

## Study of the Thrombolytic Activity of an Aqueous Extract of Leaves of *Hibiscus sabdariffa* (Malvaceae) L. Consomed in Cote D'Ivoire

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### Abstract

### Original Research Article

Treatment of thrombosis relies on the use of anticoagulants, which poses a risk of hemorrhage. The objective of this study is to provide a useful database for therapeutic recommendations in Africa. The antithrombotic effect of an aqueous extract of the leaf of *Hibiscus sabdariffa* (Malvaceae) L. was therefore evaluated. Its protective effect, anticoagulant activity, anticoagulant capacity, and blood clot-dissolving power were determined in vitro in rabbits. The results reveal that the cytotoxic activity of the extract against red blood cells is nontoxic ( $11 \pm 0.92$  %;  $12 \pm 0.61$  % and  $14 \pm 1.05$  % hemolysis, respectively, for concentrations of 0.01 mg/mL, 0.1 mg/mL, and 1 mg/mL). The anti-hemolysis tests carried out showed moderate anti-hemolytic activity ( $5 \pm 1.01$  %;  $22 \pm 2.1$  % and  $59 \pm 3.11$  % for respective concentrations of 0.01mg/mL; 0.1mg/mL and 1 mg/mL). The biological tests, carried out in vitro, indicate an anticoagulant effect at high concentration ( $0.83 \pm 0.18$  respectively for 0.001mg/mL; 0.01mg/mL and 0.1 mg/mL of the extract;  $0.9 \pm 0.19$ ,  $1.1 \pm 0.18$  and  $1.3 \pm 0.2$  respectively for 0.5 mg/mL, 1 mg/mL and 1.5 mg/mL of the extract). Similarly, the TCA extract/TCA control ratio shows that the coagulant effect of the extract is at low concentration and that the anticoagulant effect is at high concentration. The data collected for the antithrombotic effect of the aqueous extract demonstrated an antithrombotic effect of the extract proportional to the incubation time ( $40.2 \pm 16$  % after 1 hour and  $57 \pm 21$  % after 2 hours for 0.5 mg/mL; 1 mg/mL and 1.5 mg/mL respectively of the extract). In short, the aqueous extract would indeed contain elements that can combat coagulation and could therefore be associated with the treatment of thrombosis.

**Keywords:** Hibiscus sabdariffa, thrombosis, hemorrhagic, anticoagulants, Côte d'Ivoire.

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## 1. INTRODUCTION

Thrombosis is the abnormal presence of a thrombus or blood clot in an artery or vein. This clot results from a clotting disorder that prevents blood from circulating normally. The most common manifestations of this disease are deep vein thrombosis of the lower limbs and pulmonary embolism (Soya E *et al.*, 2020).

Thrombosis is one of the leading causes of death worldwide, with an annual incidence of 60 to 100 per 100,000 (Bentounes N K *et al.*, 2024). In Africa, particularly in Côte d'Ivoire, it is estimated at 5.4% of hospitalized patients (Soya E *et al.*, 2020). Treatment of thrombosis relies on the use of anticoagulants such as heparin, fondaparinux, vitamin K antagonists, and argatroban (Cazes N *et al.*, 2025). In the last decade, new, more specific anticoagulants have been used. These are called direct oral anticoagulants (Bentounes *et al.*, 2024). They act as thrombin or factor Xa inhibitors, responsible

for the lysis of arteries or veins. However, the use of these molecules carries a significant risk of bleeding (Bentounes N K *et al.*, 2024; Cazes N *et al.*, 2025).

In Côte d'Ivoire, *Hibiscus sabdariffa* (Malvaceae) L. is used to treat several diseases (Samaké E S *et al.*, 2020). This plant contains a considerable number of chemical molecules, including anthocyanins, phenolic acids, tannins, lignans, and flavonoids, which have antithrombotic properties (Akpo J M K *et al.*, 2023). The use of this plant could be combined with existing drug therapy to reduce the risk of bleeding.

The objective of this study is to build a useful database for therapeutic recommendations. The antithrombotic effect of an aqueous extract of the leaf of *Hibiscus sabdariffa* (Malvaceae) L. was therefore evaluated in vitro in rabbits. To achieve this objective, this work proposes to study its protective effect, its

anticoagulant activity, to measure its anticoagulant capacity, and to determine its blood clot-lysis power.

## I. MATERIALS AND METHODS

### II.1. MATERIALS

#### II.1.1 Biological material

##### II.1.1.1 Plant

The plant material consists of fresh leaves of *Hibiscus sabdariffa* (Malvaceae), harvested in the commune of Adjamé (Abidjan, Ivory Coast). This plant was identified at the National Floristic Center of the Félix Houphouët-Boigny University (Abidjan, Côte d'Ivoire). The herbarium number is UCJ011895.

##### II.1.1.2 Animal

The study was conducted using blood collected from *Oryctolagus cuniculus* rabbits (Leporidae) weighing between 1.5 kg and 2.5 kg.

##### II.1.1.3 Technical equipment

The technical equipment consisted of a CYANCoag coagulometer (Belgium) to determine clotting time and a centrifuge to isolate red blood cells from plasma.

##### II.1.1.3.1 Reference substances

The reference substances used in this study were sodium chloride (Merck, Germany) at a concentration of 0.9 % (NaCl 0.9 %), which served as a negative control in the cytotoxicity and antihemolytic assays. Vitamin C or ascorbic acid (Biomérieux, France) served as a positive control for the protection of the red blood cell membrane. 2,2-azobis-2-amidopropane dihydrochloride (AAPH), supplied by Biomérieux (France), enabled the attack and lysis of the red blood cell membrane in the antihemolytic assay. Cephalin-kaolin solution and prothrombin reagent (Belgium) were used in the tests exploring the intrinsic pathway (PTA) and the extrinsic or exogenous pathway (PT) of coagulation, respectively. Platelet-poor plasma served as the biological substance for the hemostasis test.

### II.2. METHODS

#### II.2.1 Preparation of the aqueous extract

A quantity of 100 g of fresh leaves, previously washed with distilled water, was finely chopped, ground, and boiled for 15 minutes in 1.5 L of distilled water. The resulting decoction was filtered with Wattman paper and absorbent cotton. The collected filtrates were dried at 50°C for 48 hours. After drying, 2 g of powder was obtained, yielding an extraction yield of 2 %.

#### II.2.2. Blood collection

The various blood samples were collected from the marginal and saphenous veins of the rabbit *Oryctolagus cuniculus* (Leporidae). The blood sample used to study the thrombolytic activity of our extract was collected in dry tubes (without EDTA). For the cytotoxicity and hemostatic tests, tubes containing citrate and those containing EDTA were used.

#### II.2.3. Cytotoxicity test

##### Principle:

The protocol followed to study the cytotoxicity of the aqueous extract of *Hibiscus sabdariffa* is that of Okoka and Ere (2012). The extract is tested at increasing concentrations. The cytotoxicity of the extract, against rabbit red blood cells, was assessed by measuring the percentage of hemolysis. The results are expressed as percentage of hemolysis in the control samples [negative control (NaCl 0.9 %) and positive control (distilled water)] and the samples treated with the extract as follows:

$$\% \text{ Hemolysis} = (\text{AE}/\text{AC}) \times 100$$

AE: Absorbance of the sample

AC: Absorbance of the positive control (hypotonic solution)

#### II.2.4. Antihemolytic test

##### Principle

The antihemolytic effect of the extracts was evaluated in vitro using the AAPH (2,2-azobis-2 amidino-propane-dihydrochloride) method reported by Zhang X *et al.*, (2021). To demonstrate the protective effect of *Hibiscus sabdariffa* extracts on the preservation of cellular integrity, which is primarily related to red blood cell membranes, erythrocytes were subjected to oxidative stress conditions by the addition of AAPH (2,2-azobis-2 amidino-propane-dihydrochloride). The thermal decomposition of this compound produces free radicals at a constant rate that attack the red blood cell membrane. When endogenous antioxidants are depleted, the red blood cell membrane ruptures and intercellular hemoglobin is released. Quercetin was used as a standard. Hemolysis monitoring is quantitatively assessed by spectrophotometric assay of hemoglobin levels in the supernatant at 540 nm.

##### Procedure:

To a volume of 200 µL of red blood cells (20%) are added 200 µL of extract at different concentrations. This solution is incubated at 37° C for 30 min. Then 400 µL of AAPH (200 mM) is added. This last solution is incubated at 37° C for 2 h. After this last incubation, a volume of 3 mL of PBS is added. The mixture is centrifuged at 1500 rpm for 10 min and the OD is read at 540 nm. The protective effect of different extracts is evaluated as a percentage of hemolysis inhibition according to the following formula:

$$\% \text{ Inhibition of hemolysis} = (1 - \text{AE}/\text{AC}) \times 100$$

AE: Sample Absorbance

AC: Positive Control Absorbance (Complete Hemolysis)

#### II.2.5 Anticoagulant activity

The tests to explore the intrinsic and extrinsic pathways of coagulation were performed according to the method proposed by Rizzo F *et al.*, (2008) with some modifications.

#### II.2.5.1. Platelet-poor plasma preparation (PPP)

Platelet-poor plasma was obtained at least 2 hours after collection. Citrated tubes containing blood were centrifuged at 3000 rpm for 15 min. The resulting plasma was collected and placed in a flask tube and centrifuged again under the same conditions as before.

#### II.2.5.2. Endogenous coagulation pathway test (ACT)

A mixture consisting of 43 µL of plasma and 7 µL of sample at different concentrations is prepared in a test tube. This mixture is incubated at 37°C for 5 minutes. A solution containing a volume of 50 µL of cephalin-kaolin is incubated at 37° C for 2 minutes and then added to the previously obtained mixture. This mixture is reincubated at 37° C for 3 minutes and coagulation is then triggered by the addition of 50 µL of a 0.025 M CaCl<sub>2</sub> aqueous solution. The clotting time is then determined using a coagulometer from the addition of calcium (starting the timer) to the formation of the fibrin clot (stopping the timer). Distilled water is considered the negative control.

#### II.2.5.3. Test to explore the extrinsic or exogenous Coagulation pathway (PT)

A 43 µL volume of plasma is introduced into a cuvette used for coagulation tests. A 7 µL volume of the test sample is added to this aliquot of plasma in a test tube. This mixture is incubated at 37° C for 5 minutes. Next, a 100 µL volume of Prothrombin reagent (rabbit brain extract + calcium chloride), preheated to 37° C for 10 minutes, is added. The clotting time is then recorded. A tube containing the plasma and distilled water serves as the negative control.

#### II.2.6. Antithrombotic activity

##### II.2.6.1. Thrombus preparation

A 5 mL volume of blood is collected from the rabbit saphenous vein in a dry tube. The blood sample is kept at room temperature for 1 hour. After thrombus formation, the supernatant is completely removed, and the thrombus is gently removed from the tube. It is divided into two equal parts and weighed.

##### II.2.6.2. Effects of the extract

In each of the two test tubes containing the thrombus, 1 mL of extract is added. 0.9 % NaCl and streptokinase are then added to each tube. The tubes are incubated at 37° C for 45 minutes in a water bath. The thrombus is then removed from each tube and weighed after 1 hour and 2 hours. The thrombi placed in the tubes containing NaCl and streptokinase, respectively, serve as

negative and positive controls. The percentage decrease in thrombus mass is obtained using the following formula:

$$DC (\%) = \frac{(M1 - M2)}{M1} (\times 100)$$

DC: Percentage of clot reduction

M1: Initial clot mass

M2: Final clot mass

#### II.2.7. Statistical analysis

The results presented in this document are in the form of tables and graphs. Graph Pad Prism 8 software was used for statistical analyses and calculation of means and standard deviations. Analysis of variance (ANOVA) followed by the Newman-Keuls multiple comparison of means test were used to rank and compare means. Means are always followed by their standard deviations. Two means are significantly different if the probability resulting from the statistical tests is less than or equal to 0.05 ( $P \leq 0.05$ ).

## III. RESULTS AND DISCUSSION

### III.1. RESULTS

#### III.1.1. Study of the protective effect of *Hibiscus sabdariffa* (Malvaceae) L. aqueous extract (EAHS) on red blood cells

To evaluate the protective effect of *Hibiscus sabdariffa* (Malvaceae) L. aqueous extract (EAHS) on red blood cells, two tests were performed: the cytotoxicity test and the antihemolytic test.

##### III.1.1.1. Study of the cytotoxicity

Incubation of *Hibiscus sabdariffa* (Malvaceae) L. (EAHS) at increasing concentrations of 0.01 mg/mL, 0.1 mg/mL, and 1 mg/mL induced a non-significant increase in the percentage of hemolysis ( $P > 0.05$ ) compared to that of isotonic saline (0.9 % NaCl). Indeed, in the presence of 0.9 % NaCl (negative control), the percentage of hemolysis is  $10 \pm 0.79$  %. EAHS caused for concentrations of 0.01mg/mL, 0.1mg/mL and 1mg/mL respectively  $11 \pm 0.92$  % ;  $12 \pm 0.61$  % and  $14 \pm 1.05$  % of hemolysis. At the concentration of 2 mg/mL of body weight, the extract induced a hemolysis rate of  $56 \pm 2.05$  %. In the presence of a hypotonic solution (distilled water) with red blood cells, the hemolysis rate is  $90 \pm 0.38$  %. This medium constitutes the positive control. The hemolysis rate of our extract is statistically similar to that of the isotonic medium at concentrations of 0.01 mg/mL, 0.1 mg/mL and 1 mg/mL (Figure 1).

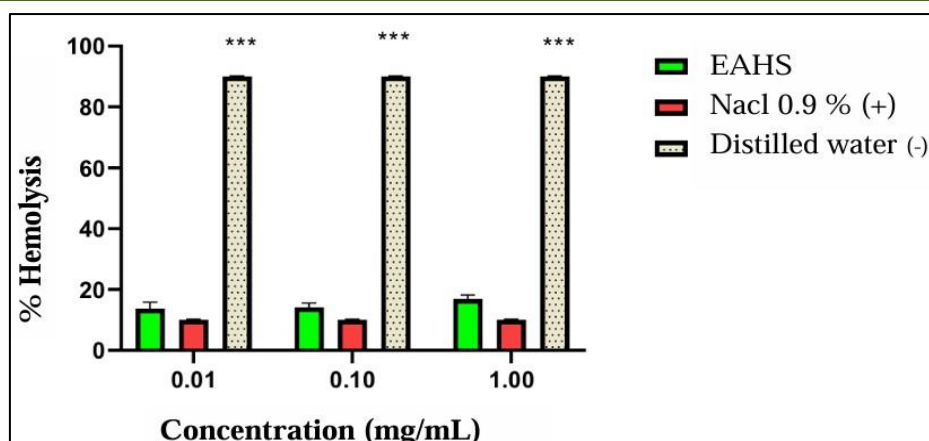


Figure 1: Percentage of red blood cell hemolysis as a function of *Hibiscus sabdariffa* (Malvaceae) L. EAHS concentration

### III.1.1.2. Antihemolytic activity

The inhibition of red blood cell hemolysis by AAPH was tested in the presence of increasing concentrations (0.01 mg/mL; 0.1 mg/mL and 1 mg/mL) of EAHS and ascorbic acid (reference radical-protecting molecule). At these different concentrations, EAHS showed concentration-dependent protective effects. These effects are statistically weak compared to those of ascorbic acid ( $P < 0.001$ ). Indeed, for concentrations of

0.01 mg/mL; 0.1 mg/mL and 1 mg/mL EAHS inhibited red blood cell hemolysis by AAPH by  $5 \pm 1.01$  %,  $22 \pm 2.1$  %, and  $59 \pm 3.11$  %, respectively. For the same concentrations, ascorbic acid induced hemolysis inhibition by  $5 \pm 2.3$  %,  $40 \pm 1.41$  %, and  $80 \pm 2.8$  %. The percentage of hemolysis inhibition as a function of the concentration of the samples tested for the three experiments is shown in (Figure 2).

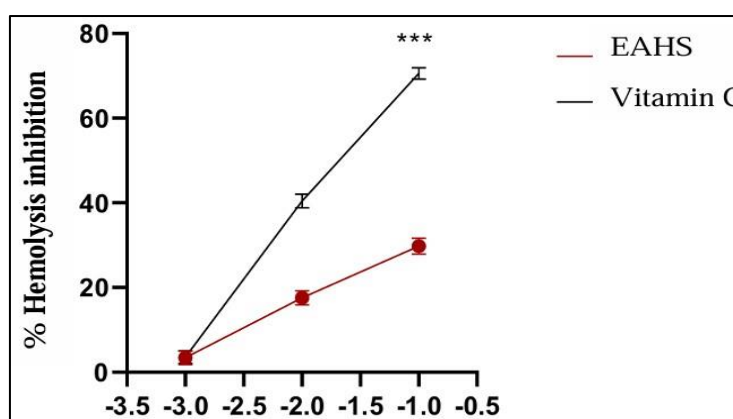


Figure 2: Anti-hemolysis effect of the aqueous extract of the leaf of *Hibiscus sabdariffa* (Malvaceae) L. (EAHS).

### III.1.2. Anticoagulant Effect

The mean values obtained after the anticoagulant activity tests of *Hibiscus sabdariffa* aqueous extract (EAHS) ( $n=3$ ) were used to plot histograms representing the ratios of extracted TP to control TP according to the different concentrations of *Hibiscus sabdariffa* aqueous extract (EAHS) and the coagulation pathway. These histograms are shown in Figures 3 and 4.

### III.1.3. Extrinsic Coagulation Pathway (TP)

For concentrations of 0.001 mg/mL, 0.01 mg/mL, and 0.1 mg/mL, the ratio of extracted TP to control TP did not vary; it remained constant at  $0.83 \pm 0.18$  (Figure 3). However, for concentrations of 0.5 mg/mL, 1 mg/mL, and 1.5 mg/mL, a statistically significant increase ( $p < 0.05$ ) was observed, the concentration of the extracted TP to control TP ratio being  $0.9 \pm 0.19$ ,  $1.1 \pm 0.18$ , and  $1.3 \pm 0.2$ . This means that our extract, at these concentrations, has an anticoagulant effect (Figure 4).

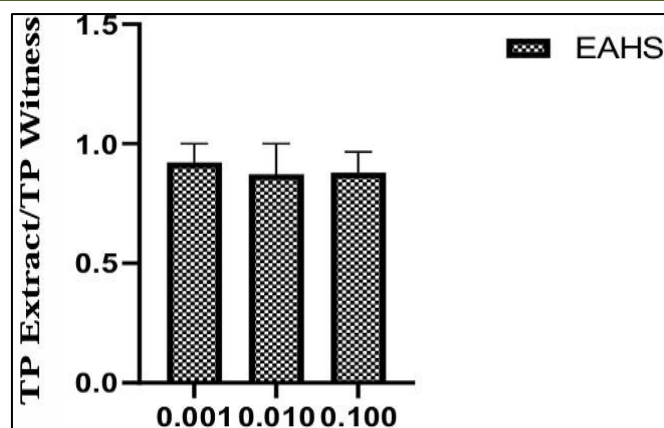


Figure 3: Coagulant capacity of aqueous extract of *Hibiscus sabdariffa* (Malvaceae) L. (EAHS) according to the exogenous route

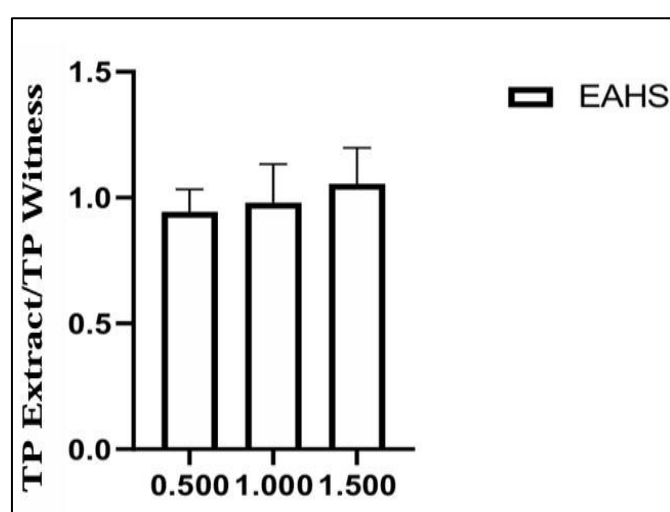


Figure 4: Coagulant capacity of aqueous extract of *Hibiscus sabdariffa* (Malvaceae) L. (EAHS) according to the exogenous route

#### III.1.4. Intrinsic TCA Coagulation Pathway

For concentrations ranging from 0.001 mg/ml, 0.1 mg/ml, and 1 mg/ml, of the aqueous extract of *Hibiscus sabdariffa* (EAHS), a significant decrease ( $P < 0.05$ ) in concentrations dependent on the ratio of TCA extracted to TCA control was observed. These were 0.8

$\pm 0.1$ ;  $0.72 \pm 0.3$ ; and  $0.54 \pm 0.012$ , respectively (Figure 5). However, for concentrations ranging from 0.5 mg/mL, 1 mg/mL, and 1.5 mg/mL, the aqueous extract of *Hibiscus sabdariffa* (EAHS) resulted in a significant increase in the ratio of TCA extracted to TCA control, i.e.,  $1.1 \pm 0.03$ ;  $1.2 \pm 0.18$ ; and  $1.5 \pm 0.2$  (Figure 6).

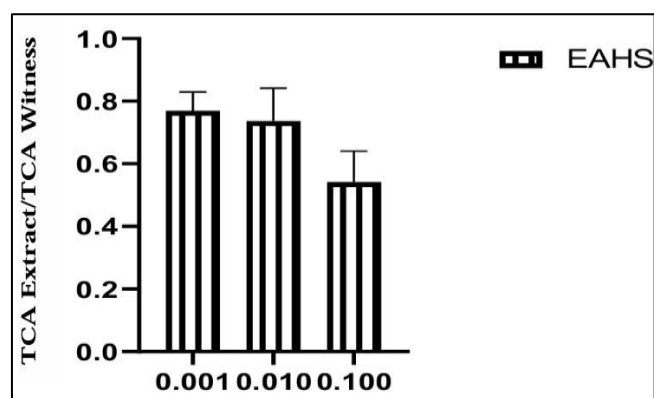
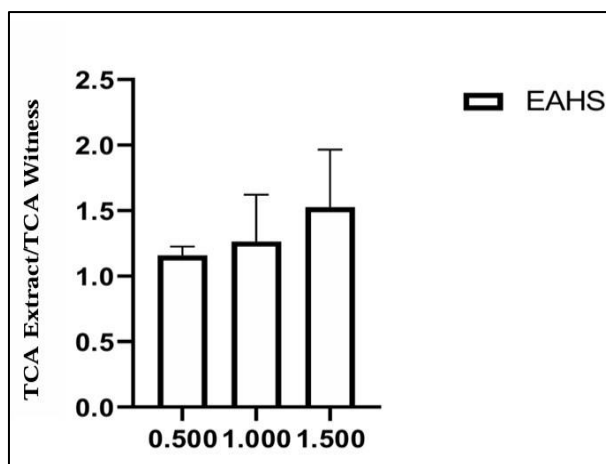


Figure 5: Coagulant capacity of aqueous extract of *Hibiscus sabdariffa* (Malvaceae) L. (EAHS) according to the intrinsic pathway





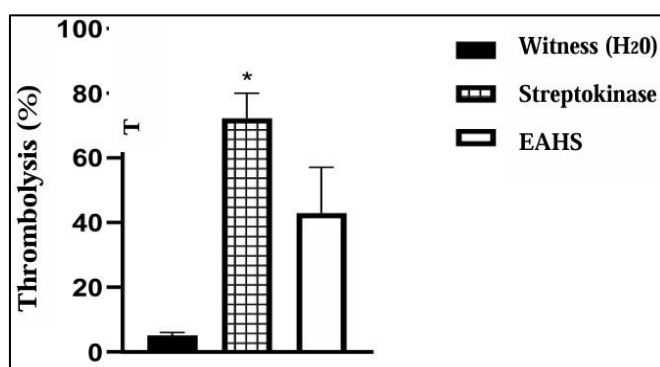
**Figure 6: coagulant capacity of aqueous extract of *Hibiscus sabdariffa* (Malvaceae) L. (EAHS) according to the intrinsic pathway**

### III.1.5. Thrombotic Activity of EAHS

The thrombotic activity of the aqueous extract of *Hibiscus sabdariffa* against the clot was evaluated after 1 h and 2 h of activity.

### III.1.6. Thrombotic Activity of EAHS after 1 h

For increasing concentrations (0.5 mg/mL; 1 mg/mL and 1.5 mg/mL) of the aqueous extract of *Hibiscus sabdariffa*, the percentage of clot lysis increased in a dose-dependent manner, to  $40.2 \pm 16\%$ . Streptokinase, the reference molecule, lyses the clot at  $78 \pm 0.2\%$  after 1 h. For the negative control NaCl 0.9%, the clot lysis was  $5 \pm 0.1\%$  for the same time (Figure 7).

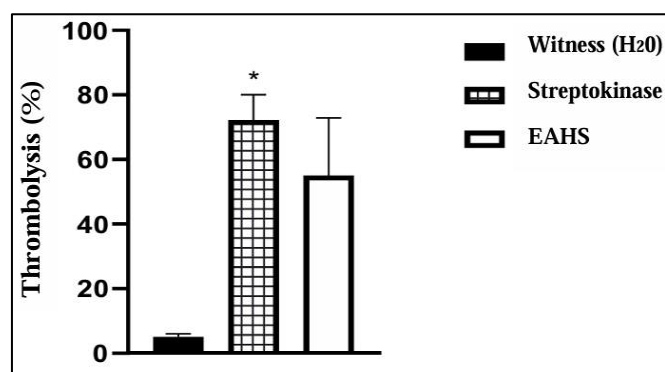


**Figure 7: Percentage of thrombolysis as a function of the concentration of *Hibiscus sabdariffa* (Malvaceae)L**

### III.1.7. Thrombotic activity of EAHS after 2 hours

Increasing concentrations of 0.5 mg/mL, 1 mg/mL, and 1.5 mg/mL of the aqueous extract of *Hibiscus sabdariffa* caused clot lysis of  $57 \pm 21\%$ .

Streptokinase and 0.9% NaCl lysed the clot by  $78 \pm 0.2\%$  and  $5 \pm 0.1\%$ , respectively. These values are identical to those obtained at lysis after 1 hour (Figure 8).



**Figure 8: Percentage of thrombolysis as a function of the concentration of *Hibiscus sabdariffa* (Malvaceae) L**

### III.2 DISCUSSION

The results presented on the cytotoxicity of the aqueous extract of *Hibiscus sabdariffa* (Malvaceae) L. leaves against red blood cells were performed using the method of Okoka T & Ere D, (2012). These results demonstrate a maximum percentage of hemolysis, i.e.,  $90 \pm 0.001$  with the positive control (distilled water) and  $13.733 \pm 2.167$  and  $14.14 \pm 1.35$  for the concentrations of 0.01 mg/ml and 0.1 mg/ml of the aqueous extract of *Hibiscus sabdariffa* (Malvaceae) L. leaves compared to the negative control medium NaCl 0.9 %. These results demonstrate that *Hibiscus sabdariffa* (Malvaceae) L. is not cytotoxic. These results are comparable to those obtained for the aqueous extract of *Hibiscus sabdariffa* (Malvaceae) L. consumed in central and northern Benin (Akpo J M K *et al.*, 2023). Indeed, this could be explained by the presence of flavonoids, which have no cytotoxic activity, since the toxicity of flavonoids is due to factors such as their possible glycolization (Dai X *et al.*, 2019).

The toxic effects of plants, which have been recognized for several years and contain one or more substances harmful to humans or animals, cause various disorders. This is what led us to study the in vitro antihemolytic effect of this plant. The percentage of antihemolytic activity of the extract and vitamin C increases with concentration. The results indicate that the extract of our plant exhibits moderate hemolytic activity, ranging from  $5 \pm 0.1$  % to  $29.01 \pm 0.2$  %. These results are corroborated by those of Ujianti *et al.*, (2023). Indeed, these authors showed that *Hibiscus sabdariffa* (Malvaceae) L. slowed the development of liver damage in laboratory animals fed a diet deficient in vitamin B12. These results are consistent with those of Lee C *et al.*, (2012) who demonstrated that *Hibiscus sabdariffa* extract could repair liver damage caused by paracetamol in laboratory animals.

These results could be explained by the action of phenolic compounds with antioxidant activity against cell lysis (Dai X *et al.*, 2019). This suggests that *Hibiscus sabdariffa* extract maintains oxidant-antioxidant homeostasis, thus preventing or limiting tissue damage (Prasomthong J *et al.*, 2022). The anticoagulant activity of our aqueous extract and its main constituents was evaluated in vitro with respect to the two coagulation pathways (the endogenous pathway and the exogenous pathway) on a pool of normal deplateleted plasmas and using two global chronometric tests, the cephalin-kaolin time test and the prothrombin time test. This allows exploring the activity of factors II, V, VIII, IX, X, XI and XII of the endogenous pathway and the common coagulation pathway (Rizzo F *et al.*, 2008).

The results obtained show a constant value for the ratio TP extract/TP control for low concentrations on the exogenous pathway. Indeed, our extract does not indicate any effect on the clotting time (TP). However, the increase in time for the ratio TP extract/TP control at

high concentration causes the dissolution of the callus by the activation of the different anticoagulant factors. In addition, our extract exhibited anticoagulant activity at high concentrations. Thus, our work can be compared to that of Hsieh C L *et al.*, (2007) who reported that certain flavonoid compounds of *Flaveria bidentis* (Asteraceae) caused a significant prolongation of Quick times. The decrease in the TCA extract/TCA control ratio rate expresses the coagulant effect of our extract at these low concentrations. On the other hand, the increase in the TCA extract/TCA control level as a function of concentrations expresses an anticoagulant effect of our extract. The anticoagulant effect of our extract by prolongation of clotting times (TCK) could be due to the lack of factors involved in the exogenous and endogenous coagulation pathway or the presence of inhibitors of these factors, or by the action of tannins and flavonoids (Kpahé Z F *et al.*, 2022).

Indeed, tannins have a therapeutic action, particularly hemostatic (Agunu A *et al.*, 2005). Flavonoids are also particularly active in maintaining good blood circulation, possess strong antioxidant or anti-radical, antiproliferative and anticarcinogenic potential and inhibit the tendency of small blood cells or platelets to group together and form blood clots (Adedapo A *et al.*, 2013; Ujianti I *et al.*, 2023). Furthermore, Guglielmone *et al.* (2002) reported that the anticoagulant activity of flavonoids may be due to their inhibitory action on the endogenous coagulation pathway.

The data collected for the antithrombosis effect of the aqueous extract of *Hibiscus sabdariffa* (Malvaceae) L. shows an activity expressed as a percentage of thrombolysis. This thrombolytic activity of the extract increases over time, with an effect of  $4.2 \pm 16$  % at one hour and an effect of  $57 \pm 21$  % after 2 hours, unlike the percentages of thrombolysis of streptokinase and NaCl, which are constant at  $78 \pm 0.2$  % and  $5 \pm 0.1$  % after 1 hour and 2 hours, respectively. These results demonstrate a dose-dependent antithrombotic activity of our extract. This antithrombotic activity could be due to the presence of certain metabolite compounds in our extract, notably tannins (Kpahé Z F *et al.*, 2022).

Indeed, tannins are antithrombotic and anticoagulant metabolites that exert a strong inhibition of thrombin. These results are also consistent with the work of (Kee N *et al.*, 2008) which revealed a strong inhibition of thrombin in aqueous extracts of leaves of *G. superba* (5.30 mg/mL), *L. leonurus* (9.69 mg/mL), *S. frutescens* (2.23 mg/mL), and *Z. aethiopica* (4.74 mg/mL). Raj N K *et al.*, (2001) reported the antithrombotic effect, particularly the antiplatelet effect, of certain flavonoid components (quercetin, 3-methyl quercetin, dihydroquercetin, and flavones). This property is linked to their inhibitory effect by binding to platelet cell receptors. Guerrero G A *et al.*, (2005) reported that some flavonoids such as apigenin effectively inhibit platelet

aggregation in vitro by inhibiting TXA2 (Arslan R *et al.*, 2011). Furthermore, flavonoids (catechin and quercetin) synergistically inhibit platelet production of hydrogen peroxide in vitro (Pearson D A *et al.*, 2002).

#### IV. CONCLUSION AND OUTLOOK

Evaluation of the cytotoxic activity of *Hibiscus sabdariffa* (Malvaceae) L. extract against red blood cells showed that the aqueous extract of *Hibiscus sabdariffa* is nontoxic. Antihemolysis tests showed that the aqueous extract of *Hibiscus sabdariffa* (Malvaceae) L. exhibited moderate antihemolytic activity. In vitro biological tests of the ratio of TP extract to control TP indicate that the anticoagulant effect of the extract is present at high concentrations. Similarly, the TCA extract/TCA control ratio shows that the coagulant effect of the extract is at low concentration and that the anticoagulant effect is at high concentration. The data collected for the anti-thrombotic effect of the aqueous extract of *Hibiscus sabdariffa* (Malvaceae) L. allowed us to highlight that the anti-thrombotic effect of this extract is proportional to the incubation time. In short, the aqueous extract of *Hibiscus sabdariffa* (Malvaceae) L. would indeed present elements that can fight against coagulation. However, it would be interesting to deepen this study on large quantities of samples as well as other tests and assay techniques on thrombotic activity in vivo.

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