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Evaluation of *in-vitro* coagulation activity of *Acalypha wikesiana* extract on blood plasma of poultry bird

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Abstract
Haemostasis is the process of forming clots in the walls of damaged blood vessels to prevent abnormal bleeding and to maintain intravascular blood in a fluid state. There is an increasing need to source for pharmacological and medicinal materials from plant source. The present study comprises the possible coagulation effect of *Acalypha* leaf chloroform and methanol extract having *in vitro* coagulant activity by using blood samples of poultry bird. *In vitro* coagulation effect of *Acalypha* leaf extract in different concentrations were examined in the blood samples of poultry bird by measuring Prothrombin Time (PT) in respect of the standard drug. The extracts were found to have coagulation activity and significantly prothrombin time in a dose-dependent manner. The principle involved in this extract is in different concentrations for the clot formation and decrease prothrombin time. This is also a subject for further studies on efficacy and safety, it also can be used in the future as a supplementary coagulant agent in blood vessels.

Keywords: Haemostasis, *in vitro* coagulant activity, prothrombin time, intravascular, blood

Introduction
Haemostasis is an interaction process between coagulation and anticoagulants that retains the blood within the injured vascular system during periods of injury. Haemostasis comprises a complex mechanism that contains three major steps:

1. Vasoconstriction,
2. Temporary blockage of a break by a platelet plug, and
3. Blood coagulation, or formation of a fibrin clot.

The coagulation mechanism is a complex cascade mechanism involving the conversion of precursor enzymes (zymogens, procoagulants, and proenzymes) into the active enzymes. Mostly, substances that are necessary for coagulation are present in an inert form and converted to an activated state. Once, one active enzyme is formed it converts the next inactive zymogen to its active enzyme. This series process continues until a fibrin meshwork clot is formed. Protein cofactors, membrane phospholipids surfaces and calcium ions play an active role in the development of the fibrin clot [1]. Cardiovascular disorders include hypertension, cerebral haemorrhage, coronary thrombosis, arteriosclerosis, and congestive heart failure are caused by blood circulatory system as blood clotting disorders constitute a serious medical problem. The prothrombin time (PT) test also known as pro-test or PT test used to screen the extrinsic pathways and detects the deficiencies in Factors V,VII, X and Thrombin. 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 V, VII, X and Thrombin. Thus, one or more of these clotting factors (VII and X) deficiency indicated by a prolonged PT and considered as abnormal [2-5]. In India, the use of plants with widespread medicinal purposes for the prevention and/or treatment of various ailments is one of the most ancient traditional remedial forms of primary health care [6, 7].
Besides, the pharmaceutical properties coagulant drugs show serious side effects and also expensive. Hence, therefore, it is necessary to explore alternative coagulants. Since the plants are the safer source of medicine, this study is a preliminary attempt to investigate the *in vitro* coagulant activities of *Acalypha* leaf extract using standard experimental models in the blood samples of normal individuals.

Materials and Method

Collection of plant material

The leaf of *Acalypha Wikesiana* were collected from Chakdaha, Nadia District, and West Bengal, India and were shade-dried, cut into small pieces and coarsely powdered followed by sediment in indirect sunlight and preserved in dark corner of lab. The coarse powder was used for extraction with solvent. Calcium Chloride was purchased from Merck India Pvt. Ltd.

Preparation of plant extract

Acalypha Wikesiana leafs were air dried at room temperature and crushed with a mortar vessel grinder. This plant material again dried at room temperature for two days. This plant material was soaked by suspending powder of *Acalypha wikesiana* leaf in 50ml chloroform and 70ml methanol by the process of percolation. After 24 hours the suspension was filtered through No.1 Whatman filter paper. The solvent was removed at low temperature (50-70 °C) under water bath by using moist heat process. They were preserved into sterile bottle kept in a refrigerator until used for further analysis.

Phytochemicals

Each extract (chloroform and methanol) of the leaf of *Acalypha wikesiana* were subjected to a preliminary phytochemical analysis for the detection of different phytochemical constituents present in extract using the different phytochemical test. Different crude extracts were dissolved in respective solvent and used for qualitative phytochemical constituent's confirmation such as alkaloids, flavonoids, tannins, phenols, saponins and glycosides.

Determination of PT

Collection of blood and separation of plasma

About 5 ml of blood was collected from healthy poultry bird (having no medicine consumption history) by intravenous injection. To the 9 µl volume of blood, 1 µl volume of 3.8% trisodium citrate solution uses added to avoid natural coagulation process. Immediately centrifugation was carried out for 15 min at a rate of 3000 rpm to separate the blood cells from plasma for prothrombin time (PT) test.

Plasma sample was divided into seven groups:

Standard: 0.2 ml plasma + 0.1ml Tranexamic acid solution (Marketed name Pause) + 0.3 ml $CaCl_2$

Blank: 0.2 ml plasma + 0.1ml Normal saline solution + 0.3ml $CaCl_2$

Sample 1: plasma 0.2 ml + extract 0.1ml +0.3ml $CaCl_2$
Sample 2: plasma 0.4 ml + extract 0.2ml +0.6ml $CaCl_2$
Sample 3: plasma 0.6 ml + extract 0.4ml +0.9ml $CaCl_2$
Sample 4: plasma 0.8 ml + extract 0.8ml +1.2ml $CaCl_2$
Sample 5: plasma 1ml + extract 1ml +1.5ml $CaCl_2$

All the tubes are shaken vigorously and tilted at an angle of 45° for every 30 seconds to measure the frothing time. Stop watch was used for measuring the froth formation. Prewarmed (37 °C) test tubes and calcium chloride reagents (37 °C), sample temperature maintained not longer than 5 minutes.

Result and Discussion

Table 1: Frothing Results for Different groups

Group	Test	Sample	Result
Standard: 0.2 ml plasma + 0.1ml marketed drug solution + 0.3 ml $CaCl_2$	Control		Formation of froth sustained in high amount
Blank: 0.2 ml plasma + 0.1ml saline solution + 0.3ml$CaCl_2$	-	-	No froth formation had been seen
Sample 1: plasma 0.2 ml + extract 0.1ml +0.3ml $CaCl_2$	1st Test Group	Plant Extract	Very small quantity froth observed and not sustained
Sample 2: plasma 0.4 ml + extract 0.2ml +0.6ml $CaCl_2$	2nd Test Group	Plant Extract	Froth observed but not sustained in duration time
Sample 3: plasma 0.6 ml + extract 0.4ml +0.9ml $CaCl_2$	3rd Test Group	Plant Extract	Froth observed in high amount but not sustained in duration period
Sample 4: plasma 0.8 ml + extract 0.8ml +1.2ml $CaCl_2$	4th Test Group	Plant Extract	Highly amount of froth observed and sustained and retained after duration period
Sample 5: plasma 1ml + extract 1ml +1.5ml $CaCl_2$	5th Test Group	Plant Extract	Highly amount of froth observed and sustained and retained after duration period.

In all the above experiment heating shock time is 1 min. and cooling shock time is 30 sec.

Table 2: Phytochemical screening of plant

Chemical Constituents	Chemical Test	Result
Alkaloids	Dragendorff reagent	Positive
Saponins	H_2O test	Positive
Flavonoids	Ammonia test	Negative
Tannins	$FeCl_3$	Negative
Phenols	10% NaOH solution	Positive
Carbohydrate	Molisch's test	Positive
Protein	Biuret test	Positive
Glycoside	Keller–Kiliani Test	Positive

Discussion

Coagulation is a process that occurs mainly due to the complex interaction of cellular and molecular components. Initially clotting involves common pathways both intrinsic and extrinsic pathway but lately it's found to be due to a balance between the procoagulants and anticoagulants. The present study signifies that sample no 3.4 and 5 definitely promised to show the coagulant activity under the heading of naturally occurring source of drug that may be less toxic in respect with synthetic coagulant drug which is subjected for the future experimentation.

Conclusion

The evaluation of Invitro coagulation activity of *Acalypha wikesiana* extract on blood plasma of poultry bird was experimented and the result was found to be positive.The coagulation activity of *Acalypha wikesiana* was not reported yet and this report was found to be the first investigation for PT test. Hence further identification and characterisation of active molecule responsible for the activity can be carried out in future.

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