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Evaluation of *in-vitro* anticoagulant activity of *Molineria recurpata* leaf extract

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ABSTRACT

Moleneria recurpata is an herb (also known as palm grass). This herb is available in the hilly areas of Tripura. The plant is well known for it's different folk medicines like leaves are used in bone fracture, in wound healing and as analgesic, anthelmintic. It was observed that the people of Tripura especially Tribes are use the juice of matured leaf in worm. The economic importance of this plant is, fibers that are collected from this plant have been used for purposes such as making nets, and the fruit is edible. The present study was carried out to evaluate the anti-coagulant activity of Molineria recurpata (Family: Hypoxidaceae) leaf extract (Methanol) on fresh human blood. Both the extracts were found sufficient anti-coagulant activity. But the concentration of 2g/ml methanolic leaf extract showed the maximum effect with respect to others. The isolation of active constituents those are responsible for different activities are going on in our laboratory.

Keywords: Anti-coagulant activity, *Molineria Recurpata*. Blood Coagulation, human plasma

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INTRODUCTION

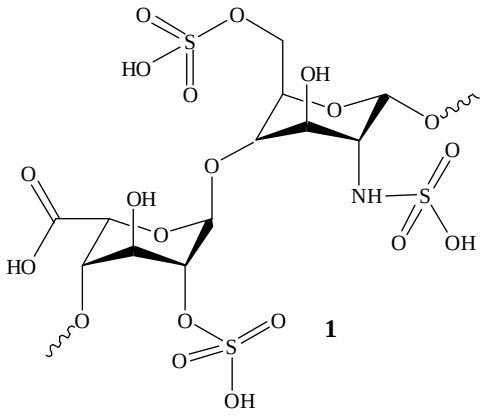
Haemostasis is the process that retains the blood within the vascular system during periods of injury. The coagulation mechanism may be thought of as a complex series of cascading reactions involving development of enzymes from their precursor (zymogens, procoagulants proenzymes). Most of the substances which are necessary for coagulation are present in an inert form and must be converted to an activated state. As one enzyme is formed it then becomes available to convert the next zymogen to its activated enzyme (serine protease). This process continues until a fibrin meshwork clot has formed. In addition to the zymogens, protein cofactors and membrane phospholipids surfaces, calcium ions play an active role in the final development of the fibrin clot [1].

Most adult cardiovascular disorders involving hypertension, cerebral hemorrhage, coronary thrombosis, arteriosclerosis and congestive heart failure are caused by problems in the blood circulatory system as blood clotting disorders which constitute a serious medical problem. A number of non-steroidal anti-inflammatory drugs (NSAIDs) such as aspirin and indomethacin have been used as antithrombotic agents. These drugs *in vitro* and *in vivo* cause inhibition of platelet aggregation and thromboxane formation [2-5].

The prothrombin time test (also known as the pro test or PT test) is a useful screening procedure for the extrinsic coagulation mechanism including the common pathway. It detects deficiencies in factor II, V, VII, and X. The prothrombin time test is frequently used to follow oral anticoagulant therapy that inhibit factors II, VII, IX and X. Thromboplastin activates the extrinsic coagulation system in plasma in the presence of calcium ions. The subsequent clotting time is dependent on the concentration of factors II, V, VII and X. Thus prolongation indicates a deficiency in one or more of these factors [6, 7].

The normal prothrombin time is 11-15 seconds. Each prothrombin time within this range indicates that the person has normal amounts of clotting factors VII and X while prolongation in prothrombin time is considered abnormal [8].

Except the NSAIDs some of the important anti-coagulants are heparin (1), dicoumarol (2), coumarins (3), 4-hydroxycoumarin (4), warfarin (5)



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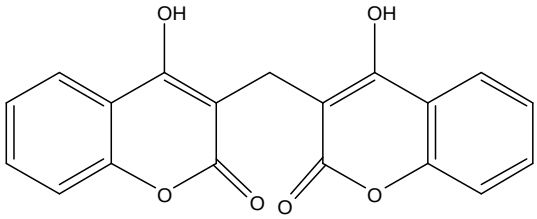

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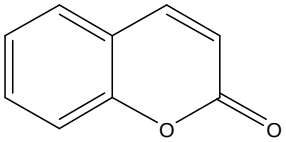

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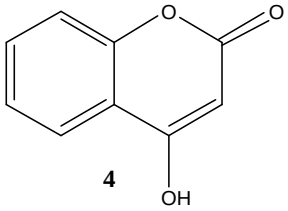

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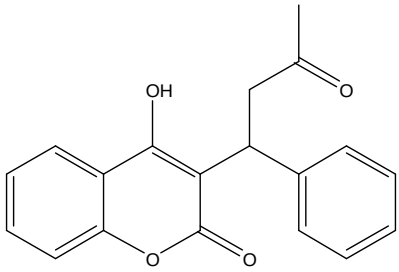

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METERIALS AND METHODS

Plant material collection

Molineria recurpata was collected from different parts of the state. The leaves were washed with fresh water and dried under shade at room temperature. The leaves were powdered and stored. 60g of powdered drug was extracted separately with methanol, petroleum ether by continuous hot percolation in soxlet apparatus and with water by cold maceration for 3 days respectively. All the extracts were filtered and evaporated using a rotary evaporator. Dried extracts were stored at 20˚c until used.

Phytochemical screening

Dried extracts were subjected for the presence of different phytoconstituent like alkaloid, steroid, flavonoid, tannin, glycoside etc [9].

Study population

Blood samples obtained from my dear batchmates, were used to assess the anticoagulant effects of *Molineria recurpata*. Participants were 20-25 years old. They had been chosen for this study according to the following

criteria: having normal prothrombin time, not suffering from any cardiovascular diseases (hypertension, congestive heart failure, coagulation disorders such as, Hemophilia A or B) or diabetes, not recently using nonsteroidal anti inflammatory drugs, not obese or smokers and free from dyslipidemic disorders.

Collection of blood samples

The blood samples were obtained from normal individuals by using sterile syringes, withdrawn from vein of right arm of each individual and placed separately in containers containing tri-sodium citrate to prevent the clotting process. Centrifugation (15 minutes at rate 3000 rpm) was carried out to separate the blood cells from plasma in order to obtain pure platelet plasma (ppp) for prothrombin time test. The obtained plasma sample of each individual were poured separately in plane containers using automatic pipette and stored at room temperature [10, 11].

Collection of blood and Plasma re-calcification

0.2 ml plasma, 0.1 ml of crude extract of different concentration and different volume of $CaCl_2$ (25 *m*M) were added together in a clean fusion tube and incubated at 37^0C in water bath. For control experiment extract solution was replaced by same volume of 0.9% saline water. The clotting time was recorded with stopwatch by tilting the test tubes every 5 seconds. This time is called the prothrombin time [12, 13].

Table: 1 Determination of coagulation time using aq. Extract

Name of plant	Amount of plasma	Amount of extract	Calcium chloride solution	Time of coagulation
Control	0.2 ml	0.1 ml	0.3 ml	70 Sec
M.recurpata (200 µg/ ml)	0.2 ml	0.1 ml	0.3 ml	8:32 min
M.recurpata (400 µg/ ml)	0.2 ml	0.1 ml	0.3 ml	22:56 min

Table: 2 Determination of coagulation time using methanol extract

Name of plant	Amount of plasma	Amount of extract	Calcium chloride solution	Time of coagulation
Extract solution (0.5 µg ml)	0.2 ml	0.1 ml	0.5 ml	8:37 min
Extract solution (1 µg/ml)	0.2 ml	0.1 ml	0.5 ml	15:28 min

RESULTS AND DISCUSSION

The percentage yields of MLE, ALE, were 10.65% w/w and 15.98% w/w respectively. The primary phytochemical screening revealed the presence of alkaloid, steroid, flavonoid, tannin, glycoside etc. Anti-coagulant activities of aqueous and methanolic extract of *M. recurpata* were carried out. From the present study it is proved that both the extract have remarkable anti-coagulant activity than the control solution. Further study is under progress to isolate the pure component fraction.

CONCLUSION

From the above experiment it has found that the aqueous and methanolic extracts *M. recurpata* may be useful as anticoagulant.

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