Research Article **www.ijcps.com**

International Journal of
Chemical and Pharmaceutical Sciences
2019, Mar., Vol. 10 (1)

ISSN: 0976-9390
IJCPS

Phytochemical screening and evaluation of anti coagulant activity of methanolic extract of flowers of *Nerium oleander Linn.*

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Received: 12th Dec 2018, Revised and Accepted: 16th Dec 2018

ABSTRACT

Purpose: This article mainly deals about the anti coagulant activity of Methanolic extract of flowers of *Nerium oleander linn.*

Process: Hemostasis is the process of formation of clots within the walls of damaged blood vessels. To prevent abnormal bleeding and to maintain intravascular blood in a fluid state, in this study we aimed to evaluate the possible anticoagulant effect of flower extracts of *Nerium oleander linn.*

Method: The Methanolic extract was tested for the following chemical constituents like Proteins,Carbohydrates, Aminoacids, Alkaloids, Glycosides, terpenoids, Steroids, Saponins and Phenols by using different types of chemical tests and The anti coagulant activity of flower extract was studied time taken for PT elongation by method using blood plasma.

Results: The present work deals with the study of flowers of *Nerium oleander Linn* for their photochemical screening and anticoagulant activity.

Conclusion: The anticoagulant activity of Nerium oleander methanolic flower extract was not yet reported and this report was found to be the first investigation for PT.

Keywords: Nerium oleander linn, Anti coagulant activity, Phytoconstituents, Methanolic extract, Haemostasis.

1. INTRODUCTION

Hemostasis is an interaction process between coagulation and anticoagulants that retains the blood within the injured vascular system during periods of injury. 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. Protein cofactors, membrane phospholipids surfaces and calcium ions play an active role in the development of the fibrin clot. Cardiovascular disorders include hypertension, cerebral hemorrhage, 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 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.

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.[4-7] Besides, the pharmaceutical properties anticoagulant drugs show serious side effects and also expensive. 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 *Nerium oleander linn* flower extracts using standard experimental models in the blood samples of normal individuals.

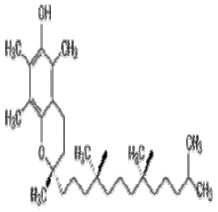
2. MATERIALS AND METHODS

2.1. Collection of Plant Materials

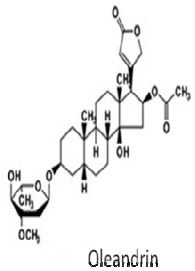
The flowers of *Nerium oleander linn* were collected from Aswani Medicinal garden, Hindu college of pharmacy, Amaravathi Road, Guntur, Andhra Pradesh, India. The *Nerium oleander linn* species were voucher specimen has been identified by the P. Satyanarayana Raju, Department of Botany and Microbilogy, Acharya Nagarjuna University, Guntur, Andhra Pradesh, India.. The Fresh flowers of *Nerium oleander linn* were allowed to macerate on Methanol for about 2 weeks.

2.2. Extraction of Plant

Fresh flowers were collected and soaked in Methanol for 48 hrs. The extract was collected by using Muclin cloth and concentrated by using Rotavapourizer at temperature 22^{0}C. The residue is stored for further use.



α-tocopherol



Oleandrin

Flowers of Nerium Oleander Linn

Nerium Oleander linn Shrub

Figure - 1: Flowers of *Nerium oleander linn.*

2.2.1. Phytochemicals

The Methanolic extract was tested for the following chemical constituents like Proteins,Carbohydrates, Aminoacids, Alkaloids, Glycosides, terpenoids, Steroids, Saponins and Phenols by using different types of chemical tests.

2.2.1.1. 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.

2.2.2. 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.

2.2.3. 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.

2.2.4. 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.

2.2.5. 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.

3. PROCEDURE FOR DETERMINATION OF PT

3.1. *Collection of blood and separation of plasma*

About 10 ml of blood was drawn from healthy volunteers (having no medicine consumption history) by making vein puncture. 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 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 $CaCl_2$

Group II: Positive control group 0.2 ml of plasma + 0.1 ml of 50 mg/ml of EDTA + 0.3 ml of $CaCl_2$ (0.5 g/ml)

Group III: 0.2 ml of plasma + 50 µg/ml of plant extract + 0.3 ml of $CaCl_2$

Group IV: 0.2 ml of plasma + 25 µg/ml of plant extract + 0.3 ml of $CaCl_2$

Group V: 0.2 ml of plasma +100 µg/ml of plant extract + 0.3 ml of $CaCl_2$.

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

3.2. Tested Extract

Methanol extract of flowers of *Nerium oleander linn* were investigated for their anticoagulant activity. The extract was prepared in the concentrations of 25, 50, 100 µg/ml with Methanol.

4. RESULT AND DISCUSSION

The present work deals with the study of flowers of *Nerium oleander Linn* for their photochemical screening and anticoagulant activity. Physicochemical studies were carried out for methanolic extract by following qualitative tests. The results are tabulated in Table No.3. Methanolic extract contains alkaloids along with other polar constituents like Glycosides, Steroids, Saponins, Flavonoids and Phenols. In Table No.1"+" sign indicating positive result and "-"sign indicating negative result.

The anti coagulant activity of flower extract was studied time taken for PT elongation by method using blood plasma. The result showed that the Methanolic extract of flowers of Nerium oleander has excellent Anti-coagulant activity.

The Methanolic extract of *Nerium oleander* linn showed a significant reduction in clotting time as similar to standards, inhibited the clot formation with a prolonged PT. It is also noticed that is, as concentration increases, the methanolic extract of Nerium oleander strongly inhibited the coagulation process and also increased the PT.

4.1. SPECTRAL DATA

The IR – spectrum (**Fig.No- 3**) of ethanolic extract was observed by using FT-IR, in which ethanol is used as blank. The results are tabulated in Table.no-3.

Table No - 1: Results of phytochemical screening of Methanolic extract of flowers.

Phytoconstituents	Methanolic extract
Steroids	+
Flavonoids	+
Glycosides	+
Phenols	--
Saponins	+
Alkaloids	+
Amino acids	+
Carbohydrates	+
Proteins	+

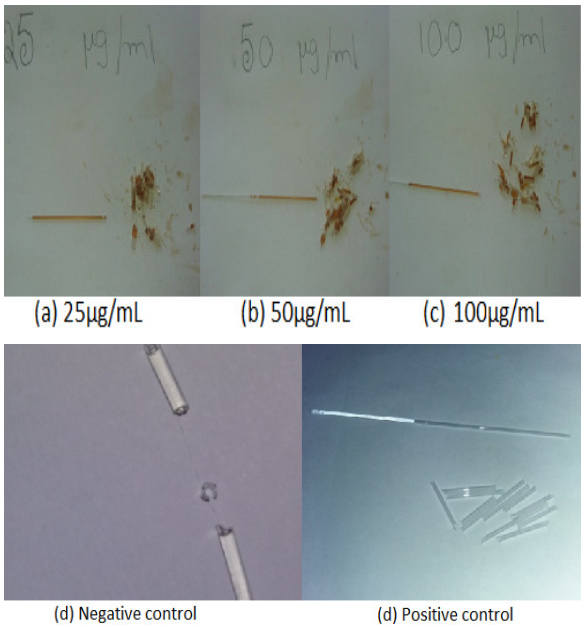


Figure – 2: Anticoagulant activity of methanolic extract of flowers of *Nerium oleander linn.*

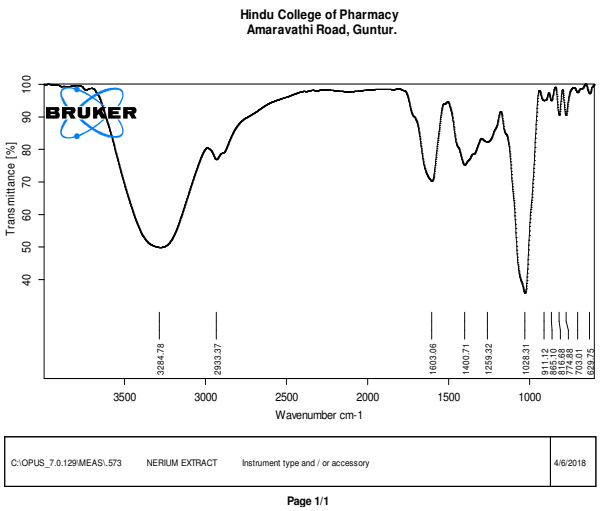


Figure - 3: IR Spectrum of Methanolic extract of flowers of NOL.

Table No - 2: Report on coagulation time

Plant extracts (*Nerium oleander linn*)	Methanol	Group I (negative control)	Group II (positive control)	Group III (25 µg/ml)	Group IV (50 µg/ml)	Group V (100 µg/ml)
Time taken for coagulation	1.27 sec	1.26 sec	NA	30 min 15 sec	45 min 32 sec	65 min 12 sec

Table No - 3: IR Bands of Methanolic extract of flowers of NOL

Functional group	IR- Range	IR-band
Amine (N-H)	3300-3500	3284.78
Alkane (C-H)	2850-2970	2933.37
Aromatic (C=C)	1600-1680	1603.06
Amines(C-N)	1020-1360	1259.32

5. CONCLUSION

The anticoagulant activity of Nerium oleander methanolic flower extract was not yet reported and this report was found to be the first investigation for PT which gave a positive response. Hence, further identification and characterization of active molecules responsible for activity was to be found out in future in detail.

ACKNOWLEDGEMENT

I, hereby, Ch. Naga Manasa, want to acknowledge N.Vijaya Lakshmi M.pharm (Ph.D) Department of Pharmaceutical Chemistry and Avinash, tahseen, Rosemary, Hindu college of Pharmarcy, Achaya Nagarjuna University, Amaravathi road,Guntur for their assistance in this work.

CONFLICT OF INTEREST

We, The authors hereby declare that we have no conflicts of interest

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