



## Evaluation of *Clinacanthus nutans* leaves extract on Prothrombin Time and Activated Partial Prothrombin Time test

Nur Farahhim Abd Rahman, Azlina Muhsin, Norhaida Che Azmi\*

Universiti Kuala Lumpur, Institute of Medical Science Technology (UniKL MESTECH), 43000 Kajang, Selangor, Malaysia

### Article History:

Received on: 19.08.2019

Revised on: 05.11.2019

Accepted on: 20.11.2019

### Keywords:

Clinacanthus nutans,  
PT,  
aPTT

### ABSTRACT

Limitations in existing anticoagulants have prompted a search for the natural origin of anticoagulants. Since there is an increasing need to source for pharmacological and medicinal materials from a plant source, thus an exploratory effort towards identifying and characterizing new anticoagulants from plants is worthwhile. *Clinacanthus nutans* (*C. nutans*) commonly known as Sabah snake grass belongs to Acanthaceae family was chosen since it has therapeutic and medicinal benefits. This plant possessed anti-oxidant, anti-microbial, anti-inflammatory, and anti-diabetic activities. This study was carried out to identify the anticoagulant activities of *C. nutans* leaves in aqueous and methanol extracts at different concentrations based on prothrombin time (PT) and activated partial thromboplastin time (aPTT). The dried leaves of *C. nutans* were ground into a fine powder and extracted using aqueous and methanol. Anticoagulant assays of PT and aPTT were done on three different concentrations of *C. nutans* aqueous and methanol extracts (10 mg/mL, 20 mg/mL, and 30 mg/mL) and control group respectively. PT and aPTT of plasma samples were prolonged in both types of extracts ( $p < 0.05$ ). This study highlights that the anticoagulant activity of aqueous and methanol extracts of *C. nutans* affects the intrinsic and extrinsic pathway, possibly due to the inhibitory action of the clotting factors. These results suggested that aqueous and methanol extracts of *C. nutans* exhibited anticoagulant activity at certain concentrations.



### \*Corresponding Author

Name: Norhaida Che Azmi

Phone: +60 17 437 1138

Email: [norhaida@unikl.edu.my](mailto:norhaida@unikl.edu.my)

ISSN: 0975-7538

DOI: <https://doi.org/10.26452/ijrps.v11i1.1813>

Production and Hosted by

IJRPS | [www.ijrps.com](http://www.ijrps.com)

© 2020 | All rights reserved.

### INTRODUCTION

Thrombosis or formation of blood clotting is commonly related to disruption and blockage of blood flow. The impact of this blood clotting contributes

to the development of many diseases such as stroke, atherosclerosis, and heart attack. Blood coagulation is one of the components of haemostasis (Omodamiro and Ikekamma, 2016). The indicators of blood coagulation are PT and aPTT (Cordier et al., 2012). PT is used to evaluate the coagulation factors V, VII, and X in the extrinsic pathway, while aPTT is used to evaluate the coagulation factors such as VIII, IX, XI, XII, and prekallikrein in the intrinsic coagulation pathway of the coagulation cascade. Current available treatment, such as heparin and warfarin, acts as an anticoagulant in order to treat blood clotting disease (Harther et al., 2015). However, these drugs can cause adverse effects such as bleeding, especially in the elderly, patient with antiplatelet therapy, and patient with other diseases such as cancer, renal, and liver problem (Ageno et al., 2012). Meanwhile, to overcome

these problems, plants can become natural remedies and alternative sources to treat blood coagulation (Harter *et al.*, 2015). Thus, many studies have been explored on the anticoagulant activity of various medicinal plants with the hope to find new and more effective anticoagulant agents (Félix-Silva *et al.*, 2014; Cordier and Steenkamp, 2012). In this study, *Clinacanthus nutans* (hereafter referred to as *C. nutans*) belongs to Acanthaceae family was chosen since it has potential therapeutic and medicinal benefits. According to Alam *et al.* (2016), *C. nutans* is known as 'Sabah snake grass' or 'belalai gajah' in Malay and 'Phaya Yo' in Thai. In Thailand, *C. nutans* is useful in treating skin rashes, insect and snake biting, herpes simplex virus (HSV) and varicella-zoster virus (VZV) lesions (Sakdarat *et al.*, 2009). The phytochemical studies demonstrated that *C. nutans* plant has rich sources of active compounds such as flavonoids, glycosides, glycolipids, cerebrosides, and monoacylmonogalatosyl glycerol that serve as anti-inflammatory, antiviral, antioxidant and anti-diabetic activities (Alam *et al.*, 2016). As of today, there is no study reported on *C. nutans* anticoagulant activities. Therefore, this study was conducted to evaluate the effect of aqueous and methanol extraction of *C. nutans* leaves extract on prothrombin time (PT) and activated partial thromboplastin time (aPTT) test on platelet-poor plasma samples.

## MATERIALS AND METHODS

### Preparation of plant material

*C. nutans* leaves were collected and identified from Alor Setar, Kedah, and used freshly for extraction. The leaves were washed with fresh water and dried under shade at room temperature. The leaves were ground into a powder and stored in a dry, dark place prior to anticoagulant testing.

### Preparation of plant extracts

#### *C. nutans* aqueous extraction

The aqueous extract of *C. nutans* was prepared according to the method by Intan *et al.* (2015) with some modification. 800 g of *C. nutans* leaves were cut into small pieces and let to be dried under the sunshade. The complete dried of the leaves pieces were ground into a fine powder. 100 g of the fine powder was weighed on a balance scale (AY220, Shimadzu, Japan) and soaked in 1 L of distilled water (1:10 w/v) at room temperature. Then the mixture was stirred by using magnetic stirrer (IKA C-Mag HS7) in a conical flask and left overnight. After that, the mixture was filtered by using Whatman No.1 filter paper (Whatman, England), and the super-

natant was collected. In order to produce a thick syrupy mass crude extract, rotary evaporator (R-215, Butchi rotavaporator, Switzerland) was used to evaporate the filtrates under vacuum reduced pressure at 60°C. The crude extract was kept at -20°C in Scott's bottle prior to further analysis

#### *C. nutans* methanol extraction

The methanol extract of *Clinacanthus nutans* was prepared according to the method by (Rahim *et al.*, 2016) with some modification. 500 g of *C. nutans* leaves were cut into small pieces and let to be dried at room temperature. The complete dried of the leaves pieces were ground into a fine powder. 100 g of the fine powder was weighed on a balance scale (AY220, Shimadzu, Japan). The fine powder was soaked in 1 L of 90% methanol at room temperature. Then the mixture was stirred by using magnetic stirrer (IKA C-Mag HS7) in a conical flask and left overnight. After that, the mixture was filtered by using Whatman No.1 filter paper (Whatman, England), and the supernatant was collected. In order to produce a thick syrupy mass crude extract, rotary evaporator (R-215, Butchi rotavaporator, Switzerland) was used to evaporate the filtrates under vacuum reduced pressure at 60°C. The crude extract was kept at -20°C in Scott's bottle prior to further analysis.

#### *C. nutans* solution preparation of Aqueous and Methanol Extraction

The stock of aqueous and methanol extraction of *C. nutans* were prepared and diluted with sterile distilled water and normal saline, respectively, to a final concentration of 10, 20, and 30mg/ml.

#### Platelet Poor Plasma Preparation

The platelet-poor plasma (PPP) was obtained by purchasing the normal control plasma (Diagnostica Stago, France). The samples of normal PPP were stored at -20°C prior to further analysis. Different concentrations of 10, 20 and 30 mg/mL of *C. nutans* extracts were used to mix with the plasma and incubated in water bath (Mettler, USA) at 37°C for 7 minutes in order to measure the prothrombin time (PT) and activated partial thromboplastin time (aPTT) within 3 hours of sample preparation.

#### In vitro Anticoagulant Activity

The sample of normal platelet-poor plasma (PPP) (Diagnostica Stago, France) was divided control group and experimental group. For the negative control group of normal prothrombin time (PT) and normal activated partial thromboplastin time (aPTT), there is no anticoagulant such as heparin or extracts was added, whereas, for positive control,

the tube contained heparin. For the experimental group, the PPP were spiked with different concentrations of *C. nutans* (10, 20, and 30 mg/mL). The sample was tested in a water bath at 37°C.

The PT test was carried out using STA-Neoplastine® CI Plus (Diagnostica Stago, France) that consists of calcium thromboplastin while the aPTT test using thromboplastin reagent C.K. Prest® 2 (Diagnostica Stago, France) containing a standardized amount of cephalin and silica. Both reagents were reconstituted by adding activator and was mixed gently. Coagulation assay was then performed manually according to the manufacturer's instruction.

### Statistical Analysis

The data of PT and aPTT are shown as mean  $\pm$  S.E.M. ( $n=3$ ), and analysis was performed using a one-way analysis of variance (ANOVA), followed by post hoc Dunnett's multiple range tests to determine the means of significance from the control (SPSS version 20.0). The student t-test was used for independent samples to determine the significant difference of clotting time among different extraction methods. The results with a p-value less than or equal to 0.05 were considered significant (\*),  $p \leq 0.01$  is highly significant (\*\*), and  $p \leq 0.001$  is very highly significant (\*\*\*).

## RESULTS AND DISCUSSION

### PT and aPTT Clotting time of *C. nutans* extraction

Table 1 showed the PT (in seconds) for aqueous and methanolic extract at three different concentrations (10, 20 and 30 mg/mL) were significantly higher in comparison to positive control ( $29.0 \pm 0.0$ ) and negative control ( $12.0 \pm 0.0$ ) with  $p < 0.001$ . While the aPTT (in seconds) for aqueous and methanol extraction at three different concentrations were also significantly higher in comparison to a positive control ( $34.0 \pm 0.0$ ) and negative control ( $30.0 \pm 0.0$ ) with  $p < 0.001$ .

Table 1 Depicts the aqueous and methanol extract of PT and aPTT at three different concentrations of 10, 20, and 30 mg/mL, respectively. By using the ANOVA and post hoc analysis with Dunnett multiple comparisons test, both aqueous and methanol extract were significantly prolonged ( $p < 0.001$ ) the PT and APTT in a dose-dependent manner in comparison to the normal control plasma.

### Prothrombin Time Between Aqueous And Methanolic Extract

Figure 1 shows the comparison between aqueous and methanol extract of *C. nutans* on PT (in seconds) at three different concentrations (10, 20, and

30 mg/mL). There was no significant difference of *C. nutans* extract at 10 mg/mL with p-value 0.3. *C. nutans* methanolic extract at 20 mg/mL and 30 mg/mL showed a significant prolongation of PT compared to aqueous extract at similar concentrations with a p-value of 0.03 and p-value 0.02, respectively.

### Activated partial thromboplastin time of aqueous and methanolic extract

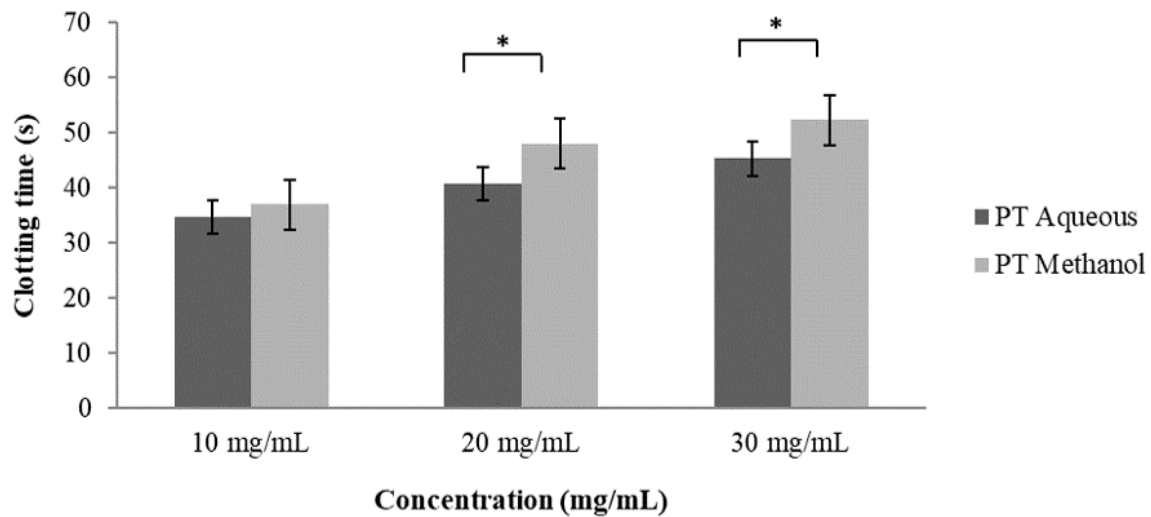
Figure 2 shows the comparison between aqueous and methanol extract of *C. nutans* on aPTT (in seconds) at three different concentrations. There was a significant prolonged aPTT of *C. nutans* methanolic extract in comparison with aqueous extract; where at 10 mg/mL p-value was 0.001. For concentration 20 mg/mL the p-value was 0.008. At concentration 30 mg/mL the p-value was 0.03.

In the present study, the aqueous and methanol extract of *C. nutans* leaves exhibited a prolonged PT and aPTT at concentrations of 10, 20, and 30 mg/ml. This finding could be associated with interference in the one or more clotting factors of intrinsic and extrinsic coagulation pathways, respectively (Hood and Eby, 2008; Adams and Bird, 2009; Cordier et al., 2012). The efficiency of the extraction and the anticoagulant activity obtained from the extracts were affected by the polarity of the solvents (Chew et al., 2011). The study also mentioned that solvents have characteristics such as low viscosity, low density, and high diffusivity allows them to diffuse easily into the pores of plant materials, thus causing the bioactive components to leach out. The extraction of plants depends on the type of solvent used in the extraction procedure. In addition, methanol has a lower boiling point, which is 64.7 °C compare to water that has a high boiling point, which is 100 °C (Chew et al., 2011). Methanol is a polar solvent that easily dissolved bioactive compounds in plants. Based on the results obtained, both aqueous and methanol solvents exhibited anticoagulant activities, but methanol exhibited a longer anticoagulation time than aqueous solvent. Thus, methanol is a better solvent to identify the anticoagulant activities of *C. nutans* compare to the aqueous solution.

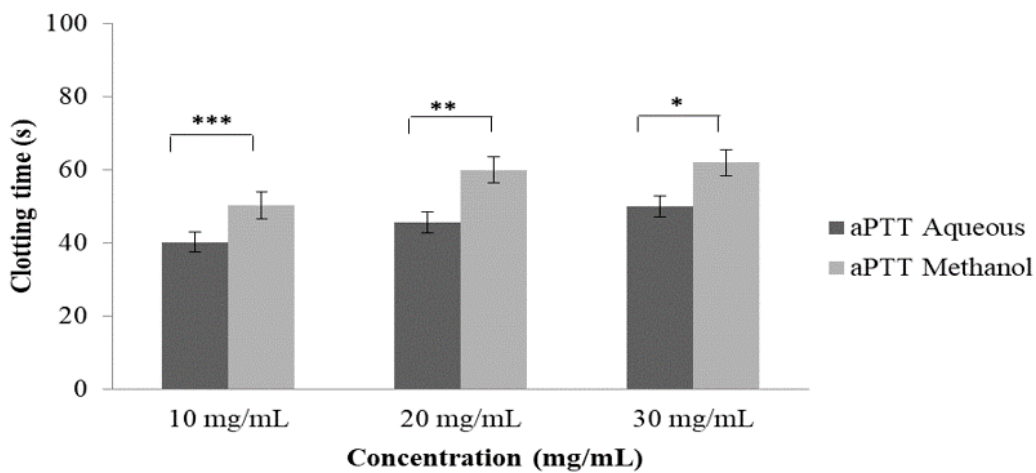
According to Khoo et al. (2014), bioactive compounds such as hexuronic acids, polysaccharides, and polyphenolics contribute to anticoagulant activities. Meanwhile, Kee et al. (2008) reported that tannins also displayed anticoagulant and antithrombotic effects. Besides, saponins, starch, alkaloids, and flavonoid also contribute to anticoagulant activities (Ughachukwu et al., 2012). Previous study by Tu et al. (2014) revealed that steroids, triterpenoids, cerebrosides, glycolglycero-

**Table 1: PT and aPTT of aqueous and methanol extraction of *C. nutans* at three different concentrations**

	PT	Aqueous aPTT	PT	Methanol aPTT
10 mg/mL	34.7 ± 2.1	40.3 ± 1.5	37.0 ± 3.0	50.3 ± 1.5
20 mg/mL	40.7 ± 1.2	45.7 ± 1.2	48.0 ± 3.6	60.0 ± 5.0
30 mg/mL	45.3 ± 1.5	50.0 ± 2.0	52.3 ± 2.5	62.0 ± 6.2
Positive control (heparin)	29.0 ± 0.0	34.0 ± 0.0	29.0 ± 0.0	34.0 ± 0.0
Negative control	12.0 ± 0.0	30.0 ± 0.0	12.0 ± 0.0	30.0 ± 0.0
p-value	<0.001***	<0.001***	<0.001***	<0.001***



**Figure 1: PT clotting time of plasma on methanol and aqueous extraction of *C. nutans*. Significance differences of PT test for both aqueous and methanol extraction at three different concentrations (10, 20, and 30 mg/mL) were determined by the Student t-test, whereby p<0.05 was considered significant**



**Figure 2: aPTT clotting time of plasma on methanol and aqueous extraction of *C. nutans*. Significance differences of aPTT test for both aqueous and methanol extraction at three different concentrations (10, 20 and 30 mg/mL) were determined by Student t-test where by p<0.05 was considered significant, \*p<0.05, \*\*p<0.01 and \*\*\*p<0.001**



lipids, glycerides, sulfur-containing glycosides were isolated from *C. nutans*, whereas Intan *et al.* (2015) mentioned that *C. nutans* contains phenolic, saponins, flavonoid and tannin which exhibited anticoagulant effects.

## CONCLUSIONS

The anticoagulant activities of *C. nutans* were shown in both aqueous and methanol extraction. In this study, the methanol extraction showed higher clotting time compared to aqueous extraction. The PT and aPTT for both extracts exceeded the reference range, thus indicates this plant has an effective anticoagulant effect. In conclusion, *C. nutans* may possess as alternative anticoagulants properties in the future. However, further works such as isolation of bioactive compounds that responsible for the anticoagulation activity need to be done to validate this observation.

## ACKNOWLEDGEMENT

The authors would like to express their gratitude to the Clinical and Biomedical Science Section, Universiti Kuala Lumpur, and FYP research grants for funding this project.

## REFERENCES

- Adams, R. L. C., Bird, R. J. 2009. Coagulation cascade and therapeutics update: Relevance to nephrology. Part 1: Overview of coagulation, thrombophilias, and history of anticoagulants. *Nephrology*, 14(5):462-470.
- Agno, W., Gallus, A. S., Wittkowsky, A., Crowther, M., Hylek, E. M., Palareti, G. 2012. Oral Anticoagulant Therapy- Antithrombotic therapy and prevention of thrombosis, 9th ed: American College of Chest Physicians evidence-based clinical practice guidelines. *Chest*, 141(2):44-88.
- Alam, A., Ferdosh, S., Ghafoor, K., Hakim, A., Juraimi, A. S., Khatib, A., Sarker, Z. I. 2016. Clinacanthus nutans : A review of the medicinal uses, pharmacology, and phytochemistry. *Asian Pacific Journal of Tropical Medicine*, 9(4):402-409.
- Chew, K. K., Khoo, M. Z., Ng, S. Y., Thoo, Y. Y., *et al.* 2011. Effect of ethanol concentration, extraction time, and extraction temperature on the recovery of phenolic compounds and antioxidant capacity of Orthosiphon stamineus extracts. *International Food Research Journal*, 18:571-578.
- Cordier, W., Cromarty, A. D., Botha, E., Steenkamp, V. 2012. Effects of selected South African plant extracts on haemolysis and coagulation. *Human & Experimental Toxicology*, 31(3):250-257.
- Cordier, W., Steenkamp, V. 2012. Herbal remedies affecting coagulation: A review. *Pharmaceutical Biology*, 50(4):443-452.
- Félix-Silva, J., Souza, T., Camara, R. B. B. G., Cabral, B., Silva-Júnior, A. A., Rebecchi, I. M. M., Fernandes-Pedrosa, M., De, F. 2014. In vitro anticoagulant and antioxidant activities of Jatropha gossypifolia L. (Euphorbiaceae) leaves aiming therapeutical applications. *BMC Complementary and Alternative Medicine*, 14(1):405-405.
- Harter, K., Levine, M., Henderson, S. 2015. Anticoagulation Drug Therapy: A Review. *Western Journal of Emergency Medicine*, 16(1):11-17.
- Hood, J. L., Eby, C. S. 2008. Evaluation of a Prolonged Prothrombin Time. *Clinical Chemistry*, 54(4):765-768.
- Intan, S. C. S., Mahiran, B., Kim, W. C., Siti, E. A., Hamid, R. F. M., Maznah, I. 2015. In vitro antioxidant, cytotoxic, and phytochemical studies of Clinacanthus nutans Lindau leaf extracts. *African Journal of Pharmacy and Pharmacology*, 9(34):861-874.
- Kee, N. L. A., Mnonopi, N., Davids, H., Naudé, R. J., Frost, C. L. 2008. Antithrombotic/anticoagulant and anticancer activities of selected medicinal plants from South Africa. *African Journal of Biotechnology*, 7(3):217-223.
- Khoo, L. T., Abas, F., Abdullah, J. O., Tohit, E. R. M., Hamid, M. 2014. Anticoagulant Activity of Polyphenolic-Polysaccharides Isolated from Melastoma malabathricum L. Evidence-Based Complementary and Alternative Medicine. pages 1-8.
- Omodamiro, O., Ikekamma, C. 2016. In vitro Study of Antioxidant and Anticoagulant Activities of Ethanol Extract of Pandanus tectorius Leaves. *International Blood Research & Reviews*, 5(1):1-11.
- Rahim, A., Zakaria, M. H., Sani, Z. A. M., Omar, M. H., *et al.* 2016. Methanolic Extract of Clinacanthus nutans Exerts Antinociceptive Activity via the Opioid/Nitric Oxide-Mediated, but cGMP-Independent. *Pathways. Evidence-Based Complementary and Alternative Medicine*, pages 1-11.
- Sakdarat, S., Shuypprom, A., Pientong, C., Ekalaksananan, T., Thongchai, S. 2009. Bioactive constituents from the leaves of Clinacanthus nutans Lindau. *Bioorganic & Medicinal Chemistry*, 17(5):1857-1860.
- Tu, S. F., Liu, R., Cheng, Y. B., Hsu, Y. M., Du, Y. C., El-Shazly, M., Chang, F. R. 2014. Chemical Constituents and Bioactivities of Clinacanthus nutans Aerial Parts. *Molecules*, 19(12):20382-20390.

Ughachukwu, P. O., Ezenyeaku, C. C. T., Ogamba, J. O., Onwurah, W. O., Ifediata, Ughachukwu, P. O. 2012. Comparative studies on anticoagulant properties of aqueous leaf extract of *Euphorbia heterophylla* and ethylene diamine tetra acetic acid (EDTA). *Journal of Medicinal Plants Research*, 6(17):3388-3392.