



***In vitro* Measurement of Anticoagulant Effect of Acacia Nilotica Aqueous Extract on Normal Human Plasma**

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Aims: To determine the anticoagulant effect of Acacia Nilotica aqueous extract on normal human plasma.

Study Design: This is an experimental study.

Place and Duration of the Study: Department of Haematology, Faculty of Medical Laboratory sciences, Alzaiem Alazhari, Khartoum – Sudan, during the period from July – August 2021.

Methodology: A total of 20 human blood samples were collected for this study from apparently healthy subjects following ethical considerations. Samples were collected in tri-sodium citrate and platelets poor plasma (PPP) were immediately prepared by centrifugation. Aqueous solution of Acacia nilotica was prepared with distilled water (D.W), three concentrations of the solution (50%, 75% and 100%) were prepared. Prothrombin Time (PT) and Activated partial thromboplastin time (APTT) were tested by manual method before and after adding different concentrations of Acacia Nilotica solution. Statistical tests were performed by using SPSS version 16.

Results: The results of study showed that Acacia Nilotica significantly prolonged the PT and APTT results of human plasma (*P* values for 50%, 75% and 100% solutions were 0.000, 0.000 and 0.000 while for APTT 0.036, 0.000 and 0.000 for PT respectively). Also the study showed no significant

correlation between control and *A. nilotica* concentrations among coagulation profile, except a significant positive correlation between control and 75% *Acacia nilotica* on APPT.

Conclusion: *Acacia Nilotica* solutions had anticoagulant effect in human plasma, and could be nominated as a potential preventive agent for thromboembolic diseases.

Keywords: *Acacia nilotica*; aqueous extract; activated partial thromboplastin time; prothrombin time.

1. INTRODUCTION

More than 21,000 plants are classified by the World Health Organization (WHO) as being used for a variety of therapeutic purposes around the world [1]. They discovered that in current medicine, 74 percent of 119 plant-derived pharmaceutical medications are employed. It is also estimated that 4 billion people (or 80% of the world's population) use herbal medicine for health care today [2]. Herbal remedies created from medicinal plants, minerals, and organic matter have been the mainstay of health care for around 75–80 percent of the world's population for hundreds of years, gaining appeal in both industrialised and developing countries [3]. The presence of active principles such as alkaloids, volatile essential oils, glycosides, resins, oleoresins, steroids, tannins, terpenes, and phenols in herbs confers therapeutic properties [4]. Herbal medicine has seen exponential growth in recent years due to its natural origins, ease of availability, efficacy, safety, and lack of side effects in the treatment of age-related disorders such as memory loss, osteoporosis, immune disorders, and others for which no modern medicine exists [5,6].

Researchers studying medicinal plants pursued numerous objectives, including the production of low-cost therapeutic chemicals and the discovery of prototypic medicines [7]. *Acacia nilotica* is also known as the Prickly Acacia, Gum Arabic tree, Babul, Egyptian thorn, or Prickly Acacia. *Acacia* is a nitrogen-fixing tree legume that can be used for a variety of purposes. It can be found from sea level to over 2000 metres above sea level and can tolerate strong temperatures (>50 °C) and air dryness, but it is sensitive to frost while young [8]. It is widely spread in subtropical and tropical Africa from Egypt to Mauritania southwards to South Africa, and in Asia eastwards to Pakistan and India [9,10].

Given the importance of blood in life, coagulation studies are crucial [11]. These findings are critical for understanding how medications can stop bleeding in small blood vessels [12] as well as the anti-thrombotic action of pharmaceuticals used to prevent atherosclerotic disorders

including myocardial and cerebral infarction. Antiplatelet medicines like aspirin and ticlopidine hydrochloride are used to reduce primary hemostasis, and anticoagulants like warfarin and heparin are used to suppress the coagulation system [13]. The present study examined the effect of *A. nilotica* on the coagulation system of normal human plasma by measuring Prothrombin Time (PT) and Activated Partial Thromboplastin Time (APTT).

2. MATERIALS AND METHODS

This was an experimental study carried out in Al-Zaiem Al-Azhari University, Khartoum State-central Sudan, during the period from July – August 2021. The study included normal blood samples from apparently healthy individuals. Hemolyzed, jaundiced and contaminated samples were excluded from the study. The study received ethical approval from Research committee at faculty of medical laboratory sciences in the University of Al-Zaiem Al-Azhari.

2.1 Preparation of the Aqueous Extract

Extraction was carried out according to method described by Umaru et,al [14]. The plant of *A.nilotica* had been collected from Al-khartoum market. The crushing of the pods was done by using pestle and mortar, after which it was ground into powder. Two hundred grams (200gm) of the powdered pod was weighed and introduced into a conical flask and 1 litre of distilled water was added thereafter . The mixture was then shaken and allowed to stand for 30 minutes, after which it was boiled (at 100°C) for one hour, cooled and shaken vigorously, before filtration using whatman No.1 filter paper. The filtrate was concentrated by placing in water bath at 95°C for three days until complete water evaporation and stored at 4°C until used. (50%, 75%, 100%) of the aqueous pod extract (crud) was prepared by using D.W.

2.2 Coagulation Testing

Determination of PT and APTT were performed by manual method. Briefly 10 ul of each aqueous extract solution were added to 90 ul of the

plasma; then the clotting time was determined for the 100 ul mixture after adding 100 ul Thromboplastin reagent (for PT), 100 ul Koalin and 100 ul Calcium Chloride reagents (for APTT).

2.3 Data Analysis

Descriptive statistics were used to summarize data. Numerical variables were expressed as mean \pm standard deviation (SD). Comparisons of mean values were conducted by one-way analysis of variance (ANOVA) and Pearson's correlation coefficient was applied to test the correlation between clotting time and the concentrations of A.nilotica solution. For all tests, P-values < 0.05 were considered statistically significant. All statistical analysis were conducted by SPSS version 16.

3. RESULTS

In this study, Acacia nilotica were tested for blood anti-coagulation effects in normal human plasma at three different concentrations (50%, 70% and 100%). Results showed significant prolongation of Prothrombin Time (PT) and Activated Partial Thromboplastin Time (APTT) of the human plasma (Tables 1 and 2). Results of diluent control revealed that PT and APTT were not affected by the addition of DW.

Also the results of study showed no significant correlation between control and A. nilotica concentrations among the coagulation profile (Table 3), except a significant positive correlation between control and 75% Acacia nilotica on APPT (Fig. 1).

Table 1. Comparisons of mean PT in difference concentration of A. nilotica

Variables (I)	Variables (II)	Mean of PT (I)	Mean of PT (II)	P value
Control	50 %	14.1 \pm 0.9	18.1 \pm 2.3	0.036*
(Plasma without extract addition)	75 %		29.1 \pm 5.0	0.000*
	100 %		61.4 \pm 10.6	0.000*
50 %	75 %	18.1 \pm 2.3	29.1 \pm 5.0	0.000*
	100 %		61.4 \pm 10.6	0.000*
75 %	100 %	29.1 \pm 5.0	61.4 \pm 10.6	0.000*

*P value < 0.05 is significant

Table 2. Comparisons of mean APTT in difference concentration of A. nilotica

Variables (I)	Variables (II)	Mean of APTT (I)	Mean of APTT(II)	P value
Control	50 %	31.8 \pm 2.6	66.1 \pm 11.2	0.000*
(Plasma without extract addition)	75 %		83.5 \pm 17.9	0.000*
	100 %		115.0 \pm 35.8	0.000*
50 %	75 %	66.1 \pm 11.2	83.5 \pm 17.9	0.010*
	100 %		115.0 \pm 35.8	0.000*
75 %	100 %	83.5 \pm 17.9	115.0 \pm 35.8	0.000*

*P value < 0.05 is significant

Table 3. Correlations between control and difference concentration of A. nilotica among PT and APTT

			50 %	75 %	100 %
PT	Control	Pearson Correlation	-.049	-.242	-.171
		Sig. (2-tailed)	.838	.304	.472
		N	20	20	20
APTT	Control	Pearson Correlation	.402	.576*	.396
		Sig. (2-tailed)	.079	.008	.084
		N	20	20	20

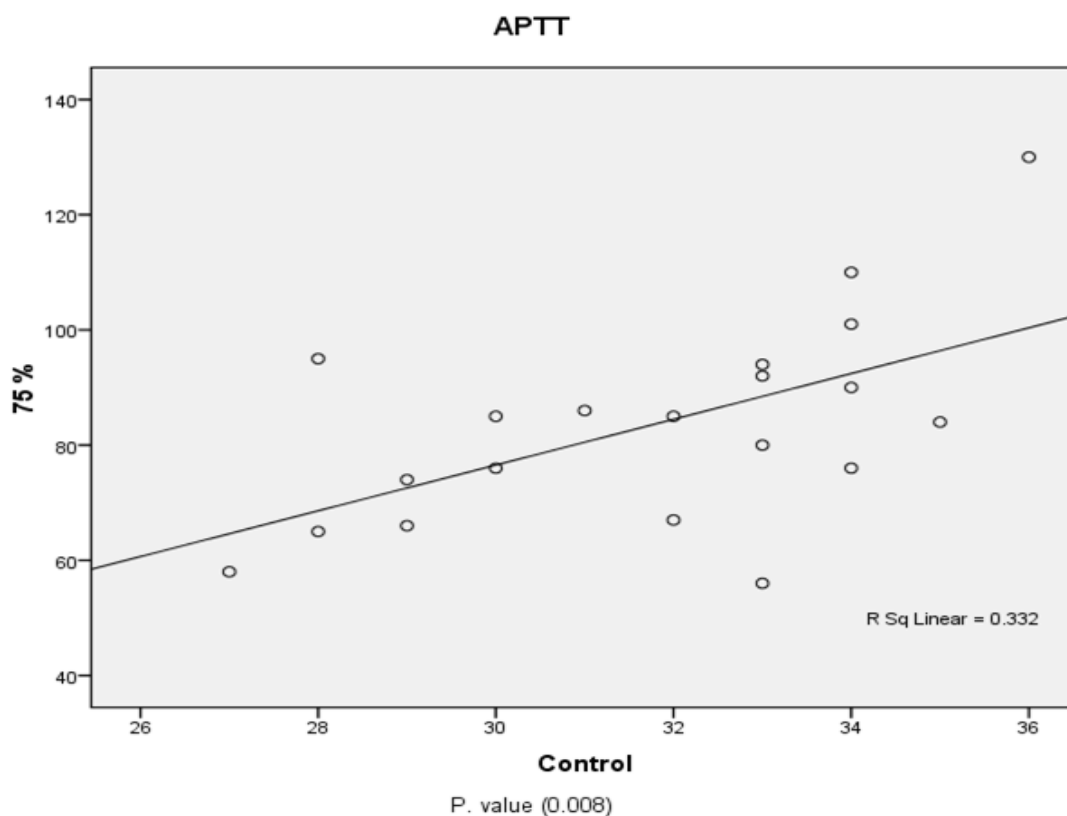


Fig. 1. Correlations between control and 75 % of *A. nilotica* among APTT

4. DISCUSSION

One of the leading causes of cerebral infraction, acute myocardial infraction, and other cardiovascular disorders is thrombosis [13]. Anticoagulants are types of drugs widely used in prevention and treatment of thrombotic disorders [15]. In this content, many epidemiological studies have indicated that, diet rich in polyphenol compounds may prevent the occurrence of cardiovascular disease [16]. The search for anticoagulant agents from natural herbal medicine, on the other hand, is an active research topic, because anticoagulant medications reduce blood coagulation, which is an important means of preventing thrombotic illnesses [17].

In This study, different concentrations of *Acacia nilotica* are investigated for their in-vitro anticoagulant activity in 20 samples of human plasma through the general tests of coagulation: activated partial thromboplastin time (aPTT) and prothrombin time (PT). the aPTT explore the endogenous pathway of coagulation, while PT explore the exogenous pathway of coagulation [18,19]. Results of this study showed that, *Acacia*

nilotica caused significant prolongation of PT and aPTT of plasma in concentration dependent manner (P values < 0.05) at all tested concentration compared to control. Data of present study clearly showed that *A. nilotica* had an important inhibition effect on both intrinsic and extrinsic coagulation pathways. Hence, these effects could be attributed to their fermented metabolite (acetate, propionate and butyrate). This can support the theoretical mechanism of action of *A. Nilotica* and indicted that the influence of fermentation products is on multiple target with in the coagulation cascade.

On the other hand, another finding of this study is that is moderate significant correlation between control and 75% concentration of *A. Nilotica*, while no correlation between the remaining concentrations among APTT and PT. In the literature, to the best of our knowledge no studies till now had demonstrated systematically correlation of coagulation parameters with the concentration of *Acacia Nilotica*.

The limitation of this study is that few concentrations of the extract were used. A wider view of the effect of *Acacia nilotica* could be

obtained by using more concentrations of the extract for the coagulation testing. Moreover additional tests needs to be conducted in order to assess the extent of anticoagulation and any negative impact.

5. CONCLUSION

The concentrations of *A. nilotica* used in this study showed marked anticoagulant effect as indicated by the prolongation of PT and APTT.

CONSENT AND 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." Informed consent was taken from all participants before enrolment in the study.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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