International Research Journal of Biological Sciences ISSN 2278-3202
Vol. **14(1),** 32-37, February **(2025)** *Int. Res. J. Biological Sci.*

In vitro anticoagulant and antioxidant activities of stem bark extracts of *Piliostigma thonningii* (Schumach.) Milne-Redh

Kouassi Konan Armand Marcelin[1*], Golly Koffi Julien[2], N'guessan Elouaflin Ettienne De Bessel[1] and N'guessan Jean David[1]

[1]Laboratory of Biology and Health, Unit of –Biochemical-Pharmacodynamics, Félix Houphouët-Boigny University of Abidjan, Côte d'Ivoire, PO BOX 582 Abidjan 22, Côte d'Ivoire

[2]Pasteur Institute of Côte d'Ivoire, Immunity Biology Pole, PO BOX 490 Abidjan 01, Côte d'Ivoire

kouassikonan@yahoo.fr

Available online at : www.isca.in, www.isca.me

Received 30th October 2024, revised 16th November 2024, accepted 19th December 2024

Abstract

Thrombotic diseases, i.e. diseases related to blood clotting, are currently a major health problem and one of the main causes of mortality in the world. In this study, the anticoagulant and antioxidant activities of stem bark extracts of Piliostigma thonningii were evaluated. The anticoagulant activity was evaluated on citrated and depleted plasma using two chronometric tests: prothrombin time (PT) and activated partial thromboplastin time (aPTT). The phytochemical screening of extracts was carried out using specific reagents and the antioxidant activity was determined by the 1,1-Diphenyl-2-picrylhydrazyl (DPPH) assay. The results indicate that the aqueous stem bark extract of Piliostigma thonningii at 80 and 100mg/mL showed significant anticoagulant activity in aPTT test, while no significant effect was observed in PT test. Concerning the hydroethanolic one, the clotting time was significantly prolonged in PT and aPTT tests at concentrations of 80 and 100 mg/mL. The phytochemical analysis revealed the presence of polyphenols, flavonoids, tannins, quinones and alkaloids in aqueous and hydroethanolicstem bark extracts of Piliostigma thonningii. Moreover, both extracts exhibited DPPH scavenging activity with antiradical power (AP) of 5.32 ± 0.03µmol/mg for aqueous (IC_{50} = 18.8 ± 0.12µg/mL) and 8.6 ± 0.33 µmol/mg for hydroethanolic extract (IC_{50} = 11.67 ± 0.44µg/mL).

Keywords: Anticoagulant activity, clotting time, antioxidant activity, *Piliostigma thonningii.*

Introduction

Thrombosis occurs when a blood clot (thrombus) develops within a vein or artery, obstructing the normal flow of blood. When dislodged, these clots can block blood flow to vital organs, potentially leading to serious conditions such as ischemic heart disease[1], and cerebrovascular disease[2]. Thrombotic disorders represent a significant global health concern and are among the leading causes of death worldwide. They arise due to disruptions in the blood coagulation system, resulting in various severe health complications, including venous thromboembolism, which may manifest as deep vein thrombosis and/or pulmonary embolism[3].

Reactive oxygen species (ROS) are naturally occurring free radicals produced within cells, playing crucial roles in numerous cellular biochemical processes including signal transduction, gene transcription, and regulation of soluble guanylate cyclase activity[4,5]. One significant characteristic of ROS is their capacity to induce oxidative damage to DNA, proteins, and lipids. Overproduction of these species can result in oxidative stress, leading to impaired cellular function and, ultimately, cell death through apoptosis or necrosis[6,7]. Oxidative stress can also directly impact the blood coagulation system. It can trigger the activation of the sympathoadrenal medullary system, which subsequently stimulates both the coagulation cascade and the fibrinolytic system, ultimately resulting in hypercoagulability[8].

Antioxidants are substances that can shield the body from damage induced by oxidative stress. They possess the ability to mitigate or prevent harm caused by free radicals by intercepting, delaying, inhibiting their functions, or disrupting the oxidation chain reaction[9].

The treatment for blood clot embolism typically involves medications such as antiplatelet, anticoagulant, or thrombolytic agents. Heparin has served as a cornerstone in anticoagulant therapy for acute thrombotic conditions for many years. However, its clinical utility is hindered by certain limitations, including ineffectiveness in patients with antithrombin deficiency, risk of bleeding complications, potential development of heparin-induced thrombocytopenia, as well as side effects such as immunosuppression and osteoporosis with prolonged use[10,11]. Therefore, there is a pressing need to explore safer and more efficacious treatment options derived from natural sources.

Medicinal plants represent a potential source of natural bioactive molecules, and are the focus of scientific studies aimed at discovering new therapeutic molecules effective

against various diseases including thrombotic diseases. *Piliostigma thonningii* (homotypic synonym: *Bauhinia thonningii*) is a plant used for various medicinal purposes in African countries. Its root and twig have been used for the treatment of dysentery, fever, infections, snake bites, hookworm and skin disease as well as laxative, antihelmintic and anti-inflammatory agents[12]. Roots are also used traditionally as a hemostatic and to promote healing[13]. The leave extract of this plant was reported to protect against the accumulation of cholesterol and triglycerides in the blood[14]. It was also reported that leave extract of *Piliostigma thonningii* appears to cause constriction of blood vessels and reduces bleeding time in rats[15]. The present study was carried out to evaluate the anticoagulant and antiradical activities of aqueous and hydroethanolic stem bark extracts of *Piliostigma thonningii.*

Materials and Methods

Plant materials: The plant materiel consists of dried powdered stem barks of *Piliostigma thonningii* (Schumach.) Milne-Redh. (Fabaceae). Fresh barks of this plant were collected in Bouaké, North-Central of Côte d'Ivoire and samples were identified and authenticated by comparison with specimens deposited at the National Floristic Center, Félix Houphouët-Boigny University of Abidjan, under number UCJ009483. The collected plant material was shade at room temperature for 3 weeks and dried samples were later pulverized using a mechanical grinder.

Chemicals: Reagent kits for prothrombin time test (Dade®Innovin®) and activated partial thromboplastin time test (Actin®FS) were purchased from Siemens Healthcare Diagnostics (France). Heparin® was obtained from Ceplapharm (France). 1,1-Diphenyl-2-picrylhydrazyl (DPPH) was purchased from Sigma Chemical Co. (St. Louis, USA). All the reagents used were of analytical grade.

Extract preparation: One hundred (100) grams of *Piliostigma thonningii* stem barks powder were shaken in 2 L of distilled water for six cycles of homogenization using an electronic mixer. The mixture was then filtered using a Buchner funnel and Whatman filter paper (3 mm). Filtrate was concentrated to dryness under reduced pressure at 30°C using a rotary evaporator (BÜCHI).The resultant extract constitutes the aqueous extract[16]. The hydroethanolic extract was prepared following the same procedure except that plant powder was in this case shaken in ethanol-water (70:30, V/V). These dried extracts were weighed and then stored at 4°C for analysis.

Blood collection and preparation of plasma: Bloodwas taken from 15adult healthy volunteers of both sexes aged 18 to 35 years old. Sick people, people with hemorrhagic diseases, including those who had been transfused in the previous two months or who had taken anticoagulant drugs did not participate in this study. Blood samples were collected over sodium citrate in blue capped tubes and were centrifuged at 3200 rpm for 5 min. The plasma resulting from this operation was then collected in hemolysis tubes.

Determination of anticoagulant activity: Prothrombin time (PT) and activated partial thromboplastin time (aPTT) assays were used toevaluated the anticoagulant activity of the extracts of *Piliostigma thonningii*, according to the method of Caquet[17].

Prothrombin time test: Prothrombin time (PT) test was carried out using commercial reagent kits (Dade®Innovin®). The calcium thromboplastin reagent was prepared with distilled water according to the manufacturer's instructions and pre-warmed at 37°C for 10 min in a water bath. Plasma (90 µL) was mixed with 10µL of samples (20-100 mg/mL) and incubated at 37°C for 5 min. Then, 200µL of PT assay reagent (calcium thromboplastin) was added to each sample range and the clotting time was recorded by a coagulometer (SYSMEX CA-104). Plasma alone (only with NaCl 0.9%) was used as control (absence of anticoagulant activity). Heparin® (1 IU/mL) was used as positive control.

Activated partial thromboplastin time (aPTT) test: The test was carried out using commercial reagent kits (Actin® FS). The partial thromboplastin reagent (Kaolin Platelet substitute mixture), a mixture of Kaolin and Phospholipid, was prepared according to the manufacturer's instructions. The resulting suspension and a calcium chloride solution (0.025M) were separately pre-warmed at 37°C for 10min in a water bath. Plasma (90µL) was mixed with 10µL of samples (20-100 mg/mL) and incubated at 37°C for 5 min. Then, 100µL of partial thromboplastin reagent was added to each sample range and the contents rapidly mixed. After addition of 100 µL of pre-warmed calcium chloride, the mixture was further incubated for 2 min and the clotting time was recorded by a coagulometer (SYSMEX CA-104). Plasma alone (only with NaCl 0.9%) was used as control (absence of anticoagulant activity). Heparin® (1 IU/mL) was used as positive control.

Determination of antioxidant activity: The antioxidant activity of aqueous and hydroethanolicstem bark extracts of *Piliostigma thonningii* was determined using DPPH radical scavenging assay[18]. Different concentrations (1.5µg-100mg/mL) of each extract were added at an equal volume to methanol solution of DPPH (100µM). The mixture was allowed to react at room temperature in the dark for 15 min. The absorbance was then measured spectrophotometrically at 517 nm. The experiment was repeated for three times. Ascorbic acid was used as standard control. The ability to scavenge DPPH radical was calculated by the following equation:

DPPH inhibition (%) = $1-(A_1/A_0) \times 100$
Where: A_0 is the absorbance of DPPH radical without sample extract and A_1 is the absorbance of DPPH radical with sample extract.

The concentration of extract required to scavenge 50% of DPPH radicals (IC_{50}) (µg extract/mL reaction solution) was

determined. The latter is used to calculate the efficient concentration 50 (EC_{50}) (mg extract/µmol DPPH) using the formula:

$$EC_{50} = IC_{50} / \text{concentration of DPPH solution}$$

This last parameter gives the antiradicalar power (AP)=$1/EC_{50}$ expressed as µmol DPPH scavenged/mg extract.

Phytochemical screening: Phytochemical screening was carried out to characterize some secondary metabolites contained in the plant extracts. The large chemical groups of pharmacological interest namely sterols, polyterpenes, flavonoids, tannins, quinone compounds, saponosides and alkaloids were researched. This study is carried out by a qualitative method in test tubes in the presence of specific reagents[19].

Statistical analysis: Statistical analysis of data was performed using Graph Pad Prism 7.0 program. Results were expressed as mean with standard errors on the mean (Mean ± ESM). Differences between means were determined using one way Analysis of Variance (one-way ANOVA) followed by Tukey's multiple comparisons. P-values less than 0.05 ($P<0.05$) were considered statistically significant.

Results and Discussion

Effect of *Piliostigma thonningii* on prothrombin time: In the prothrombin time (PT) test, the results show that the clotting time varies from11.23 ± 0.25 to 31.57 ± 1.81 s with the aqueous extract of *Piliostigma thonningii* (20-100 mg/mL). The PT of the negative control (NaCl 0.9%) being 10.85±0.45 s, these variations correspond to elongation times of 0.38 to 20.72 s. The statistical analysis indicated that the PT of the aqueous extract is not significantly different to that of negative control (Figure-1).

Regarding the hydroethanolic extract (20-100mg/mL), the clotting time varies from 48.79 ± 15.46 to 212.1 ± 10.68 s, with elongation times of 37.94 to 201.22 s. The PT of this extract at concentrations of 40, 80 and 100mg/mL is significantly different ($P<0.05$) to that of the negative control (Figure-1). Heparin®, used as positive control, presented a significant PT (361.4 ± 6.45 s) with an elongation time of 350.55 s.

Effect of *Piliostigma thonningii* on activated partial thromboplastin time: The results of activated partial thromboplastin time (aPTT) test showed that in presence of aqueous extract of *Piliostigma thonningii* (20-100mg/mL), the clotting time varies from 51.37±2.62 to 147.7±2.0 s with elongation times of 15.6 to 111.93s. In fact, the aPPT of the negative control (NaCl 0.9%) recorded is 35.77±2.62 s. Aqueous extract shows significant aPTT at 80 and 100mg/mL (Figure-2).

In presence of hydroethanolic extract (20-100mg/mL), the clotting time varies from 58.83 ± 10.89 to 203.1 ± 4.40 s with elongation times of 23.06 to 167.36 s. This extract shows significant aPTT at 80 and 100 mg/mL compared to that of the negative control (NaCl 0.9 %) which is 35.77 ± 2.62 s (Figure 2). Heparin® also presented a significant aPTT (311.6 ±3.07 s) with an elongation time of 275.83.s.

DPPH radical scavenging activity: The radical scavenging activity of extracts increased with increasing concentration (Figure-3). IC_{50}, EC_{50} and antiradical power (AP) for DPPH radical scavenging activity were reported in Table-1. A lower IC_{50} value and a higher antiradical power correspond to a larger scavenging activity. Extracts showed scavenging activity significantly less ($P<0.05$) than that of Ascorbic acid. Hydroethanolic extract of *Piliostigma thonningii* (AP = 8.6 ± 0.33 µmol/mg) showed the best activity among both extracts.

Table-1: DPPH scavenging activity of stem bark extracts of *Piliostigma thonningii.*

Extracts	IC_{50} (µg/mL)	EC_{50} (mg/µmol)	AP (µmol/mg)
AEPt	$18.8 \pm 0{,}12^{a}$	0.188 ± 0.001^{a}	5.32 ± 0.03^{a}
HEPt	$11.67 \pm 0{,}44^{b}$	0.117 ± 0.004^{b}	8.6 ± 0.33^{b}
Vitamin C	$6.2 \pm 0{,}23^{c}$	0.062 ± 0.02^{c}	16.17 ± 0.6^{c}

AEPt: aqueous extract of *P. thonningii;* HEPt: hydroethanolic extract of *P. thonningii;* IC_{50}: inhibitor concentration 50; EC_{50}: efficient concentration 50; AP: antiradical power. Values in the same column with different superscripts are significantly different ($P < 0.05$).

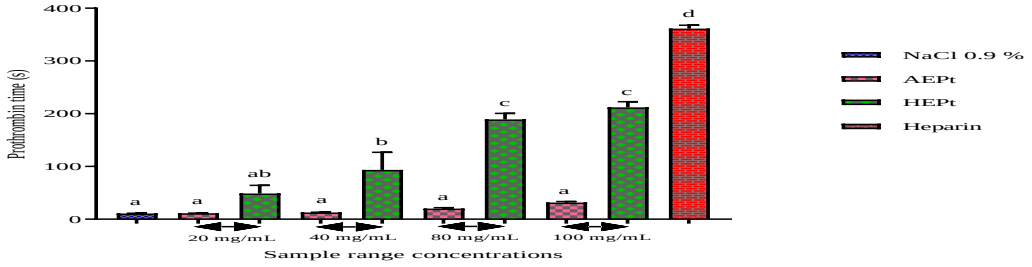

Figure-1: Effect of *Piliostigma thonningii* extracts and heparin on prothrombin time. AEPt: aqueous extract of *P. thonningii;* HEPt: hydroethanolic extract of *P. thonningii.* Results are mean ± SEM values (n=15). Letters represent statistical significance. Mean prothrombin times with different letters are significantly different ($P < 0.05$).

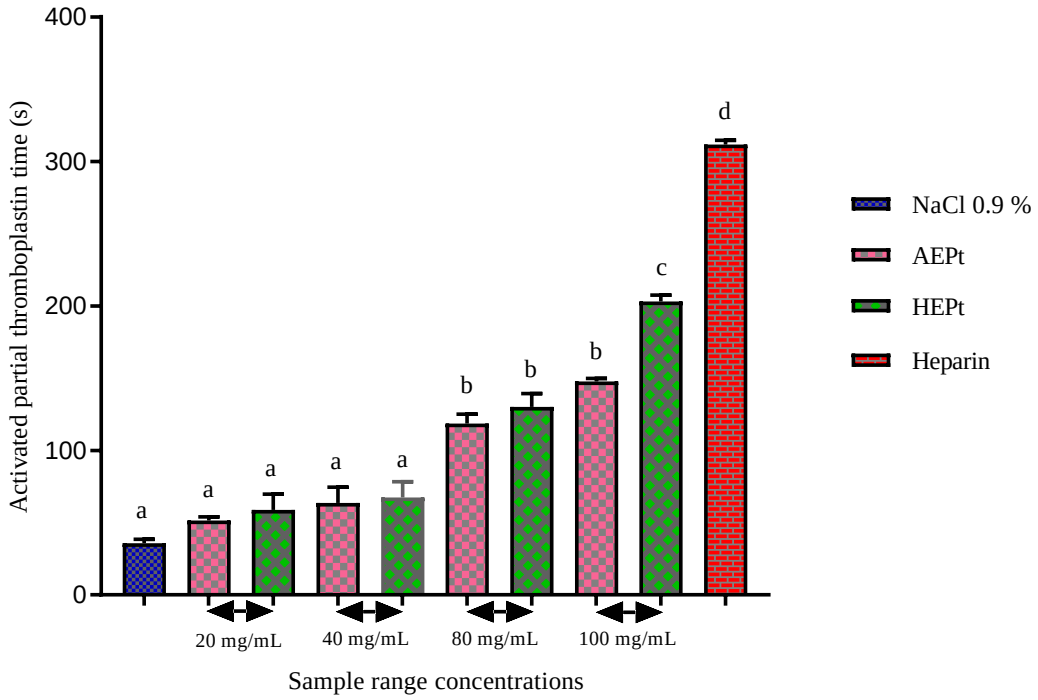

Figure-2: Effect of *Piliostigma thonningii* extracts and heparin on activated partial thromboplastin time. AEPt: aqueous extract of *P. thonningii;* HEPt: hydroethanolic extract of *P. thonningii.* Results are mean ± SEM values (n=15). Letters represent statistical significance. Mean activated partial thromboplastin times with different letters are significantly different ($P < 0.05$).

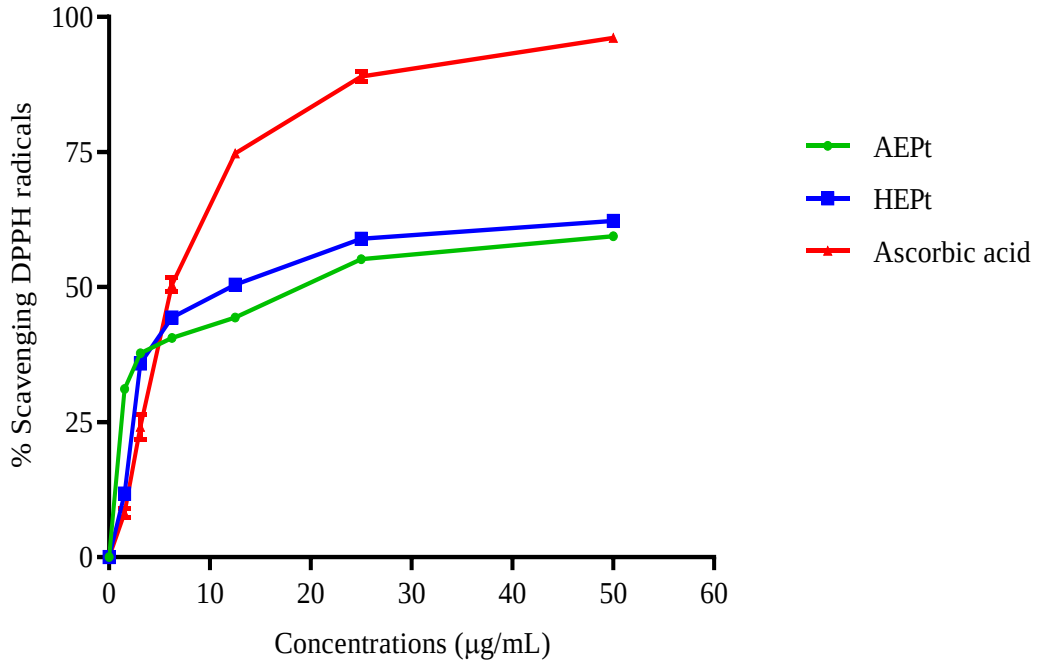

Figure-3: DPPH radical scavenging activity of stem bark extracts of *Piliostigma thonningii* and Ascorbic acid. AEPt: aqueous extract of *P. thonningii;* HEPt: hydroethanolic extract of *P. thonningii.* Results are expressed as percentage of scavenging of DPPH radicals. Values expressed as mean ± SEM (n = 3).

Phytochemical screening: The phytochemical analysis revealed the presence of polyphenols, flavonoids, tannins, quinones and alkaloids in both extracts of *Piliostigma thonningii*. Saponinsare present in the hydroethanolic extract, but absent in the aqueous one (Table-2).

Table-2: Phytochemical compounds of stem bark extracts of *Piliostigma thonningii.*

Chemical groups		Aqueous extract	Hydroethanolic extract
Sterols and polyterpenes		+	+
Polyphenols		+	+
Flavonoids		+	+
Tannins	Gallic	-	-
	Catechic	+	+
Quinones		+	+
Alkaloids		+	+
Saponins		-	+

(+) Presence of chemical group, (-) Absence of chemical group.

Discussion: The ethnopharmacological use of medicinal plants in various disorders, diseases and injuries has motivated the researchers for inspecting the observed effects by scientific studies. The present study aims to assess the anticoagulant and antioxidant activities of stem bark extracts of *Piliostigma thonningii*. The *in vitro* anticoagulant activity was evaluated through the determination of two coagulation parameters, namely the prothrombin time (PT) and the activated partial thromboplastin time (aPTT). The results show that the aqueous and hydroethanolic stem bark extracts of *P. thonningii* prolong the prothrombin time (PT). However, the hydroethanolic extract at 40 to 100mg/mL showed significant anticoagulant activity in PT test compared to the negative control (NaCl 0.9%). No significant effect was observed in PT test with aqueous extract at all concentrations. The activated partial thromboplastin time (aPTT) results indicate that both extracts at concentrations of 80 and 100mg/mL significantly prolonged the clotting time compared to the negative control (NaCl 0.9%).

However, the clotting time of heparin, used as positive control, is better in PT and aPPT tests than plant extracts. The coagulation system comprises a complex sequence of reactions involving many proteins[20]. These reactions convert fibrinogen into fibrin, which forms a clot (thrombus) with platelets. The initiation of the coagulation cascade is split into two pathways: the intrinsic and extrinsic pathways. The extrinsic pathway is primarily responsible for the initial activation of factor X (factor Xa), induced by the factor VIIa/tissue factor complex.

The intrinsic pathway involves coagulation factors XII, XI, IX, and VIII. Activation of the intrinsic pathway amplifies Factor Xa, a key component in the coagulation cascade's central, or common, pathway[20]. The aPTT and PT tests assess the blood's ability to clot within a normal time frame; if any of these factors are impaired or inhibited, the test results will be prolonged. The aPTT test specifically evaluates coagulation factors VIII, IX, XI, and XII in the intrinsic pathway, while the PT test assesses the extrinsic clotting factors, including Factor VII. Additionally, both tests measure the common pathway factors I, II, V, and X within the clotting cascade.

In this study, the significant prolongation of aPTT, but not PT, by aqueous stem bark extract of *P. thonningii* indicates the inhibition of coagulation factors in the intrinsic coagulation pathway[21]. The present results suggest that aqueous extract inhibit preferentially intrinsic pathways of coagulation. The anticoagulant activity of this extract may involve coagulation factors VIII, IX, XI or XII. Elsewhere, hydroethnolic extract which significantly prolonged both aPTT and PT induced the inhibition of coagulation factors in the two coagulation pathways.

Phytochemical investigations of stem bark extracts of *P. thonningii* indicated the presence of polyphenols, flavonoids, catechic tannins, quinones and alkaloids in the hydroethanolic extract and in the aqueous one. This results are in accordance of those of Dieng *et al*.[22] who showed the presence of flavonoids, tannins and saponosides in the hydroethanolic extract of bark from this plant.

Given that anticoagulant activity combined with antioxidant properties may be beneficial for various cardiovascular diseases, the radical-scavenging activity of aqueous and hydroethanolic stem bark extracts of *P. thonningii* was also examined. The results showed that both extracts exhibited DPPH scavenging activity with antiradical power (AP) of 5.32±0.03 µmol/mg for aqueous extract and 8.6±0.33µmol/mg for hydroethanolic extract.

This activity is better than that of *Acacia auriculiformis* (AP = 1.96µmol/mg), *Emblica officinalis* (AP = 3.62 µmol/mg), *Caesalpinia Mexicana* (AP=6.49µmol/mg) fruit extracts obtained by Prakash *et al.*[23]. The free radical scavenging activity of these extracts can be attributed to polyphenols, flavonoids and tannins found whose presence has been revealed.

Conclusion

The present study showed that stem bark extracts of *Piliostigma thonningii* are able to delay blood coagulation *in vitro* by inhibiting coagulation factors. Aqueous extract preferentially inhibits intrinsic pathways of coagulation, while the hydroethanolic one acts on both blood coagulation pathways. Both extracts also have antioxidant capacity by scavenging DPPH free radicals.

Acknowledgements

The authors are thankful to the management of the National Floristic Center, Félix Houphouët-Boigny University of Abidjan, for identification and authentication of the plant, and the Immunity Biology Pole, Pasteur Institute of Côte d'Ivoire, for providing facilities to conduct this research work.

References

1. Ambrose, J. A., & Weinrauch, M. (1996). Thrombosis in ischemic heart disease. *Archives of internal medicine*, 156(13), 1382-1394.

2. Gorelick, P. B. (1986). Cerebrovascular disease: Pathophysiology and diagnosis. *Nursing Clinics of North America*, 21(2), 275-288.

3. Waldo, A.L. (2009). Anticoagulation: Stroke prevention in patients with atrial fibrillation. *Cardiol Clin*, 27(1), 125-135, ix.

4. Uttara, B., Singh, A. V., Zamboni, P., & Mahajan, R. (2009). Oxidative stress and neurodegenerative diseases: a review of upstream and downstream antioxidant therapeutic options. *Current neuropharmacology*, 7(1), 65-74.

5. Chen, K., & Keaney Jr, J. F. (2012). Evolving concepts of oxidative stress and reactive oxygen species in cardiovascular disease. *Current atherosclerosis reports*, 14(5), 476-483.

6. Nita, M., & Grzybowski, A. (2016). The role of the reactive oxygen species and oxidative stress in the pathomechanism of the age-related ocular diseases and other pathologies of the anterior and posterior eye segments in adults. *Oxidative medicine and cellular longevity*, *2016*(1), 3164734.

7. Redza-Dutordoir, M., & Averill-Bates, D. A. (2016). Activation of apoptosis signalling pathways by reactive oxygen species. *Biochimica et Biophysica Acta (BBA)-Molecular Cell Research*, 1863(12), 2977-2992.

8. Pawlak, K., Naumnik, B., Brzósko, S., Pawlak, D., & Myśliwiec, M. (2004). Oxidative stress–a link between endothelial injury, coagulation activation, and atherosclerosis in haemodialysis patients. *American Journal of Nephrology*, 24(1), 154-161.

9. Jung, H. A., Su, B. N., Keller, W. J., Mehta, R. G., & Kinghorn, A. D. (2006). Antioxidant xanthones from the pericarp of Garcinia mangostana (Mangosteen). *Journal of agricultural and food chemistry*, 54(6), 2077-2082.

10. Moll, S. and Roberts, H.R. (2002). Overview of anticoagulant drugs for the future. *Semin Hematol*, 39(3), 145-157.

11. Lapikova, E.S., Drozd, N.N., Tolstenkov, A.S., Makarov, V.A., Zvyagintseva, T.N., Shevchenko, N.M., Bakunina, I.U., Besednova, N.N. and Kuznetsova, T.A. (2008). Inhibition of thrombin and factor Xa by Fucusevanescensfucoidan and its modified analogs. *Bull Exp Biol Med*, 146(3), 328-333

12. Igoli, J.O., Ogaji, O.G., Tor-Anyiin, T.A. andIgoli, N.P. (2005). Traditional medicine practice amongst the Igede people of Nigeria. Part II. *Afr. J. Tradit. Compliment. Altern. Med*, 2(2), 134-152.

13. Burkill, H.M. (1995). The useful plants of West Tropical Africa. 2nd Ed. Vol.3, Families J-L. Royal Botanic Gardens, Kew Richmond, London, pp.1-857.

14. Ighodaro, O.M. and Omole, J.O. (2012). Effects of Nigerian *Piliostigmathonningii* species leaf extract on lipid profile in Wistar rats. *ISRN Pharmacol*, 2012, 1-4.

15. Ozolua, R.I., Alonge, P. and Igbe, I. (2010). Effects of Leaf Extracts of *Piliostigmathonningii* Schum on Aortic Ring Contractility and Bleeding Time in Rats. *J. Herbs Spices Med Plants*, 15(4), 326-333.

16. Zirihi, G.N., Kra, A.K.M. and Guédé-Guina, F. (2003). Evaluation de l'activité antifongique de *Microglossapyrifolia* (Lamarck O. KuntzeAsteraceae) « PYMI» sur la croissance *in vitro* de *Candida albicans*. *Rev Méd Pharm Afr*, 17(3), 11-19.

17. Caquet, R. (2004). 250 examens de laboratoire : prescription et interprétation. 9ème Ed., Masson, Paris, pp.388-389.

18. Koleva, I.I., Van Beek, T.A., Linssen, J.P.H., De Groot, A. andEvstatieva, L.N. (2002). Screening of plant extracts for antioxidant activity: a comparative study on three testing methods. *Phytochem Anal*, 13, 8-17.

19. Wagner, H. and Bladt, S. (2001). Plant drug analysis. A thin layer chromatography atlas. 2nd edition, Springer, Berlin, pp.1-384.

20. Palta, S., Saroa, R. and Palta, A. (2014). Overview of the coagulation system. *Indian J Anaesth*, 58(5), 515-523.

21. Mao, W., Li, H., Li, Y., Zhang, H., Qi, X., Sun, H.-H., Chen, Y. and Guo S. (2009). Chemical characteristic and anticoagulant activity of the sulfated polysaccharide isolated from *Monostromalatissimum* (Chlorophyta). *Int J BiolMacromol*, 44(1), 70-74.

22. Dieng, S.I.M., Fall, A.D., Diatta-badji, K., Sarr, A., Sene, M., Mbaye, A., Diatta, W. andBassene, E. (2017). Evaluation de l'activité antioxydante des extraits hydro-éthanoliques des feuilles et écorces de *Piliostigmathonningii* Schumach. *International Journal of Biological and Chemical Science*, 11(2), 768-776.

23. Prakash, D., Upadhyay, G. and Pushpangadan, P. (2011). Antioxidant potential of some underutilized fruits. *Indo-Global J Pharm Sci*, 1(1), 25-32.