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Decreased thrombin activity by a Congolese herbal medicine used in sickle cell anemia

Marie Miezi Nsimba [a,b], José Nzunzu Lami [b,*], Yumiko Hayakawa [c], Chika Yamamoto [a], Toshiyuki Kaji [a,d]

[a] Organization for Frontier Research, Department of Environmental Health, Faculty of Pharmaceutical Sciences, Hokuriku University, Ho-3 Kanazawa-machi, Kanazawa 920-1181, Japan
[b] Laboratory of Bio-Organic Research, Department of Medicinal Chemistry and Pharmacognosy, Faculty of Pharmaceutical Sciences, Kinshasa University, PO Box 212 Kinshasa XI, DR Congo
[c] Department of Neurosurgery, Faculty of Medicine, Toyama University, 2630 Sugitani, 930-0194, Japan
[d] Faculty of Pharmaceutical Sciences, Tokyo University of Science, 2641 Yamazaki, Noda-shi, Chiba 278-8510, Japan

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ABSTRACT

Ethnopharmacological relevance: Aqueous extracts from *Ceiba pentandra* (Malvaceae/Bombacoideae) and *Quassia africana* (Simaroubaceae) are used as crude medicines for the management of sickle cell anemia (SCA) in the Democratic Republic of Congo (DR Congo). Since it is postulated that the pathogenesis of SCA is associated with an increased blood coagulation activity, the present study is conducted to determine the effect of the two extracts on the coagulation by assessing the thrombin activity and the plasma clotting time.
Materials and Methods: Thrombin activity was measured by chromogenic assay in the presence of the aqueous extracts (10, 100 or 200 μg/ml); and plasma clotting times were measured by activated partial thromboplastin time (APTT) and prothrombin time (PT) in the presence of *C. pentandra* (10, 100 or 200 μg/ml) and *Q. africana* (5, 20 or 50 μg/ml).
Results: Reduced thrombin activity and prolonged plasma clotting time measured by APTT were observed in the presence of C. pentandra extract only. However, plasma clotting time measured by PT was not modified by the use of the two extracts.
Conclusions: This study suggests that the aqueous extract of *C. pentandra* may contain active components that reduce the thrombin activity and prolong the plasma clotting time by affecting the coagulation intrinsic pathway.

1. Introduction

The practice of traditional medicine is widespread in the world. In industrialized countries, over 50% of the population use adaptations of traditional medicines called complementary or alternative medicines (CAM) (Bodeker and Kronenberg, 2002). In China, 40% of urban patients and 90% of rural patients are using traditional medicines; and around 5000 herbs are found there (American Cancer Society, 2013; Hoareau and DaSilva, 1999). In most developing countries, especially in Africa, where access to modern medical care is difficult, up to 80% of the people use traditional medicine to deal with the diseases (Hoareau and DaSilva, 1999). Medicinal plants are almost the sources of natural products which are used for a diversity of pharmacological effects (Rates, 2001; Balunas and Kinghorn, 2005). But, most of them have not yet been scientifically studied in depth to prove their benefits.

The plant materials used in this study, *Ceiba pentandra* (L.) gaertn and *Quassia africana* (baill), are used traditionally in DR Congo to treat SCA (Bouquet, 1969). The "Centre de Phytothérapie Moderne NIECA", an officially recognized medical center located in Kinshasa (DR Congo), administers to SCA patients, since many years, crude drugs from the two plants, *C. pentandra* and *Q. africana*, under the names of BEAT-SS and DOCABE, respectively. This center asserts that the crude drugs alleviate significantly clinical manifestations of SCA patients who undergo treatment with these drugs after some period. The clinical manifestations refer especially to fatigue, acute pains, swelling in hands or feet, and skin pallor. Moreover, non-recourse to blood transfusion is done for a long period after cessation of medication.

C. pentandra, commonly known under the names of silk-cotton or kapok tree, is a big tree generally found in rainforest tropical zones, and is widely used as a herbal medicine in West and Central

* Corresponding author. Tel: +243 99 991 8243; +243 81 329 9876.
E-mail addresses: jose.lami@unikin.ac.cd, jos.lam85@gmail.com (J.N. Lami).

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Africa, South America, and West and South East Asian countries (Burkill, 1985). In African traditional medicine, it is reputed for relieving symptoms in many diseases, almost all the parts of the plant (root, seed, flower, fruit-pod, stem-bark, root-bark, and wood) are widely used for extended purposes (Burkill, 1985). In Congo, a bark-decoction is also taken orally to relieve stomach complaints, diarrhea, hernia, blennorrhea, heart-trouble and asthma; and in mouth-washes and gargles, it is used for gingivitis and toothache (Burkill, 1985). Some of the pharmacological activities have been confirmed by scientific investigations: the plant is reported to have hypoglycemic (Djomeni et al., 2006, 2007; Olusola et al., 2003), antifungal (Nwachukwu et al., 2008), anti-oxidative (Agbor et al., 2007), anti-ulcerous and anti-diarrhetic properties (Abena et al., 2008). The antidrepanocytary activity of *C. pentandra* was observed in the ethanolic extract of the leaves (Mpiana et al., 2007). Yet, the antisickling activity of the aqueous extracts of the bark of trunk and branches of *C. pentandra* and that of the roots of *Q. africana* on the sickle erythrocytes was reported in a recent study (Nsimba et al., 2012).

Q. africana (baill) is a small tree of the lowland rainforest in the transition zone from evergreen to semi-deciduous forest; and all the parts of the plant (bark, leaves, roots and wood) are used in African folk medicine (FAO, 1986). The plant has been found to have antiviral and antipaludic activities (Apers et al., 2002; Lohombo et al., 2003; Mbatchi et al., 2006). The decoction of the bark and leaves is used for gastro-intestinal conditions and as a vermifuge. The root is used to treat bronchial illness, as a febrifuge and as an anti-rheumatic (FAO, 1986).

SCA is a genetic hemoglobinopathy in which the 6th amino-acid of the beta-chain of hemoglobin molecule (glutamic acid) is substituted by valine (Pauling et al., 1949). The disease is characterized by chronic hemolytic anemia accompanied often with fever, infections and unpredicted painful crises due to vaso-occlusion of sickle erythrocytes trapped in small vessels (Pauling et al., 1949; Ingram, 1956; Murayama and Nalbandian, 1973). This is the result of hemoglobins polymerization, which causes the misshape of erythrocytes (sickle erythrocytes). The sickle erythrocytes become rigid and then lose their elasticity or ability to pass trough small vessel where they are stuck (Murayama and Nalbandian, 1973). The phenomenon can lead to congestion and smudging of vascular bed as a result, this can be followed by ischemia and infarct (Fabry et al., 1992; Rosse et al., 2000; Lonergan et al., 2001; Buchanan et al., 2004).

It is now known that SCA is also accompanied by other disorders, notably the defects on the membrane of the sickle RBC, a blood hypercoagulable state (Stathakis et al., 1975; Francis and Cage, 1991; Solovey et al., 1998), a deficit in coagulation inhibitors (Hagger et al., 1993; Westerman et al., 1999; Aysun and Yurdanur, 2001) or a deficit of the fibrinolytic system (Phillips et al., 1988, 1990). According to Francis, abnormalities implicating the increased adherence of sickle RBC to microvascular endothelium play a role in vascular occlusion (Francis, 1988a, 1988b, 1991; Francis and Cage, 1991). Increase in blood viscosity and other vascular disorders observed in SCA are also important factors initiating pathogenesis of occlusive pains (Walters, 1958; Francis, 1997; Cines et al., 1998; Bennett, 2006). Moreover, abnormalities of coagulation related to the increase of thrombin generation and the increase of the activity of blood coagulation factors are reported to amplify fibrin formation that worsen the vascular occlusion (Francis, 1991; Francis and Cage, 1991; Peters et al., 1994; Nsiri et al., 1996). Consequently, these coagulation disorders may lead to thrombotic complications in SCA patients such as pulmonary thrombosis, a life-threatening condition contributing to morbidity in children and almost considered as the leading cause of death in SCA patients (Adedeji et al., 2001). From this view, the use of anticoagulants and antiplatelet agents in the prevention of painful crises and hypercoagulability has been also estimated in the management of SCA (Francis and Cage, 1991; Neil et al., 2002). In the present study, investigations are conducted to examine the action of the aqueous extracts of *C. pentandra* and *Q. africana* on the coagulation by accessing the thrombin activity and the plasma clotting time.

2. Material and methods

2.1. Blood samples

Blood samples were collected from individuals with sickle cell anemia (Hb-SS) and healthy volunteers (Hb-AA) in test tubes containing sodium citrate 3.8% (1 volume of anticoagulant for 9 volumes of blood) and were provided under the authorization of the Congolese ethic committee (No. d'approbation: ESP/CE/048/2009) by the "Centre de Phytothérapie Moderne NIECA" and the "Centre de Médécine Mixte et d'Anémie SS," both located in Kinshasa (DRC). The samples were immediately centrifuged at 3000 rpm for 10 min. Plasma was decanted and stored at −30 °C before the experiment. Sickle cell anemia patients were all on routine medical consultation at the Centre de Médecine Mixte et d'Anémie SS (Kinshasa) and those who did not receive any blood transfusion 2 months ago were selected. All subjects including healthy volunteers were not on anticoagulant medication.

2.2. Preparation of plant extracts

Plant materials and their dried powdered parts, bark of trunk and branches of *C. pentandra* and roots of *Q. africana*, were separately provided by the "Centre NIECA" and were identified by Professor Mulavwa Habari of the "Institut d'Etudes et des Recherches Agronomiques" (INERA), Faculty of Sciences (Kinshasa University DR Congo), by comparison with specimens collected by Wagemans (for *C. pentandra*) and Breyne (for *Q. africana*), voucher specimens 2145 and 3198, respectively, deposited at the Herbarium of INERA. 1000 ml of aqueous extracts from each plant were prepared separately according to the phytotherapist know-how, as explained in previous experiments (Nsimba et al., 2012).

2.3. Measurement of thrombin activity

Thrombin activity was measured by chromogenic assay using commercial human thrombin. Assays were performed in a 96-wells plate in the presence or absence of heparin cofactor II (HC II), 35 μg/ml; and also in the presence or absence of antithrombin III (AT III), 1.25 μg/ml. In the presence of the two cofactors for thrombin inhibition (HC II and AT III), 50 μl of HC II or AT III was introduced in a well and 50 μl human thrombin (0.25 NIHU/ml) was added. After that, 50 μl of either BEAT-SS (10, 100 and 200 μg/ml), DOCABE (10, 100 and 200 μg/ml), dermatan sulfate (250 μg/ml), or heparin (25 μg/ml) were added. Then, 50 μl of assay buffer (50 mM Tris–HCl, pH 8.4; 0.15 M NaCl and 0.1% BSA) was added and incubated at room temperature for 5 min. After incubation, thrombin residual activity was determined by measuring the absorbance at 405 nm after addition of 50 μl of chromogenic substrate (S-2238). The same assay was repeated in the absence of the two cofactors (HC II and AT III): 50 μl of assay buffer was introduced in the well and 50 μl human thrombin (0.25 NIHU/ml) was added and the experiment was further proceeded as stated above. Three control samples were used for all the above assays: (a) the control sample where the thrombin activity was measured with nothing added to thrombin (50 μl); (b) the control sample where the thrombin activity was measured after addition of AT III to thrombin; and (c) the control sample where the thrombin activity was measured after addition of HC II (50 μl) to thrombin. A volume of the assay buffer was added to each sample control to make a total