

Evaluation of Hemostatic and Antihemolytic Effects of Aqueous Extract of *Garcinia kola* (Clusiaceae) Fresh Seeds

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Abstract

Objectives: *Garcinia kola* seeds (EAgk) (Clusiaceae) contain coagulant components use to pretreat aquaculture wastewater in Nigeria. This work was undertaken to assess the effects of EAgk on blood coagulation because of its contents. **Methods:** Five groups of four rats of both sexes (200 ± 5 g) received orally distilled water, phytomenadione (15 mg/kg b.w.) and *garcinia kola* seeds extract (250, 500 and 1000 mg/kg b.w.) for four days and after this period, bleeding time was measured by tail hemorrhage model. Activated partial thromboplastin time (aPTT) and platelet count were determined by coagulometer and hematological analyzer respectively. Blood red cells protection of EAgk was measured by the methods of 2,2'-Azobis(2-methylpropionamide) dihydrochloride (AAPH)-induced hemolysis and inhibition of 2,2-Diphenyl-1-picryl hydrazyl (DPPH) radical and hydrogen peroxide. **Results:** EAgk at 1000 mg/kg b.w. induced a significant decrease in bleeding time from 420 ± 27.3 s to 157.5 ± 18.9 s. The platelet count of group of rat treated with EAgk (1000 mg/kg b.w.) increased significantly ($p < 0.01$, $n = 4$). EAgk, decreased aPPT in a concentration dependent manner ($p > 0.05$, $n = 4$). EAgk inhibited hemolysis, DPPH radical and hydrogen peroxide with IC_{50} values of 1.02 ± 0.2 ; $3.2 \times 10^{-2} \pm 0.12$ and 1.01 ± 0.5 mg/mg respectively. **Conclusions:** EAgk exhibits hemostatic effects, which could justify the presence of coagulant components in the extract. Erythrocytes' protective activity of *Garcinia kola* seeds may be due to free radicals and hydrogen peroxide inhibition.

Keywords

Garcinia kola, Hemostatic, Antihemolytic, Bleeding Time, Platelet

1. Introduction

Hemostasis is a great protective mechanism that depends on an important balance of procoagulant and anticoagulant processes [1]. When a blood vessel is injured, platelets and fibrin play an essential role to build blood clot and stopping blood loss from the damaged vessel [2]. The clotting cascade (secondary hemostasis) is broken up into two pathways which are intrinsic and extrinsic. The term intrinsic refers to a series of sequential reactions involving the plasma proteins factors VIII, IX, XI and XII; and prekallikrein and high molecular weight kininogen that are required for initiation of coagulation in the activated partial thromboplastin time (aPTT) assay, while the extrinsic pathway consists of the transmembrane receptor tissue factor (TF) and plasma factor VII/VIIa (FVII/FVIIa) [3] [4].

Uncontrolled bleeding remains the leading cause of preventable deaths in many cases of hemorrhage. Over the past 15 years, pharmaceutical industries have developed new hemostatic agents [5]. Also, several studies, worldwide have focused on the hemostatic activity of many medicinal plants with the hope of finding new and more effective agents [6] [7].

Garcinia kola is widely known as Bitter kola, belongs to the family of plants called Clusiaceae. The seeds of *Garcinia kola* play an important role in traditional herbal medicine worldwide [8]. *Garcinia kola* seeds are chewed as an aphrodisiac or used to cure cough, dysentery, chest colds, liver disorders, diarrhoea, laryngitis, bronchitis, and gonorrhoea [9]. Bitter kola is used to prevent and relieve colic; it can also be used to treat headache, stomachache and gastritis [10] [11]. *Garcinia kola* seeds possess several pharmacological properties such as antioxidant, hepatoprotective, antidiabetic, antimicrobial and antipyretic activities [12] [13] [14]. The phytochemical compounds isolated from *Garcinia kola* include alkaloids, cardiac glycosides and biflavonoids [15] [16]. Seeds contain polysaccharides and can form a gel in a solution, so they can act as a coagulant to pre-treat aquaculture wastewater [17]. However, the effects of *Garcinia kola* seeds on blood coagulation still remain unknown.

The aim of the present work was to investigate *in vitro* and *in vivo* hemostatic effects of *Garcinia kola*.

2. Material and Methods

2.1. Plant Material and Extract Preparation

Garcinia kola fresh seeds were collected at Abobo large market, in the North of Abidjan in 2021. A voucher specimen was deposited at the Herbarium from the National Floristic Center of Felix Houphouet Boigny University (IBAAN-00648). After harvesting, the seeds were washed and rinsed with distilled water and finely grated and ground in a mortar with a pestle for 5 - 10 min. Two hundred grams (200 g) of ground nuts are macerated in a flat-bottomed flask containing 1.5 L of distilled water for 12 hours under a magnetic stirrer. The mixture obtained is filtered through absorbent cotton. The filtrate is dried in an oven at 40°C for 48

hours. The extract obtained, is stored at 5 °C and used for different tests.

2.2. Animals and Ethics

Rats (*Ratus norvegicus*) and rabbits (*Oryctologus cuniculus*) weighing 200 ± 5 g and 2 ± 0.4 kg respectively were used in our experiments. These animals were obtained from the Animal House of the Laboratory of Biology and Health of UFR Biosciences at Cocody University in Abidjan (Côte d'Ivoire). They were housed in constant temperature rooms with a light/dark cycle of 14/10 hours. All animals were fed and given water ad libitum until use. Experimental procedures and protocols used in this study were approved by ethical committee of Health Sciences, University Felix Houphouet-Boigny of Cocody-Abidjan. These guidelines were in accordance with the internationally accepted principles for laboratory use and care [18].

2.3. Phytochemical Screening

Phytochemical screening was performed, to highlight the major chemical group such as alkaloids, saponosids, flavonoids, polyphenols, tannins, quinons, sterols and polyterpens, using standard procedures [19] [20].

2.4. Chemicals and Reagents

2,2-Diphenyl-1-picryl hydrazyl (DPPH), 2,2'-Azobis(2-methylpropionamide) dihydrochloride (AAPH) were purchased from Sigma-Aldrich (USA). Prothrombin, cephalin-kaolin and Calcium chloride (CaCl_2 0.025 M) were obtained from Cypress Diagnostics (Belgium). Ascorbic acid was purchased from Merck (Germany). All other chemicals and reagents used were of analytical grade.

2.5. Red Blood Cell Protective Activity

2.5.1. Preparation of Rabbit Erythrocytes

Five rabbits were anesthetized with ketamine (100 mg/kg b.w.) and the blood was collected from saphena vein. Erythrocytes were isolated and stored according to the method described by [21].with slight modifications. The blood samples collected were centrifuged at 3000 rpm for 10 min. The erythrocytes were separated from the plasma and buffy coat and were washed three times by centrifugation (3000 rpm, 5 min). The supernatant and buffy coats of white cells were carefully removed with each wash. The washed erythrocytes were stored at 4 °C and used within 6 h for further studies.

2.5.2. Hemolytic Assay

The method described by [22] was used to evaluate the percentage of hemolysis of red blood cells. Two (2) concentrations of *Garcinia kola* extract (10^{-3} and 10^{-1} mg/mL), were added to 20% red blood cell solution. The saline solution NaCl (0.9%) (Positive control) and distilled water (negative control) were also added separately to the 20% red blood cell solution. The mixture of 0.2 mL of 20% red blood cell solution and 0.8 mL of *Garcinia kola* was incubated for 30 min at 37 °C

and then centrifuged at 3000 rpm for 10 min. The percentage of hemolysis determined at the longwave of 470 nm, was expressed as:

$$\% \text{ Hemolysis} = [AE/AC] \times 100$$

with AE: Absorbance of the sample and AC: Absorbance of the control (hypotonic solution).

2.5.3. Antihemolytic Activity

In vitro study of the antihemolytic effect of the extract of *Garcinia kola* was carried out according to the AAPH (2-2'-Azobis(2-methylproprionamide) dihydrochloride) method used by [23]. The standard control used for the study is ascorbic acid. Conveniently it was added to (200 μ L) extracts or ascorbic acid at different concentrations (10^{-1} - 1 mg/mL), 200 μ L of 20% red blood cells. The mixture was incubated for 30 min at 37°C. 400 μ L of AAPH (200 mM) was added to the mixture and was incubated again at 37°C for 2 h. Before centrifugation of the mixture at 1200 rpm for 10 min, 3 mL of PBS was added. The percentage of inhibition of hemolysis was determined at the longwave of 540 nm, as follows:

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

with AC: Absorbance of the positive control and AE: Absorbance of the sample.

2.5.4. DPPH Free Radical Scavenging Activity

The radical scavenging activities of the plant extracts against 2,2-Diphenyl-1-picryl hydrazyl radical were determined by spectrophotometry at a longwave of 517 nm. Radical scavenging activity was measured by a slightly modified method previously described by [24]. The following concentrations of the extracts were prepared, 0.001, 0.01, 0.1 and 1 mg/mL in ethanol. Ascorbic acid was used as the antioxidant standard at the same concentrations. 1 mL of the extract was placed in a test tube, and 2 mL of ethanol solution of DPPH (0.1 mM) was added. A blank solution was prepared to contain the same amount of ethanol and DPPH. The radical scavenging activity was calculated using the following formula:

$$\% \text{ Inhibition} = [(A_0 - A_1)/A_0] \times 100$$

with A_0 : Absorbance of control at a longwave of 517 nm and A_1 : Absorbance of tested substance at a longwave of 517 nm.

2.5.5. Hydrogen Peroxide (H₂O₂) Scavenging Activity

In this study, we used a prior method of [25], with some modifications for our experiments. A solution of 43 mM H₂O₂ was prepared in phosphate buffer solution (pH 7.4). Both extract and standard solution were prepared at four different concentrations (10^{-3} - 1 mg/mL). 2 mL of different concentrations (10^{-3} - 1 mg/mL) of *Garcinia kola* seeds extract were added to a H₂O₂ solution (1 mL, 43 mM). Absorbance was measured at 230 nm by spectrophotometer. A blank was prepared using a sodium phosphate buffer without H₂O₂. The percentage of

H₂O₂ scavenging was calculated using the following equation:

$$\% \text{ Inhibition} = [(A_0 - A_1)/A_0] \times 100$$

with A_0 : Absorbance of the control and A_1 : Absorbance in the presence of the sample.

2.6. Hemostatic Activity

2.6.1. Preparation of Platelet-Poor Plasma

Platelet-poor plasma preparation was realized according to the method described by the professional order of medical technologists of Quebec [26]. Platelet-poor plasma (PPP) was separated from citrated rabbit whole blood. At first, the whole blood was centrifuged at 2500 rpm for 15 min. The supernatant obtained, was removed without disturbing the pellet. To be sure that the plasma was devoided of platelet, second centrifugation was operated at 2500 rpm for 10 min. The new plasma was taken without cellular debris and was stored at -20°C until used.

2.6.2. Activated Partial Thromboplastin Time (aPTT) Assay

Intrinsic and extrinsic pathway of coagulation was determined according to the method described by [27], with slight modifications. Plasma (43 μL) was pipetted into clotting tubes and warmed for 2 - 3 min at 37°C . Then, 7 μL of distilled water (for control), and of plant extracts (10^{-1} - 1 mg/mL) for the test were added. Cephalin-koalin reagent (50 μL) and calcium chloride (50 μL) were added to the mixture respectively. Cephalin-koalin reagent and calcium chloride were pre-warmed at 37°C for 2 - 3 min. Phytomenadione (10^{-1} - 1 mg/mL) was used as reference drug. The coagulation time was recorded with a coagulometer (CyanCoag, Belgium).

2.6.3. Prothrombin Time (PT) Assay

To explore the extrinsic pathway of coagulation, 43 μL of plasma was pipetted into clotting tubes and incubated for 2 - 3 min at 37°C . Then, 7 μL of distilled water (for control), and of plant extracts (10^{-1} - 1 mg/mL) for the test, were added to clotting tubes. Prothrombin reagent (100 μL) pre-warmed at 37°C for 2 - 3 min was added to the mixture. Phytomenadione (10^{-1} - 1 mg/mL) was used as reference drug. The coagulation time was recorded with a coagulometer (CyanCoag, Belgium).

2.7. Acute Toxicity

The acute oral toxicity of *Garcinia kola* seeds extract was performed according to the OECD 423 [28], guidelines. Nine female Rats were divided in three groups of three animals. The first group received distilled water used as reference, and the two other groups were treated with 2000 and 5000 mg/kg b.w. of the extract. To study the behavioral changes, the treated rats were observed every 30 min for a period of two (2) hours [29]. The mortality of animals was appreciated after 24 hours and the toxicity of the extract was determined.

2.8. Bleeding Time Activity

Rat tail hemorrhage model is one of the most commonly used animal models for preclinical efficacy studies of hemostatic agents [7] [30]. *In vivo* hemostatic activity of *Garcinia kola* seeds extract was investigated as described by [30]. Twenty rats of both sexes, were divided in five groups of four animals. Distilled water was administered per os to the control group. The second group received phytonadione (15 mg/kg b.w., positive control). The three other groups were pretreated orally with 250, 500 and 1000 mg/kg b.w. of the extract for four days. The treated rats were anesthetized with ketamine (100 mg/kg b.w.) and the tip of their tails was cut to induce bleeding. The site of bleeding was gently blotted with filter paper every 30 s, till cessation of bleeding. The observation of time was limited to 20 min. Care was taken that no pressure was exerted on the tail tips that could affect homeostasis.

2.9. Hematological Assay

Blood samples were collected from rats used to achieve bleeding time assay, into heparinized tubes. Hematology Analyzer (Sysmex XN-1000, Japan) was used to determine platelet counts. This automate was able to measure several others parameters such as white blood cell counts, red blood cell count. Blood is sampled and diluted, and moves through a tube thin enough that cells pass by one at a time. Characteristics about the cell are measured using lasers (fluorescence flow cytometry) or electrical impedance.

2.10. Statistical Analysis

All statistical analyses were performed using Graph-pad Prism 5 (Graph-pad Software Inc., USA). The results were expressed as mean \pm sem of four independent measurements. Statistical analysis was determined by using One-way Analysis of Variance (ANOVA), and Turkey's multiple comparison test was also applied. Results were indicated as significant at $p < 0.05$.

3. Results

3.1. Phytochemical Screening of *Garcinia kola* Seeds Extract

The phytochemical study of the aqueous extract of *Garcinia kola* seeds revealed the presence of sterols and polyterpines, polyphenols and alkaloids (Table 1).

3.2. Hemolytic Activity of *Garcinia kola* Seeds Extract (EAgk)

Red blood cell of rabbit was used to assess hemolytic activity of *Garcinia kola* seeds extract (EAgk). EAgk induced $13.85\% \pm 4.5\%$ and $12.66\% \pm 2.3\%$ of hemolysis at 10^{-3} and 10^{-1} mg/mL respectively. Water (positive control) and saline solution (NaCl 0.9%) used as negative control, are showed percentage of hemolysis of $90.1\% \pm 10.2\%$ and of $10.04\% \pm 2.26\%$ respectively. The percentage of hemolysis of EAgk is very close to that of saline solution (isotonic solution).

Table 1. Phytochemical analysis of aqueous extract of *Garcinia kola* fresh seeds.

Phytochemicals	EAgk
Sterols and polyterpines	+
Polyphenols	+
Flavonoids	–
Saponins	–
Quinones	–
Alkaloids	+
Tannins	–

“+ means presence, – means absence”.

The extract did not show any harmful effects on erythrocytes. Water (hypotonic solution) used as reference, showed a significant hemolysis activity ($p < 0.001$, $n = 4$) (Figure 1).

3.3. Antihemolytic Activity of *Garcinia kola* Seeds Extract

2,2'-Azobis(2-methylpropionamide) dihydrochloride (AAPH) was used to study antihemolytic activity of EAgk. *Garcinia kola* seeds exhibited antioxidant activity, thereby protecting erythrocytes from hemolysis. The percentage of hemolysis inhibition of EAgk was increased in a concentration-dependent manner, with IC_{50} value of 1.02 ± 0.2 mg/mL compared with $0.65 \times 10^{-2} \pm 0.5$ mg/mL for ascorbic acid, which served as positive control. Ascorbic acid showed significant inhibition of hemolysis ($p < 0.001$, $n = 4$). The antihemolytic effects of EAgk and ascorbic acid are summarized in Figure 2.

3.4. DPPH Radical Scavenging Activity of *Garcinia kola* Seeds Extract

The aqueous extract of *Garcinia kola* seeds (EAgk) was shown to have significant concentration-dependent DPPH radical scavenging activity, with a concentration required to inhibit radical formation by 50 % (IC_{50}) value of $3.2 \times 10^{-2} \pm 0.12$ mg/mL. In comparison, ascorbic acid, as control, was used and IC_{50} value obtained was $1.4 \times 10^{-3} \pm 0.2$ mg/mL. EAgk and ascorbic acid at the unique concentration of 10^{-1} mg/mL, induced DPPH scavenging activities of $74.14\% \pm 4.21\%$ and $83.68\% \pm 2.36\%$ respectively (Figure 3).

3.5. Peroxide Scavenging Activity of *Garcinia kola* Seeds Extract

The extract scavenged hydrogen peroxide in a concentration-dependent manner with the IC_{50} value of 1.01 ± 0.5 mg/mL compared with IC_{50} value of $0.52 \times 10^{-1} \pm 0.1$ mg/mL for ascorbic acid. EAgk and ascorbic acid at the unique concentration of 10^{-1} mg/mL, induced peroxide scavenging activities of $26.85\% \pm 2.41\%$ and $32.57\% \pm 2.3\%$ respectively (Figure 4).

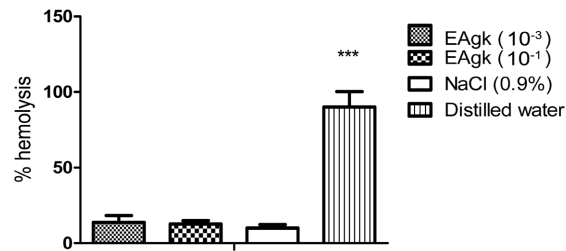


Figure 1. Hemolytic effects of EA_{gk}, distilled water and saline solution (NaCl 0.9%). EA_{gk} hemolytic activity was very close of that of saline solution (negative control). Distilled water (positive control) showed a significant hemolysis effects (**p < 0.001, n = 4).

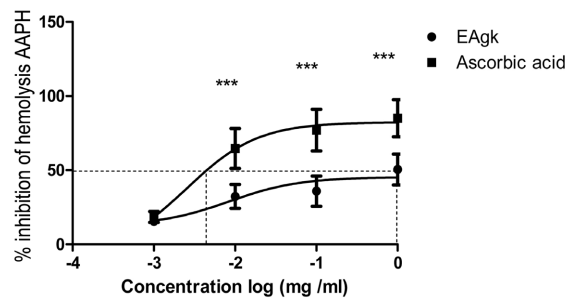


Figure 2. Antihemolytic effects of EA_{gk} and ascorbic acid on AAPH-induced hemolysis. EA_{gk} and ascorbic acid inhibited hemolysis with IC₅₀ values of 1.02 ± 0.2 and 0.65 × 10⁻² mg/mL respectively. Difference between EA_{gk} and ascorbic acid values are significant (**p < 0.001, n = 4).

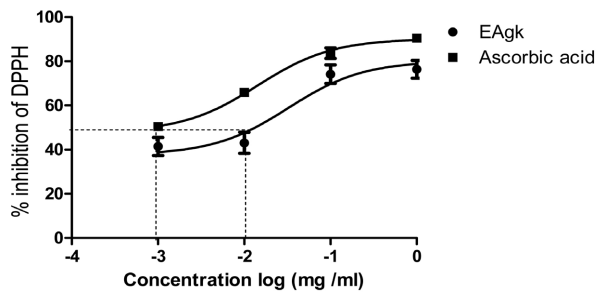


Figure 3. Scavenging effects of EA_{gk} and ascorbic acid on DPPH free radical. EA_{gk} and ascorbic acid inhibited DPPH free radical with IC₅₀ values of 3.2 × 10⁻² ± 0.12 and 4 × 10⁻³ ± 0.2 mg/mL respectively.

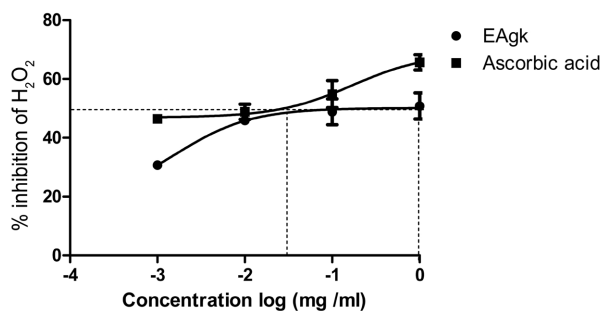


Figure 4. Scavenging effects of EA_{gk} and ascorbic acid on peroxide hydrogen. EA_{gk} and ascorbic acid inhibited peroxide radical with IC₅₀ values of 1.01 ± 0.5 and 0.52 × 10⁻¹ ± 0.1 mg/mL respectively.

3.6. Hemostatic Activity of *Garcinia kola* Seeds Extract

The plasma poor platelet was used to study the hemostatic effects of *Garcinia kola* seeds and Phytomenadione (vitamin K₁).

3.6.1. Effects of *Garcinia kola* Seeds Extract on Activated Partial Thromboplastin Time (aPPT)

The aqueous extract of *garcinia kola* seeds caused the decrease of activated partial thromboplastin time in a concentration dependent manner. The most important decrease of this time, induced by EAgk and phytomenadione (Vitamin K₁) used as positive control, were operated at the concentration of 1 mg/mL. But these decreases were not significant compared to the negative control which was distilled water ($p > 0.05$, $n = 4$). The outcomes are summarized in **Figure 5**.

3.6.2. Effects of *Garcinia kola* Seeds Extract on Prothrombin Time (PT)

The prothrombin time was not affected by the aqueous extract of *Garcinia kola* seeds and vitamin K₁. *Garcinia kola* seeds extract and vitamin K₁ effects are shown in **Figure 6**.

3.7. Acute Oral Toxicity of *Garcinia kola* Seeds Extract

EAgk at the dose of 5000 mg/kg b.w. did not cause any mortality in rat. However

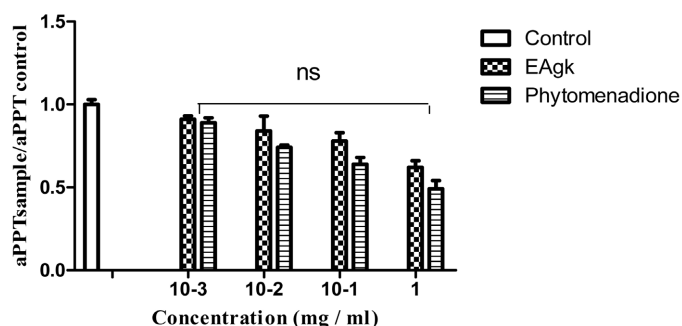


Figure 5. Effects of EAgk and Phytomenadione on activated partial prothrombin Time (aPPT) *in vitro*. EAgk and phytomenadione decreased coagulation time in concentration-dependent manner by intrinsic pathway. This decrease was not significant (ns $p > 0.05$, $n = 4$).

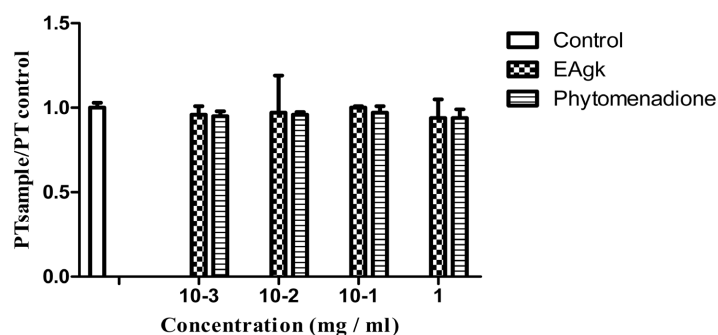


Figure 6. Effects of EAgk and Phytomenadione (vitamin K₁) on prothrombin time *in vitro*. EAgk and phytomenadione did not affect coagulation time by extrinsic pathway.

at the same dose, EA_{gk} induced a general modification of rat behavior compared to the control group. They became remarkably quiet, and remained in groups at the corner of the cage. Their spontaneous movement was reduced for 2 hours.

3.8. Effects of *Garcinia kola* Seeds Extract on Bleeding Time

Bleeding time was shortened in rats treated with different doses (250, 500 and 1000 mg/kg b.w.) of the aqueous extract of *Garcinia kola* seeds compared to the control group of rat. The decrease of bleeding time was dose-dependent. Rat groups treated with EA_{gk} (1000 mg/kg b.w.) and Phytomenadione (15 mg/kg b.w.) were shown to reduce significantly bleeding with the time values of 157.5 ± 18.9 s and 127.5 ± 25.5 s respectively, compared to control rats (420 ± 27.3 s) ($p < 0.001$, $n = 4$). The results are shown in **Figure 7**.

3.9. Effects of *Garcinia kola* Seeds Extract on Platelet Count

Platelet count was increased in rats treated with different doses (250, 500 and 1000 mg/kg b.w.) of the aqueous extract of *Garcinia kola* seeds compared to the control group of rat. This increase of platelet count was dose-dependent. Rat groups treated with EA_{gk} (1000 mg/kg b.w.) and Phytomenadione (15 mg/kg b.w.) were shown to increase significantly platelet count, compared to control group ($p < 0.1$; $p < 0.01$, $n = 4$). Outcomes are shown in **Figure 8**.

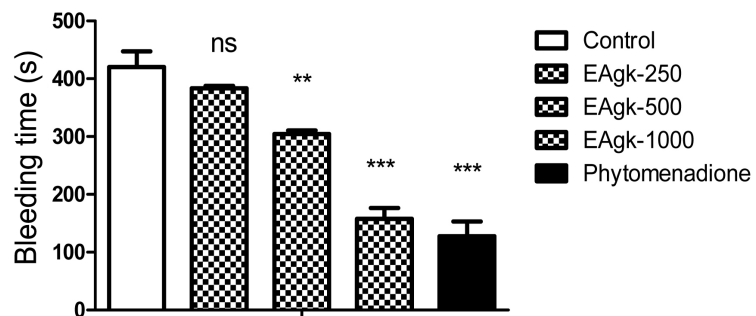


Figure 7. Effects of EA_{gk} and phytomenadione (vitamin K₁) on bleeding time *in vivo*. EA_{gk} (1000 mg/kg b.w.) and phytomenadione (15 mg/kg b.w.) shortened significantly coagulation time (*** $p < 0.001$, $n = 4$).

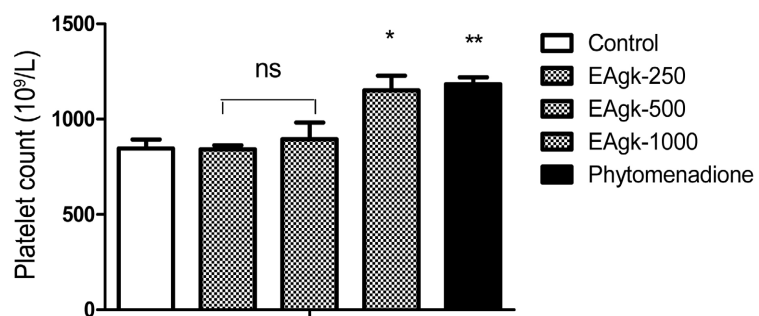


Figure 8. Effects of EA_{gk} and phytomenadione on platelet counts *ex vivo*. EA_{gk} (1000 mg/kg b.w.) and phytomenadione increased significantly platelet counts (* $p < 0.1$; ** $p < 0.01$, $n = 4$).

4. Discussion

The hemostatic effects of the aqueous extract of *Garcinia kola* seeds and phytonadione were assessed under different conditions. Phytonadione or vitamin K₁ (coagulant) was considered a reference drug. *In vivo*, *ex vivo* and *in vitro* tests were used in this study. *Garcinia kola* seeds extract as well as phytonadione shortened significantly bleeding time in rat. To further confirm the seeds extract bleeding time shortened action, we evaluated its effects on platelet count, on prothrombin time and on activated partial thromboplastin time.

In *ex vivo* experiments, *Garcinia kola* extract and vitamin K₁ increased platelet count. The correlation between platelet counts and bleeding times has been reported by several authors. Platelet count was significantly lower and bleeding time significantly prolonged in patients with preeclampsia compared with the control group [31]. Also, high platelet counts were associated with 1.8-fold risk of arterial thrombosis in the brain [32]. Thus, platelet count increase induced by *Garcinia kola* seeds extract could justify bleeding time shortened effects.

Garcinia kola extract and phytonadione shortened activated partial thromboplastin time *in vitro* whereas prothrombin time was not affected. However, the decrease of activated partial thromboplastin time was not significant. The extract could act by intrinsic pathway of the coagulation. This way involves the plasma proteins factors VIII, IX, XI and XII; and prekallikrein and high molecular weight kininogen [4].

It is well documented in the literature that phytonadione strengthens coagulation as measured by prothrombin time decrease and increases in the activity of vitamin K-dependent clotting factors and thrombin generation [33]. Vitamin K₁ effects on prothrombin time in this study are different from those obtained in our experiments. This difference may be due to experimental conditions which are not the same.

According to professional order of medical technologists of Quebec, it is strongly advised not to carry out hemostasis analysis on a hemolyzed sample [26]. Hemolytic samples are unsuitable for coagulation assays because of the release of hemoglobin, intracellular components, and thromboplastic substances from damaged blood cells [34].

Hemolytic and antihemolytic activities of *Garcinia kola* seeds extract were undertaken to appreciate the ability of the extract to protect red blood cells against hemolysis.

Preincubation of red blood cells with *Garcinia kola* seeds extract, did not show any harmful effects on cells. In addition, the extract inhibited 2,2'-Azobis(2-methylpropionamidine) dihydrochloride (AAPH)-induced hemolysis. At physiological temperature, AAPH can decompose to generate alkyl radicals to initiate lipid peroxidation and hemolysis [35]. Protective action of *Garcinia kola* seeds extract against hemolysis may be due to the ability of the extract to scavenge 2,2-Diphenyl-1-picryl hydrazyl radical and peroxide radical assessed in our study. The radical scavenging properties of *Garcinia kola* seeds extract could be attri-

buted to the presence of phenolic compounds revealed through phytochemical screening.

Polyphenols are responsible for many pharmacological activities such as scavenged free radical and hemostatic activity [36] [37].

5. Conclusion

The present study indicates that the aqueous extract of *Garcinia kola* seeds shortens bleeding time, activated partial thromboplastin time and increases platelet counts. The extract protected blood red cell against hemolysis and could accelerate blood coagulation through intrinsic pathway. *Garcinia kola* seeds possess hemostatic properties. This property is justified by the presence of coagulant components in the extract. More studies are needed to isolate the coagulant component and to provide a better insight into the mechanism of these pharmacological actions.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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