

**NELUMBO NUCIFERA (LOTUS) LEAF EXTRACTS IN VITRO ANTICOAGULANT
ACTIVITY ON NORMAL HEALTHY BLOOD PLASMA****¹Dr. Nabeela M. Khan, ²Dr. Pankaj Surve and ³Dr. Aparna Ghotankar**¹M.D.(P.G.Scholar), Department of Dravya Guna C.S.M.S.S Ayurvedic College, Aurangabad Maharashtra India.²M.D. Asso. Proffesor, Department of Dravya Guna C.S.M.S.S Ayurvedic College, Aurangabad Maharashtra India.³M.D. Phd. HOD, Department of Dravya Guna C.S.M.S.S Ayurvedic College, Aurangabad Maharashtra India.***Corresponding Author: Dr. Nabeela M. Khan**

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ABSTRACT

Formation of clots within the walls of damaged blood vessels is called haemostasis. To maintain intravascular blood in a fluid state and to prevent abnormal bleeding, in this study we aimed to evaluate the possible anticoagulant effect of leaf extracts of *Nelumbo nucifera*. **Materials and Methods:** For *in vitro* prothrombin time (PT) The aqueous, methanol, acetone, and ethyl acetate extracts of *N. nucifera* at different concentrations were tested. In different concentrations 0.5, 0.25, 0.125, and 0.062 g/ml. The *invitro* anticoagulant effect of different extracts of *N nucifera* were examined using plasma, from blood samples of normal individuals by measuring PT. Ethylenediaminetetraacetic acid (EDTA) and saline in distilled water were used as a negative and positive control, respectively. The extract plasma was subjected to anticoagulation activity and was compared with EDTA-plasma and saline plasma. **Results:** The methanol leaf extract of *N. nucifera* was found to inhibit coagulation process in 60 min: 3 s in 0.5 g/ml. The time taken for clotting at the concentration of 0.5 g/methanol leaf extract showed the moderate effect of 10 min:20 s with respect to control while ethyl acetate extract showed the least effect of 8 min: 23 s compared to control. Overall, the concentration of 0.5 g/ml of leaf extract showed a maximum effect in all the tested extracts with respect to other concentrations 0.25, 0.125, and 0.062 g/ml. Thus, *N. nucifera* methanol, ethanol, and ethyl acetate leaf extract in different concentrations inhibits clot formation and increases the PT in a dose-dependent manner. Due to the presence of certain phytochemical constituents in the crude extract prolonged prothrombin activity could be observed. Phytochemical analysis revealed the presence of tannins, flavonoids and steroid compounds in the crude extract and further, the active principles could be isolated and evaluated for clinical or physiological purposes. **Conclusion:** *In vitro*, anticoagulant activity studies results demonstrated that leaf extract *N. nucifera* possesses pharmacologically active anticoagulant components which could be helpful in preventing blood clotting disorders. Thus, as a supplementary source of natural anticoagulant in future *N nucifera* leaf could be used.

KEYWORDS: Hemostasis, phytochemical, prothrombin time, clotting factors, calcium.**INTRODUCTION**

Between coagulation and anticoagulants, haemostasis is an interaction process that retains the blood within the injured vascular system during periods of injury.^[1] Hemostasis 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 in the development of the

fibrin clot protein cofactors, membrane phospholipids surface and calcium ions plays an active role.^[1] Hypertension, cerebral hemorrhage, coronary thrombosis, arteriosclerosis, and congestive heart failure are cardiovascular disorders 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 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.^[2-4] 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.^[5]

In India, the use of plants for medicinal purposes for the prevention and treatment of various ailments is one of the most ancient traditional remedial forms of primary health care.^[6,7] Anticoagulant drugs show serious side effects and also expensive besides the pharmaceutical properties. Hence, therefore, it is necessary to explore alternative anticoagulants. Since the plants are the safer source of medicine, this study is a preliminary attempt to investigate the *in vitro* anticoagulant activities of *Nelumbo nucifera* leaf extracts using standard experimental models in the blood samples of normal individuals.

MATERIALS AND METHODS

Collection of Plant Materials

The leaves of *N. nucifera* were collected from botanical garden C.S.M.S.S ayurvedic college Aurangabad. The *N. nucifera* species were voucher specimen has been identified by the Department of Botany, Dr Babasaheb Ambedkar Marathwada University Aurangabad. The leaves of *N. nucifera* which float on water and washed to solid debris and dust particles were removed then cut into small pieces, air dried at room temperature.

Extraction of Plant

N. nucifera leaves were air dried at room temperature and crushed into powder with an electric grinder. This plant material was soaked by suspending 10 g of powdered *N. nucifera* leaf in 100 ml of ethyl acetate, ethanol, and methanol with occasional stirring for 24 h. After 24 h, the suspension was filtered 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. For further analysis they were preserved into sterile bottle kept in a refrigerator until used.

Phytochemicals

For the detection of different phytochemical constituents present in extract Each extract (ethyl acetate, ethanol, and methanol) of the leaves of *N. nucifera* was subjected to a preliminary phytochemical analysis^[8,9] using the different phytochemical tests. Different crude extracts were 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.

Steroids

Salkowski test

To extract, 2 ml of chloroform, 10 drops of acetic anhydride, and 2 drops of concentrated sulfuric acid were added. The change of color from red to blue and finally bluish indicates the presence of steroids.

Determination of PT

Collection of blood and separation of plasma

From healthy volunteers (having no medicine consumption history) about 10 ml of blood was drawn by making vein puncture. To the 9 µl volume of blood, 1 µl volume of 3.8% trisodium citrate solution was 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 and to obtain pure platelet plasma (PPP). PPP was used for PT test.

Plasma sample was divided into four groups.

- Group I: Negative control group 0.2 ml plasma, 0.1 ml of 0.9% saline water and 0.3 ml of 25 ml CaCl₂
- Group II: Positive control group 0.2 ml of plasma + 0.1 ml of 50 mg/ml of EDTA + 0.3 ml of CaCl₂ (0.5 g/ml).
- Group III: 0.2 ml of plasma + 0.062 ml of plant extract + 0.3 ml of CaCl₂
- Group IV: 0.2 ml of plasma + 0.125 g/ml of plant extract + 0.3 ml of CaCl₂
- Group V: 0.2 ml of plasma + 0.25 g/ml of plant

- extract + 0.3 ml of CaCl₂
- Group VI: 0.2 ml of plasma + 0.1 g/ml of plant extract + 0.3 ml of CaCl₂.

At an angle of 45° for every 30 s all the tubes are tilted to measure the clotting time. Stop watch was used for measuring the clot formation. This time is called as PT. Tests were repeated for 3 times and the average time was calculated.^[10]

Tested Extracts

For their anticoagulant activity, ethyl acetate extract, ethanol extract, and methanol extract of leaves of *N. nucifera* were investigated. Each extract was prepared in the concentrations of 0.062, 0.125, 0.25, and 0.5 g/ml with dimethyl sulfoxide.

RESULTS

Phytochemical

Extracts of three from *N. nucifera* leaf increased the clot time in relative to the control. The results are summarized in Table 2. The methanol extract of *N. nucifera* at concentrations of 0.5, 0.25, 0.125, and 0.062 g/ml showed an increase in PT of 60 min:3 s, 31 min:36 s, 11 min:30 s, and 1 min:20 s, respectively. The ethanol extract also produced a prolonged time duration to clot as the concentration increases 0.5 g/ml (10 min:20 s), 0.25 g/ml (7 min:2 s), 0.125 g/ml (5 min:40 s), and 0.062 g/ml (2 min:7 s). While the ethyl acetate produced an inhibitory activity at least level compared to control with the following time intervals of 8 min:23 s, 6 min:49 s, 4 min:5 s, and 1 min:10 s at the concentration of 0.5 g/ml, 0.25, 0.125, and 0.062 g/ml, respectively. From the data, it was found that methanol extract had taken a greater prolonged duration to clot formation compared to ethanol and ethyl acetate. The clot formation increases as the concentration increases from 0.062 to 0.5 g/ml.

DISCUSSION

A coagulation occurred mainly due to the complex interaction of cellular and molecular components.^[11] Clotting involves common pathway of both intrinsic and extrinsic pathways, but lately, it found to be due to a balance between the procoagulants and anticoagulants.^[11] A similar study was reported by Ikese *et al.*, 2015,^[12] whose findings reported that aqueous extract of *Tridax procumbens* reduced the clotting time. The petroleum ether extract of *T. procumbens* also showed a significant reduction in clotting time as similar to our reports.^[13] The results of this study are also similar to those results obtained by Kale *et al.*, 2008^[14] in which the ethanolic leaf extract of *T. procumbens* had specifically reduced the clotting time. A reduced clotting time of plant extract was also shown by Sowmya *et al.*, 2015^[15] as like our clotting time. Taj *et al.*, 2011,^[16] noticed a correlation between concentration of aqueous extract of *Allium cepa* and the time needed to inhibit the clot formation with a prolonged PT. That is, as concentration increases, the aqueous extract of red onion strongly inhibited the

coagulation process and also increased the PT. In this investigation, an similar correlation exists between concentration of extracts and the time taken to inhibit the clot formation. This may be attributed due to the presence of several phytochemical compounds that have been noted in the extracts of leaves.

Manicam *et al.*, 2010,^[17] studied the anticoagulant activity of aqueous leaf extract of *Melastoma malabathricum* Linn. The study revealed that the aqueous leaf extracts prolonged the coagulation time as similar to the result obtained. Many researchers also studied the anticoagulant property of some plant extracts such as *Sutherlandia frutescens* leaf extract, *Gloriosa superba*, *Zantedeschia aethiopica* leaf extract, and *Leonotis leonurus* root extract^[18] and their study revealed to have a prolonged coagulation time, as same to our present coagulation time. Further studies are desired to assess its effect and to ascertain the mode of action.

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