriosclerosis, congestive heart failure and cerebral haemorrhage are caused by blood circulatory system as blood clotting disorder constitute a serious medical problem.[3]Prothrombin time test also known as PT test or pro-test is used for screening the extrinsic pathways and to detect the deficiencies in Factors II, V, VII and X. The extrinsic pathway in coagulation system is activated by thromboplastin only in the presence of calcium ions and the subsequent clotting time depends on concentration of Factors II, V, VII and X. Thus, one or more of these clotting factors (VII and X) deficiency is indicated by a prolonged PT and considered as abnormal. The normal PT is 11-15 s. Except for the nonsteroidal anti-inflammatory drugs such as aspirin and indomethacin, some other important synthetic anticoagulant agents are heparin, ethylenediaminetetraaceticacid (EDTA), citrate and warfarin have anti-inflammatory and anti-platelet activity. [4-5]

Anticoagulants are used to prevent the formation of thromboembolism. Thromboembolism is one of the causes of cardiovascular disease in the form of blocked arteries caused by blood clots. Trauma, smoking, surgery and usage of drugs that contain estrogen are some of the causes of thromboembolism. Drugs used for prevention and treatment of thromboembolism are thrombolytic, anti-thrombocyte, anticoagulant and haemostatic groups.[6]

Medicinal plants have historically been the first source of antithrombotic and anticoagulant molecules.[6] In India, the use of plants with widespread medicinal purposes for the prevention and the treatment of various aliments is one of the most ancient traditional remedial forms of primary health care. Despitethe pharmaceutical properties provided by the anticoagulant drugs, they show serious side effects and are expensive.[7]Hence, therefore it is necessary to explore the alternatives. As the fact that plants are the safer source of medicine, this work is a preliminary attempt to investigate the invitro anticoagulant activities of *Tabebuia pallida* leaf extracts using standard experimental methods in blood samples of normal individuals.[8]

II. MATERIALS

Collection of Plant Materials

The leaves of *Tabebuia Pallida* were collected from the planted trees on the road side street near English Union High School, Jyothinagar, Karimnagar, Telangana, India. Identification and Authentication was done by certified Botanist. The collected leaves of *Tabebuia Pallida*are dried under the shade at room temperature. The dried leaves are then subjected to grinding to achieve fine powder.Powder obtained after sieving was also used to evaluate the Pharmacognostic studies.

III. METHODS

Plant Extraction:

Tabebuia Pallida leaves are collected, air dried at room temperature andwith the help of electric grinder the leaves are smashed into powder. This plant material has been soaked by suspending 100g of powdered leaves of *Tabebuia pallid* in 250 ml of methanol with extraction by maceration for 7days with occasional stirring. After 7 days, the suspension was filtered through muslin cloth and then through No.1 Whatman filter paper. The solvent was removed at low temperature (40-50^0

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C)and reduced pressure in the rotatory evaporatorto dryness. They were preserved into sterile bottle kept in a refrigerator until used for further analysis.[9]

Another method of extraction has also been carried out with same amount(100g) of dried powder of *Tabebuia pallida* leaves by the process of soxhlation up to 6 cycles with the solvent methanol.

IV. Prothrombin Time (PT) Determination

Separation of plasma from collected blood:

10 ml of blood was drawn by vein puncturing of healthy individuals. Volume of blood about 9 mu/l, volume of 1 mu/l and 3.8% trisodium citrate solutions added to prevent the coagulation process that occurs naturally. Then it is subjected to centrifugation for 15 minutes at 3000 rpm. After that blood cells gets separated from the plasma. From that pure platelet plasma was obtained which is used for prothrombin time (PT) test.[10]

Plasma sample was divided into following different groups:
Group I: Negative control group - 0.2ml of plasma + 0.1ml of saline water + 0.3 ml of 0.5 g/ml $Cacl_2$.
Group II: Positive control group–0.2 ml of plasma + 0.1 ml of 50 g/ml EDTA + 0.3 ml of $Cacl_2$.
Group IIIA: 0.2 ml of plasma + 0.1 ml of 0.125 g/ml of plant extract obtained from maceration + 0.3 ml of 0.5 g/ml $Cacl_2$.
Group IIIB: 0.2 ml of plasma + 0.1 ml of 0.125 g/ml of plant extract obtained from soxhlation + 0.3 ml of 0.5 g/ml $Cacl_2$.
Group IVA: 0.2 ml of plasma + 0.1 ml of 0.250 g/ml of plant extract obtained from maceration + 0.3 ml of 0.5 g/ml of $Cacl_2$.
Group IV B: 0.2 ml of plasma + 0.1 ml of 0.250 g/ml of plant extract obtained from soxhlation + 0.3 ml of 0.500 g/ml of $Cacl_2$.
Group V A: 0.2 ml of plasma + 0.1 ml of 0.500 g/ml of plant extract obtained from maceration + 0.3 ml of 0.5 g/ml of $Cacl_2$.
Group V B: 0.2 ml of plasma + 0.1 ml of 0.500 g/ml of plant extract obtained from soxhlation + 0.3 ml of 0.5 g/ml $Cacl_2$.

All tubes are tilted at 45^0 of angle for every 30 seconds for measuring the clotting time. This time is called PT, for measurement of clot formation stopwatch was used and turbidity was measured with the help of turbidometer.

Tested extracts:

Methanolic extracts of leaves of Tabebuia pallida was investigated for the determination of anticoagulant activity. The concentrations of preparations are 0.125 g/ml, 0.250 g/ml and 0.500 g/ml of both maceration and soxhlation was taken.

V. Results And Discussion

Both the methanolic extracts of*Tabebuia pallida* leaves obtained from maceration and soxhlation has shown increased clotting time or PT time with respect to the increased concentration of plant extracts. The following Table 1 and Figure 1represents the values obtained after performing prothrombin time (PT) test by using maceration as well as soxhlation extracts.

TABLE 1. Prothrombin Time and Turbidity of the *Tabebuia pallida* leaves

S.No.	Test Tubes	Concentration of Plant Extract	Prothrombin Time	Turbidity
1.	**Group I:**Negative control	-	27.8 sec	15.6
2.	**Group II:**Positive control	-	32.5min	89.6
3.	**Group III A:**Test (Maceration)	0.125 g/ml	2.30 min	52.5
4.	**Group III B:**Test (Soxhlation)	0.125 g/ml	2.45 min	53.7
5.	**Group IV A:**Test (Maceration)	0.250 g/ml	3.48 min	66.9
6.	**Group IV B:**Test (Soxhlation)	0.250 g/ml	3.55 min	68.2
7.	**Group V A:**Test (Maceration)	0.500 g/ml	6.26 min	72.4
8.	**Group V B:**Test (Soxhlation)	0.500 g/ml	6.45 min	73.9
9	**Group VI A:**Test (Maceration)	1 g/ml	10.4 min	81.4
10	**Group VI B:**Test (Soxhlation)	1 g/ml	10.5 min	82.6

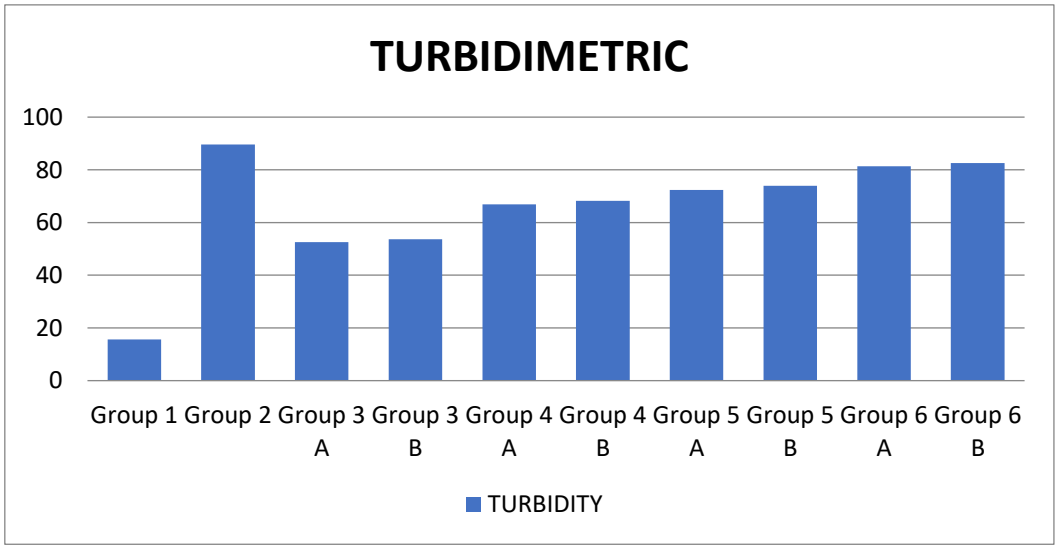

Figure1. Turbidity measurement of *Tabebuia pallida* leaves

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Discussion:

The formation of interaction between cellular and molecular components of coagulation, clotting forms based on intrinsic and extrinsic pathways. The equivalence between anticoagulants and procoagulants has found to have most effective anticoagulant effect.

There were many similar studies which used PT test to determine the anticoagulant activity of a plant extract. PT test is most used method of determination for measuring clotting time, it enables us to know the time that prolongs the process of coagulation and the ability of the Methanolic extract of Maceration and Soxhlation has showed almost the same activity, of a specific plant to do so. Generally, it is considered that polyphenolic compounds and flavonoids present in the plant shows anticoagulant properties.

VI. CONCLUSION

We conclude that the methanolic extracts of plant *Tabebuia pallida* was shown to have anticoagulant activity and as there is no report that this plant has anticoagulant properties, this part of work is the first investigation of anticoagulant activity of *Tabebuia pallida* through Prothrombin Time (PT) test.

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Corresponding Author:
Radhika Batchu,
Associate professor, Department of pharmacognosy, Vaageswari college of pharmacy, Karimnagar

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