*Original Research Article*

# DETERMINATION OF IN VITRO ANTICOAGULANT EFFECT OF ACACIA NILOTICA EXTRACT ON NORMAL HUMAN BLOOD SAMPLES



**ABSTRACT**

Herbal medicines are attractive as therapeutic agents for treating different disease either alone or in combination with chemical drugs. Among these, Acacia nilotica have activities such as antimalarial, antioxidant, antifungal and many other activities. This study aimed to determine the effect of Acacia nilotica on coagulation parameters including whole blood clotting time, clot lysis and clot retraction with different concentrations of aqueous extract of Acacia nilotica. The study was conducted in Al-Zaiem Al–Azhari University among 20 apparently healthy individuals during the period between August to September 2021. Blood samples were collected from all participants without anticoagulant. The serum was mixed with different concentrations of Acacia nilotica aqueous extract (50%, 75% and 100%) then coagulation parameters were measured. The results revealed that; the whole blood clotting time was significantly increased after the addition of the extract in relation to the concentration (*P* value for 75% = 0.012, and 100% = 0.000). Moreover; clot retraction test results were significantly affected by the addition of various concentrations of the extract (*P* values < 0.05), while no significant effect was observed in clot lysis test results after the addition of various concentrations of A.nilotica extract. The study concluded that the aqueous extract of A. nilotica have an in vitro anticoagulation effect on human plasma. This effect is related to the concentration of the extract.

***Keywords:*** *Acacia nilotica, clot lysis, clotting time, retraction.*

## 1. INTRODUCTION

Herbal medicine (also herbalism) is the study of pharmacognosy and the use of medicinal plants, which are a basis of traditional medicine [1].There is limited scientific evidence for the safety and efficacy of plants used in 21st

century herbalism, which generally does not provide standards for purity or dosage [2] .The scope of herbal medicine commonly includes fungal and bee products, as well as minerals, shells and certain animal parts. Herbal medicine is also called phytomedicine or phototherapy [3]. Medicinal plants have been used as source of medicine in virtually all cultures. During the last decade, the use of traditional medicine (TM) has expanded globally and continues to gain more popularity. It has continued to be used not only for primary health care of the poor in developing countries, but also in countries where conventional medicine is predominant in national health care system [4]. Almost one fourth of pharmaceutical drugs are derived from plant. Herbal medicine is used to treat many conditions, such as asthma, eczema, premenstrual syndrome, rheumatoid arthritis, migraine, menopausal symptoms, chronic fatigue, irritable bowel syndrome and cancer [5].

Acacia nilotica is a single stemmed plant, grows to 15-18 m in height and 2-3 m in diameter. Pods and seeds: pods are 7-15 cm long green and to Mentos (when immature) or greenish black (when mature). Seeds are 8-12 per pods, compressed, ovoid, dark brown shining with hard taste [6]. Acacia species contains secondary metabolites including amines and alkaloids such as cyanogenic glycosides, cyclitols, fatty acids and seed oils, fluoroacetate, gums, no protein amino acids, trepans, flavonoids and condensed tannins [7].

In a previous study which was carried on the extract of acacia showed that it has anti-thrombotic activity through its capacity to block platelet aggregation mediated by platelet agonist [8].Other study the effect on aqueous pod extract of acacia on platelets , white blood cells and clotting time in albino rats; the result showed the aqueous extract of A.nilotica in treated rats found significantly ($p<0.05$) increase WBcs count when compared to control group [9].

Thrombosis is a common pathology underlying ischemic heart disease, and venous thromboembolism that is responsible of mortality for about one out of four deaths worldwide. Anticoagulant therapies are used for the treatment and prevention of such events, but their efficiency in the protection against hyper coagulable diseases is variable and at the other hand there is a risk of drug side effects. This study aimed to evaluate the anticoagulant effect of A.nilotica aqueous extract on normal blood samples.

## 2. MATERIAL AND METHODS

This experimental study was conducted in Alzaiem Al-Azhari University during period from August to October 2021. The study was conducted on normal blood samples from apparently healthy individuals. This study received ethical clearance from the Research Board at the faculty laboratories sciences university of Al-zaiem Al- zhari. Informed consent was taken from each subject before blood collection

Extraction was carried out according to method described by UMAN,B et al [9]. Acacia nilotica was obtained from the market in Khartoum state. The pods of acacia nilotica were air dried at room temperature for three weeks .The crushing of the pods was done in the laboratory using pestle and mortar. 200g of powdered pod was weight and mixed with distil water in conical flask. The mixture was shaken and boiled for 1 hour before it was filtered. The filtrate was then concentrated in a rotatory evaporator and stored at 4°C until used.

### 2.1. IN VITRO WHOLE BLOOD CLOTTING TIME:

100 ul of extract were added to three test tubes . Additional one test tube was included as control. Venous blood (5.0 ml) was collected, 1.5 ml was added in each tube; incubated in water bath at 37 ºC.

## 2.2. IN VITRO CLOT RETRACTION TIME:

100 ul of extract were added to three test tubes Additional one test tube was included as control. One ml of blood was added to each tube.

The tubes were incubated in water-bath at 37 ºC and time monitored for clot retraction.

## 2.3. IN VITRO CLOT LYSIS TEST:

Whole blood sample was collected from each participant. Then incubated at 37ºC for 45 minutes, then serum was discharged and the weight of the clot was measured. 100 microliter of extract was added, then the clot weight was re-measured the difference between the two weights was calculated. Weight of clotted blood (ΔW) was taken by subtracting the pre-weight (W1)

From the weight of clot containing tube (W2) as, ΔW = W2-W1. The equation for calculating weight of clot is given below:

Clot weight = weight of tube containing clot - weight of empty tube.

- 100 µL of extract added to the clot containing tubes for test.
- 100 µL of D.W added to the clot containing tube for the control tube.
- Nothing is added to the clot containing tube for normal test.

All the tubes were incubated at 37°C for 90 mins and weighed again for getting the weight variation among the pre weight and final weight for the determination of clot lysis.

Descriptive statistics were used to summarize data. Data were expressed as mean and standard deviation (S.D). Comparisons of means were performed by Analysis of variance (ANOVA) and chi square tests. For all tests, P values of less than 0.05 were considered statistically significant. All data analysis were conducted by SPSS (Statistical Package for Social Sciences) version 16.0.

## 3. RESULTS

In this study, for all concentrations of A.nilotica (50% ,75% ,100% ) there was significant variation in clot retraction test (*P* value <0.05) (Table 2).

In contrast, for concentration of A. nilotica (75%, 100%), there was significant variation in whole blood clotting time when compared with D.W (*P* values = 0.012 and 0.000 for 75% and 100% respectively).

Further, for all concentrations of Acacia nilotica (50%, 75%, and 100%), there was no significant variation in clot lysis test when compared with D.W (*P* value > 0.05).

**Table (1): Clotting time and clot lysis tests results**

| | N | Minimum | Maximum | Mean | Std. Deviation |
|---|---|---|---|---|---|
| | | | **Clotting Time (minutes)** | | |
| D.W | 20 | 6.09 | 9.44 | 7.3 | 0.6 |
| 50 % | 20 | 6.10 | 22.15 | 10.2 | 4.3 |

| | | | | | |
|---|---|---|---|---|---|
| 75 % | 20 | 6.00 | 52.00 | 16.5 | 11.8 |
| 100 % | 20 | 7.21 | 56.00 | 30.9 | 18.6 |
| | | | **Clot Lysis (%)** | | |
| D.W | 20 | 13.00 | 100.00 | 80.1 | 28.7 |
| 50 % | 20 | 50.00 | 100.00 | 88.6 | 18.8 |
| 75 % | 20 | 11.00 | 100.00 | 88.5 | 24.8 |
| 100 % | 20 | 20.00 | 100.00 | 81.9 | 27.9 |

**Table (2) : Comparisons of mean clotting time results in different concentrations of Acacia nilotica**

| Variables (I) | Variables (II) | Mean of clotting time (I) | Mean of clotting time (II) | *P* value |
|---|---|---|---|---|
| D.W | 50 % | 7.3 ± 0.6 | 10.2 ± 4.3 | 0.428 |
| | 75 % | | 16.5 ± 11.8 | 0.012* |
| | 100 % | | 30.9 ± 18.6 | 0.000* |
| 50 % | 75 % | 10.2 ± 4.3 | 16.5 ± 11.8 | 0.077 |
| | 100 % | | 30.9 ± 18.6 | 0.000* |
| 75 % | 100 % | 16.5 ± 11.8 | 30.9 ± 18.6 | 0.000* |

***P* value < 0.05 indicates significant difference**

**Table (3): Comparisons of mean clot lysis results in different concentrations of Acacia nilotica**

| Variables (I) | Variables (II) | Mean of clot lysis (I) | Mean of clot lysis (II) | *P* value |
|---|---|---|---|---|
| D.W | 50 % | 80.1 ± 28.7 | 88.6 ± 18.8 | 0.290 |
| | 75 % | | 88.5 ± 24.8 | 0.295 |
| | 100 % | | 81.9 ± 27.9 | 0.823 |
| 50 % | 75 % | 88.6 ± 18.8 | 88.5 ± 24.8 | 0.990 |
| | 100 % | | 81.9 ± 27.9 | 0.403 |
| 75 % | 100 % | 88.5 ± 24.8 | 81.9 ± 27.9 | 0.410 |

**Table (4): Distribution of clot retraction results in different concentrations of Acacia nilotica**

| Variables | | Frequency | Per cent |
|---|---|---|---|
| **D.W** | Good | 20 | 100.0 |
| **50 %** | Good | 14 | 70.0 |
| | Bad | 6 | 30.0 |
| | Total | 20 | 100.0 |
| **75 %** | Good | 12 | 60.0 |
| | Bad | 8 | 40.0 |
| | Total | 20 | 100.0 |
| **100 %** | Good | 9 | 45.0 |
| | Bad | 11 | 55.0 |

| Variables | Frequency | Per cent |
|---|---|---|
| Total | 20 | 100.0 |

**Table (5): Association of clot retraction and different concentrations of Acacia nilotica**

| Variables | Clot retraction | | *P* value |
|---|---|---|---|
| | Good | Bad | |
| D.W | 20 (100.0%) | 0 (0.0%) | 0.002* |
| 50 % | 14 (70.0%) | 6 (30.0%) | |
| 75 % | 12 (60.0%) | 8 (40.0%) | |
| 100 % | 9 (45.0%) | 11 (55.0%) | |
| Total | 55 (68.8%) | 25 (31.2%) | |

## 4. DISCUSSION

Acacia nilotica consist of different chemicals such as: calcium, magnesium, ascorbic acid, tannin and other components. Immature pods of A.nilotica act as anticancer, anti-platelet and antioxidants. It has been demonstrated that, the extract of A.nilotica blocks platelets aggregation mediated by platelet agonists.

The aim of the current study was to determine the effect of A.nilotica on coagulation tests including whole blood clotting time, clot lysis and clot retraction. The results showed significant increase in clotting time with different concentration of A.nilotica (75%, 100%). This result agree with an American study which showed that, the addition of aqueous extract with concentrations of (100% ,200% and 400%) caused elevated mean clotting time of normal blood samples (9.6 ,20 ,9.5) respectively[9].

Another fining of this study was the significant change of clot retraction after the addition of aqueous extract of A.nilotica with different concentrations. This result agree with study done by Mariyam R in India, which showed that it have thrombotic activity through its capacity to block platelet aggregation mediated by platelet agonist [8].

Regarding clot lysis test, the results of this study clearly showed that increase concentration of A.nilotica had no effect on the test result. This result is agree with study done by Mayada Mohammed in Sudan 2021 on extract of A. nilotica , which showed no significant in clot lysis test [10].

## 5. CONCLUSION

Results of this study showed in vitro significant effect of A.nilotica extract on coagulation tests whole blood clotting time and clot retraction, related to the concentration of the extract.

**CONSENT**

All authors declare that 'written informed consent was obtained from all subjects. A copy of the written consent is available for review by the Editorial office/Chief Editor/Editorial Board members of this journal

## ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the Faculty of Medical Laboratory Sciences at Alzaeim Alazhari university and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki."

## REFERENCES


1. Chang –Xiao, Liu. Medical model plants breaking the traditional medicine research methods.*J.Chines herbal medicine*.2016; 8(1); 53-58.

2. Lack CW, Rousseau J. *Critical Thinking, Science, and Pseudoscience: Why We Can't Trust Our Brains*. . New York. Springer Publishing Company. 2016.

3. Herbal medicine. Complementary and alternative therapy. Cancer Research UK. https://www.cancerresearchuk.org/aboutcancer/cancer-in-general/treatment/complementary-alternative/therapies/individualtherapies/herbal-medicine. Published February 2015. Assessed October 2021.

4.Hailu, T., Endris, M., Kaleab, A. and Tsige, G.M. Antibacterial activities of some selected traditional Ethiopian medicinal plants used in treatment of skin disorders. *J. Ethnopharmacol*. 2005; **100** (1-2):168-175.

5. Steven, D. and Ehrlich, N.M.D. Solutions Acupuncture, a private practice specializing in complementary and alternative medicine, phoenix, AZ. Review provided by *J. VeriMed Healthcare Network. Health.* 2011; **2**(11): 1-8.

6.Keshaw R A, Anand B, Sudha S, H, Bhupendra N T. Free radical scavenging activity and reducing power of Acacia nilotica wood lignin. *International J of Biological Macromolecules*. 2014; **67**: 220–227

7. Pande, M.B. Note on the nutritive value of babul (Acacia nilotica L) seeds (extracted). *Ind. J.Anim.Sci*. 1981; **51**(1):107-108

8. Mariyam R, wajeeha B , Rumaiza J , acacia arabica(Babool).A Review on Ethobotanical and unani traditional uses as well as phytochemical and pharmacological properties, *international J.of pharmaceutical and phytopharmacological Research*, 2015; **4**(6):315-321.

9. UmaruB. SAKA. ,MAHRE M.B. DOGO H.M, effect of aqueous pod extract of acacia nilotica on white blood cells, platelets,and clotting time in albino Rats , *American J. of pharmacology and pharmacotherapeutics*,2016 ;**3**(1):001-006.

10. Mohammed M , Badawi M , Yousif O , In vitro effect of acacia nilotica aqueous extract on coagulation parameters among healthy people [B.Sc. Thesis] .Alzaiem Al-azhari University, 2021.