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A Review on "The In-vitro Study of Anticoagulant Activity of Different Plant Extract"

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Abstract: *The aim of this review is to evaluate the plant extract which show highest anticoagulant activity. There are different plant extract to show the anticoagulant activity. On the basis of this study to know about which plant show maximum anticoagulant activity and which plant has minimum anticoagulant activity. The plants used are naturally obtained. They have other pharmacological properties but some plant have significant anticoagulant activity. The plant used for studied are Myrciaria plinioides, Terminalia bellerica, Gymnema sylvestre, Jatropa gossypiifolia, Allium sativum , Curcuma longa,Rubus ulmifolius, Gymnema sylvestre. Among these plant the Gymnema sylvestre has maximum anticoagulant activity, and the other plant show a significant anticoagulant activity. The prothrombin test, activated prothrombin test , thrombin test, recalcification method were used for the in vitro test of anticoagulant activity. The extract of plant were obtained by various extraction process such as maceration, soxhlet extraction, decoction method etc.*

Keywords: Anticoagulant activity, Prothrombin time, Activated Prothrombin time, Hydro-Ethanolic extract, Hydro-Methanolic extract

I. INTRODUCTION

Coagulation the process of formation of blood clot. The hemostatic system consists of coagulation ,platelet aggregation, andthrombolysis. The coagulation is a complex process which involves the positive feedback mechanism. Only few stages are include in in coagulation process. The clothing factor involved in coagulation process arethe table. Their number represent the order in which they were discovers. These clotting factor activate each other in a specific way. Resulting in the formation of prothrombin activators. The prothrombin is the first step in the final common pathway. The enzyme thrombin activate by the prothrombin and convert the inactivated fibrinogen into insoluble fibrin. As the amount of fibrin increases the platelet plug progressively established. The final common pathway can be initiated by two processes, which often occur together: the extrinsic and intrinsic pathway. The extrinsic pathway is activated rapidly within second, following tissue damage. The intrinsic pathway is slower (3-6 min) and is triggered when blood come in contact with damaged blood vessels.

Blood clotting factors:-
I Fibrinogen
II Prothrombin
III Tissue Factor (Thromboplastin)
IV Calcium(Ca^{2+})
V Labile factor, proaccelerin, Ac-globulin
VII Stable factor, proconvertin
VIII Antihaemophilic globulin(AHG) Antihaemophilic factor A
IX Christmas factor, plasma thromboplastin components (PTC), antihaemophilic factor B
X Stuart-Power factor
XI Plasma thromboplastin antecedent (PTA), antihaemophilic factor C
XII Hageman factor
XIII Fibrin stabilizing factor
Vitamin k is essential for synthesis of factor

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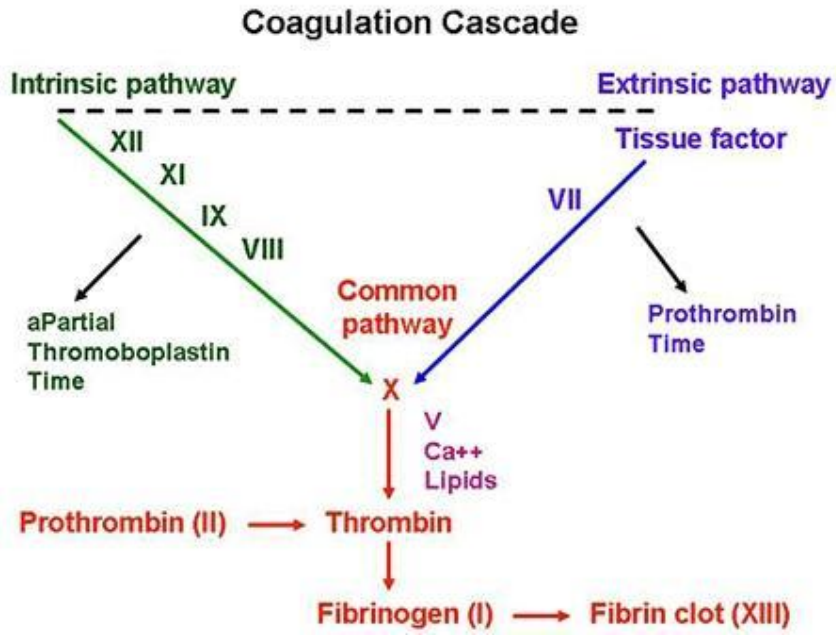

II. MATERIAL AND METHODS

Collection of information related to the plants having anticoagulant activity:

To gather a list of all the known anticoagulant compounds and plants, databases such as Google Scholar and PubMed were searched with appropriate keywords, which are listed in our previous work Accordingly, we prepared two separate lists from anticoagulant compounds and plants reported previously.

Plant showing anticoagulant activity:

To find similar compounds to the reported anticoagulant compounds, similarity search was carried out according to the name or the chemical structure of the identified anticoagulant compounds in the PubChem structure search engine. The PubChem similarity search uses Tanimoto calculation and the PubChem constructed binary fingerprint to discover related structures according to the specified threshold of similarity. In this respect, the specified threshold of similarity was set at 90% Afterward, the similar compounds found by PubChem were used to selectsuitable medicinal plants to evaluate their anticoagulant effect.

List of plants :

- Myrciaria plinioides-
- Terminalia bellerica
- Gymnema sylvestre
- Jatropa gossypiifolia
- Allium sativum
- Curcumalonga
- Rubus ulmifolius,
- Gymnema sylvestre,
- Zingiber officinale

Anticoagulant Activity of M. Plinioides Ethanolic Extract via Extrinsic Pathway:

Myrciaria plinioides D. Legrand (Myrtaceae) is a native plant of Southern Brazil, which have potential in the food industry due to its edible fruits.The therapeutic use of this plant were studied widely. The study of this plant use plasma recalcification time method study. Increasing concentrations (30, 50, 80, 120 and 180 µg/mL) of the extract were

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incubated with human plasma and the common coagulation pathway was triggered by the addition of calcium. Thehighest concentration (180 µg/mL) of the extract, which extended the onset of clotting about 3.8 times over the clotting time of extract-free plasma. The PT was prolonged by the extract up to 1.9 times . This show that the extract has anticoagulant activity in significant time.

Terminalia bellerica:

The Terminalia bellirica has 32.95%in vitro thrombolytic activity in clot lysis. The percentage of weight loss of clot after application of extract solution was taken as the functional indication of thrombolytic activity. 100 µl SK as a positive control (30,000 IU) was added to the clots along with 90 minutes of incubation at 37°C, showed 70% clot lysis Clots when treated with 100 µl sterile distilled water (negative control) showed only negligible clot lysis (2.8%). It can be show that T. bellirica has got the potential as a candidate for future thrombolytic agent.

Gymnema sylvestre:

The ethanol extracts of the stem, leaves and root of Gymnema sylvestre were studied for the in vitro anti- coagulant activity by Plasma re-calcification method. Based on the concentrations dependent manner (i.e.,) in ppm these extracts showed significant anti-coagulant activity. Among the three plant extracts, leaf has maximum anti-coagulant activity of (40.39mins) in 1000ppm and a minimum of (02:38mins) in 1500ppm of root extract.

Jatropa gossypiifolia :

Jatropha gussypiifolia L. Euphorbiaceae is a medicinal plant largely used in folk medicine. Teas from the leaves are popularly used as an antithrombotic agent and the branches are frequently employed as a "thick blood agent.The anticoagulant activity of the crude extract of J gossypiifolia (CE) was evaluated by the prothrombin time (PT) and activated partial thromboplastin time (aPTT) assays, using normal citrated human plasma. The aPTT test prolong time up to 3 times and PT test not prolong the time. Heparin was used as positive control and as expected presented significant anticoagulant activity, with PT higher than 60 s (negative control: 16.27 ±0.32 s) and APTT higher than 240 s (negative control: 35.07 ±0.03 s).

Allium sativum *:*

In this study the two type of extract were used the Methanolic extract of Allium sativum and the water extract of Allium sativum. The result show that the 100 µg/ml of aqueous extract has 0.80 min prolongation time. While the methanolic extract has 1.23 min prolongation time with same concentration. As the concentration increases the tine also increases. The 500µg/ml concentration of methanolic and aqueous extract has 6.23 min and 7.32 min prolongation time respectively. This study demonstrates that MEAS and AEAS in different concentrations (100-500 µL) inhibits clot formation and increases PT. It also shows that increasing concentrations of extracts strongly inhibits the coagulation process and increases PT, and that aqueous extract of Allium sativum have anticoagulant properties through the prevention of clot formation than compared to methanol extract. The constituents of garllicallicin and ajoene has anticoagulant activity.

Curcuma longa :-

Curcumin extend the blood clotting time as proved by the Prothrombin test and the activated prothrombin time test and thrombin time test method. Curcumin has 37.2 sec prolongation time at 0.1µM in aPTT method in in vitro test and at 50 µM has 119.8 sec bleeding time. With same concentration the Curcumin has less bleeding time in PT test method. At 0.1 µM concentration the Curcumin has bleeding time only 17.4 sec in PT test and at 50 µM concentration the bleeding time is about 35.2 sec. The in vivo bleeding time of Curcuminalso study and it show that the Curcumin at 100 mg/kg concentration have tail bleeding time 102 sec. Prolonged PT indicates that Curcumin could also inhibit the extrinsic pathway of coagulation.

Rubus ulmifolius :-

The anticoagulant activity of Rubus ulmifolius hydro-methanolic and hydro-ethanolic extracts was measured by a coagulometer in AP and APTT test. Results showed that the two examined extracts prolonged PT and aPTT differently.Two examined extracts of Rubus ulmifolius prolonged PT for 12.5 and 6.25mg/ml concentrations relative to the normal control ($P<0.01$). Except methanolic extract of Rubus ulmifolius prolonged PT for 3.125 mg/ml concentration relative to the normal control ($P<0.01$). At 12.5 and 6.25mg/ml concentrations it has got a PT and APTT prolongation times due to a deficiency or inhibition of the common pathway coagulation factors.The two extracts

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exhibited significant prolonged time of intrinsic clotting of blood at all concentrations and this effect persist at a concentration as low as 195 µg/mL but the methanolic one was shown to be the most active extract. It has been shown that an isolated PT prolongation is a possible cause of extrinsic pathway (FVII) deficiency or inhibition, but mild factor X, V, and II deficiencies are also possible causes.

Gymnema sylvestre:-

The ethanollic extracts of the stem, leaves and root of Gymnema sylvestre were used for the in vitro anti- coagulant activity by Plasma re-calcification method. These extracts showed significant anti-coagulant activity based on the concentrations dependent manner in ppm. Among the three plant extracts, leaf showed maximum anti-coagulant activity of (40:39mins) in 1000ppm and a minimum of (02:38mins) in 1500ppm of root extracts. The standard drug EDTA showed very good anti-coagulant activities of more than one hour.

Zingiber officinale:-

The anticoagulant effect of ginger aqueous extract (5%) in different volumes (25, 50, 75 and 100 µL.) was examined in vitro in blood samples of normal individuals through measuring of prothrombin time (PT). The aqueous extract of ginger inhibited coagulation process and significantly prolonged prothrombin time in a dose-dependent manner. The ginger has significantly (P=0.001) showed prolongation in the prothrombin time in a dose dependent manner as 15.8±20.12, 18.5±0.2, 20.20±0.22 and 21±0.27 seconds respectively.

III. RESULT AND DISCUSSION

Plant extract	Concentration	Time
Gymnema sylvestre	1000 ppm	40:39 min
Zingiber officinale	100 micro liter	21±0.27sec.

IV. CONCLUSION

The various plant extract were studied under anticoagulant activity. This study show that the leaf extract of Gymnema sylvestre show maximum anticoagulant activity of 40:39 min.

For 1000ppm. While the minimum anticoagulant activity shown by the Zingiber officinale extract, it has 15 sec to the 21 sec prolongation time in the aqueous extract. This result is obtained by the prothrombin time method. The ethanolic extract of many plants show the maximum anticoagulant activity. So the ethanolic extract were mostly used for study. The ethanol or methanol and the water were used as the solvent for the extraction.

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