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Invitro Anticoagulant Activity of Emblica Officinalis Leaf Extract on Normal Healthy Blood Plasma

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Abstract:- Hemostasis is a process involving in formation of clots within walls of damaged blood vessels .It prevents abnormal bleeding and helps in maintaining Intravascular blood in the form of fluid state, In present study helps to evaluate Anticoagulant activity of *Emblica officinalis* leaf extract.

- ***Materials and methods***

Methanol extract of *Emblica officinalis* at different concentrations are tests for Invitro prothrombin time (PT) test. The In vitro Anticoagulant effects of different extracts of *Emblica officinalis* in different concentrations 0.5,0.25,0.125, and 1 g/ml are examined by using plasma, which was collected from blood samples of healthy individuals by measuring PT. saline and EDTA are used as a positive and negative control respectively.

- ***Results***

Methanol leaf extract of *Emblica officinalis* was found to show coagulation process in 1g/ml.The time required for clotting of 0.5g/ml methanol leaf extract was found to be 6.14 sec. The time required for clotting of 0.25g/ml was found to be 3.39 sec. The time required for clotting of 0.125 g/ml was found to be 2.56 sec.The concentration of 1 g/ml has showed a maximum effect with respective to all other concentrations.The prolonged PT activity is due to the presence of phytochemical constituents called tannis in the crude extract.These principles could help in the Isolation and evaluation clinical and physiological purposes.

- ***Conclusion***

Invitro Anticoagulant activity of leaf extract of *Emblica officinalis* possess pharmacological active anticoagulant components called tannis which helps in preventing blood clotting disorders.

I. INTRODUCTION

Hemostasis is a process between coagulation and anticoagulant that retains the blood within the injured vascular system during injury. The opposite of hemostasis is hemorrhage. It is the first stage of wound healing. This involes coagulation,which changes blood from a liquid to a gel. The Endothelial cells of intact vessels prevent blood clotting with a heparin -like molecule and thrombomodulin and prevent platelet aggregation with nitric acid and prostacycline. Hemostasis involves three major steps (Sirridge MS, Shannon R 1993).

- Vasoconstriction
- Temporary blockage of a hole in a damaged blood vessels by a platelet plug
- Blood coagulation (formation of fibrin clot)

Fibrin clot, Cardiovascular disorders include hypertension, cerebral hemorrhage, coronary thrombosis, arteriosclerosis, and congestive heart failure is caused by blood circulatory system as a blood clotting disorder constitute a serious medical problem (Saxena R et al., 2007). The prothrombin time (PT) test also known as pro-test or PT test is used to screen the extrinsic pathways and detects the deficiencies in Factors II, V, VII, and X. In the presence of calcium ions thromboplastin activates the extrinsic pathway in coagulation system and the subsequent clotting time depends on the concentration of Factors II, V, VII, and X. Thus, one or more of these clotting factors (VII and X) deficiency indicated by a prolonged PT and considered as abnormal. The normal PT is 11-15 s. Except for nonsteroidal anti-inflammatory drugs (aspirin and indomethacin) some other important synthetic anticoagulant agents are heparin, ethylenediaminetetraacetic acid (EDTA), citrate, and warfarin have anti-inflammatory and anti-platelets activity (Quick AJ 1966; Quick AJ 1970).

In India, the use of plants with widespread medicinal purposes for the prevention and the treatment of various ailments is one of the most ancient traditional remedial forms

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of primary health care. Besides, the pharmaceutical properties anticoagulant drugs show serious side effects and also expensive (Hoffbrand AV et al., 2006). Hence, therefore, it is necessary to explore alternative anticoagulants. The plants are the safer source of medicine, this study is a preliminary attempt to investigate the in vitro anticoagulant activities of *Emblica officinalis*l leaf extracts using standard experimental methods in the blood samples of a normal individuals (Calixto JB 2000).

The active ingredient that has significant pharmacological action in Amla is designated by Indian scientists as phyllemblin . The leaves are rich in quercetin, phyllaemblic compounds, Gallic acid,tannins,flavonoids ,pectine and vit C and also contains various polyphenolic compounds (Newman DJ and Cragg GM 2012). A wide range of photochemical components including terpenoids, alkaloids,flavonoids. Apart from the useful antioxidant activity, they have anti-thrombosis properties to promote vascular health by improve blood fluidity, anti-coagulant, and antiplatelet activity, which can cause a warming sensation. Amla also supports natural immunity and digestive functions. It is not completely understood, which amla components are responsible for each activity and they may be mediated through multiple different mechanisms. The combined anti-inflammatory, anti-thrombosis, anti-coagulant, and anti-platelet activities of amla make it attractive target for the prevention of a variety of vascular disorders

Oral administration of the amla fruit extract (50 mg/kg body weight) significantly decreased the concentrations of pro-inflammatory cytokines, TNF-α and IL-6 in serum. These results suggest that amla fruit extract may be an effective anticoagulant and anti-inflammatory agent.

II. MATERIALS AND METHODS

➢ *Collection of Plant Materials*

The leaves of *Emblica officinalis* had been collected from wild Developing tree with inside the botanical garden, pharmacognosy Department, in vaageswari college of pharmacy in Thimmapur, Karimnagar, Telangana, India. Identification and Authentication had been performed by a certified taxonomist. A Specimen had been deposited with-inside the institutional Herbarium. The collected plant material had been turned into very Loose from any overseas natural matter. Leaves had been separated, coloured dried and powdered with laboratory mixer and sieved pharmacognostic studies had been performed with clean leaves and leaf powder.

➢ *Extraction of Plant*

Emblica officinalis leaves are air dried at room temperature and smashed into powder with a electric grinder. This plant material has been soaked by suspending 10 g of powdered *Emblica officinalis l*eaf in 100 ml methanol with occasional stirring for 24 h. After 24 h, the suspension was filleted through a fine muslin cloth and then through a No. 1 Whatman filters paper. The solvent was removed at low temperature (40-50°C) under reduced pressure in a rotary evaporator to dryness. They were preserved into sterile bottle kept in a refrigerated until used for further analysis (Harbome JB 1998).

➢ *Phytochemicals*

Methanolic extract of the leaves of *Emblica officinalis* is subjected to preliminary phytochemical analysis for the detection of different phytochemical constituents present in the extract by using the different phytochemical tests. Different crude extracts have been dissolved in respective solvent and used for qualitative phytochemical constituent's confirmation such as alkaloids, saponins, flavonoids, phenols, tannins, and steroids.

➢ *Alkaloids*

- *Dragendroff's reagent test*

To 1 ml of extract, a few drops of dragendroff's reagent were added to the test tube, and the development of color was noticed. Appearance of orange color indicates the alkaloids presence.

➢ *Saponins*

- *Foam test*

To 1 ml of extract, 10 ml of water was added and boiled. After few minutes, the mixture was shaken vigorously and filtered. The formation and persistence of froth (1 cm height) for 1 h indicates the presence of saponins.

➢ *Flavonoids*

- *Sodium hydroxide test*

To 1 ml of extract, 1 ml of sodium hydroxide solution was added and observed. Appearance of yellow color indicates the presence of flavonoids.

➢ *Phenolics*

- *Ferric chloride test*

To 1 ml of extract, 2 ml of distilled water was added followed by a few drops of 10% ferric chloride. The presence of phenols was indicated by the appearance of blue or green color.

➢ *Tannins*

- *Ferric chloride test*

To 2 ml of extract, 1 drop of ferric chloride was added followed by the appearance of bluish or greenish black color indicates the presence of tannins.

➢ *Stoerids:*

- *Salkowski test:*

Two drops of concentrated sulfuric acid and 10 drops of acetic anhydride and 2 ml of chloroform added to extract. Color changes from Red to blue. Presence of steroids indicates bluish colour (Trease GE, Evans WC 1989).

III. PROTHROMBIN TIME (PT) DETERMINATION

➢ *separation of plasma from collected blood:*

- By making vein puncture of healthy volunteers, 10 ml of blood was drawn. Volume of blood about 9 mu/l ,volume of 1 mu/l and 3.8% trisodium citrate solutes added .for Ignoring of coagulation process which occurs naturally. Then centrifugation done about for 15 minutes at 3000 RPM rate. Then from plasma the blood cells get separated. From that pure platelet, plasma was obtained. PT test was used for. Pure platelet plasma (PPP) (Dandjesso C et al., 2012).
- plasma sample was divided into five groups.
- **Group I:** Negative control group 0.2 ml. Plasma +0.1 ml of saline water + 0.3 ml of Cacl2(0.5g/ml)
- **Group II:** positive control group 0.2 ml of plasma + 0.1 ml of 50 mg/ml of EDTA + 0.3 ml of cacl2. (0.5mg/ml)
- **Group III:** 0.2 ml of plasma + 0.1 ml of. 0.125 g/ml of plant extract + 0.3 ml of Cacl2 (0.5g/ml)
- **Group IV:** 0.2 ml of plasma + 0.1 ml of 0.25 g/ml of plant extract + 0.3 ml of Cacl2.(0.5g/ml).
- **Group V:** 0.2 ml of plasma + 0.1 ml of 0.5 g/ml of plant extract + 0.3 ML of Cacl2 (0.5g/ml).
- **Group VI:**0.2 ml plasma+0.1 ml of 1g/ml of plant extract+0.3ml of cacl2 (0.5g/ml).

At the angle of 45 ° all tubes are tilted for every 30 seconds for measuring of clotting time. for clot formation stopwatch is used. The time is called Prothrombin time PT. This was repeated for three times Then calculation of average time was done (Hoffman M, Monroe DM 2007).

➢ *Tested extracts:*

For determination of anticoagulant activity, methanol extract of leaves of Phyllanthus *Embilica Officinalis* was investigated. The concentrations of preparations are 0.125, 0.25, 0.5 g/ml and 1 gram .

IV. RESULTS

➢ *Phytochemical: secondary metabolites identification by phytochemical screening from phyllanthus emblica officinalis extract:*

Table I: Summarizes from phyllanthus *Emblica Officinalis* leaf extracts phytoconstituents are identified. Methanolic extract was subjected to the preliminary phytochemical analysis was done. Positive results showed the presence of flavonoids, tannins, steroids etc.

➢ *PROTHROMBIN TIME (PT):*

Methanol extracts from phyllanthus emblica Officinalis leaf Results in relative to control Increase the clot time. In Table 2, the results are summarized at the concentrations of 0.125 g/ml, 0.25g/ml , 0.5g/ml , 1 g/ml Of methanol extract of Phyllanthus Emblica officinalis. Results in increased prothrombin time of 1gram Respectively.

We conclude that methanol extract has prolonged duration of Clot formation. The clot formation increases when we increase concentrations from 1 g/ml with increasing prothrombin Time 8.24sec.

V. DISCUSSION

A complex interaction between cellular and molecular components results in formation of coagulation. Clotting involves in intrinsic and extrinsic pathway.

Table1 Phytochemical and preliminary evaluation of Phyllanthus Emblica officinalis extract:

Test	Observation
Carbohydrates	+ve
Cardiac Glycosides	+ve
Flavonoids	+ve
Saponins	-ve
Anthraquinons	-ve
Tannins and phenolic compounds	+ve
Alkaloids	+ve
Proteins	+ve
Tannins	+ve

Table 2 Table1 Phytochemical and preliminary evaluation of Phyllanthus Emblica officinalis extract:

S. No	Test tubes	Concentration of Extract	Prothrombin Time	Turbidity
1	**Group-I:** Negative control	—	30.5 sec	12.0
2	**Group-II:** Positive control	—	50.6 sec	90.7
3	**Group-III** Test	0.125g/ml	2.56 min	50.9
4	**Group IV** Test	0.25g/ml	3.39 min	63.5
5	**Group V** Test	0.5g/ml	6.14 min	71.1
6	**Group VI** Test	1g/ml	8.24 min	73.5

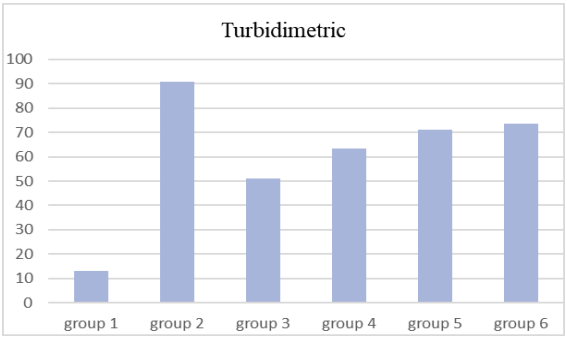

Fig 1 Turbidity Histogram

The balance in between the procaogulants/ anticoagulants. The similar study was reported Ikese et al., 2015 .And The extract of Tridox procumbens reduced the clotting time .The petroleum ether will extract of T . procumbens is also show the clotting time similar results in Kale et at.,2008 ethanolic leaf extract of T.procumbens also reduced the clotting time .A plant that also shown by Soumya et at .,2015 same like the clotting time.taj et at .,2001that noticed correlation between concentration in the aqueous extract of Alliumcepa to inhibit the clot formation with prolonged PT. Then the concentration increases and the aqueous extract red onion strongly inhibited coagulation process mean while increased the PT.In this investigation same correlation exists between between the concentration of extracts and time taken to inhibit clot formation. This was due to presence of several phytochemical compounds noticed in the extracts of leaves.

The Manicam et at.,2010 studied the anticaogulation activity of aqueous leaf extract of *Melastoma Malabathricum Linn* .this study showed the aqueous leafs extracts prolonged coagulation time similar to the result obtained.

Many reaschers studied anticoagulant property of few plant extract as Sutherlandia frutescens leaf extra,Glorioso Superna,Zantedeschia aethiopica leaf extract,and this study showed that prolonged coagulation time which is compared with the standard and control.

VI. CONCLUSION

The anticoagulant activity of Emblica officialis extract was not yet reported and this report was found to be the first invistagtion for PT.Hence, further identification and characterization of active molecules responsible for activity was to be found out in future.

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