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Blood coagulation activities of the root extracts of *Fagara xanthoxyloides* plant

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Summary

The coagulant properties of root bark and root wood extracts of *Fagara xanthoxyloides* lam plant are reported. Results of an earlier report which showed that the aqueous extract of the root shortened both the PT and PTT of normal and FVIIIC-deficient plasma are confirmed as well as the absence of many such effects on FIXC-deficient plasma. Root bark manifested twice as much potency as an equal concentration of root wood. The activity could still be demonstrated in the residue of root bark after the lyophilized aqueous extract had been successively extracted with methanol and hexane. It is suggested that these results may have clinical implications.

Résumé

La présente étude établit les qualités coagulatrices de l'écorce et du bois de racine de la plante *Fagara xanthoxyloides* lam. Elle confirme d'ailleurs le résultat de notre première étude qui démontrait d'une part que l'extrait liquide de ces racines raccourcissait les TP et TTP du plasma normal ainsi que ceux du plasma dont le FVIIIC est défectueux, et d'autre part l'absence de toute action sur le plasma manquant de FIXC. L'écorce de racine s'avérait deux fois plus active qu'une même quantité du bois de racine. Après plusieurs extractions avec méthanole et hexane, le résidu de l'écorce présentait toujours les mêmes qualités, ces résultats suggèrent des possibilités médicales.

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Introduction

The medicinal properties of *Fagara xanthoxyloides* lam plant had been known among indigenous populations in the rain forest belt of West Africa for several centuries. In depth chemical investigations of the plant extractives had yielded several chemical products such as fagaramide (Thoma & Thuwen, 1912), quinoline and phenol derivatives (Eshiet & Taylor, 1966), Coumarin (Enyinihi, 1974), sesamin and fagarol (Carnmalm, Erdthman & Pelchowicz, 1955), hesperidin (Arthur, Hui & Ma, 1956) and several other chemicals (Okogun *et al.*, 1981). However, identification of biological properties of some of these agents had lagged behind although some indications were sometimes noted. For instance, antifertility activity of hesperidin had been suggested (Sieve, 1952), and traditional medicine practitioners in parts of Nigeria had advocated use of the crude extract of some parts of the plant in the treatment of abdominal pains and tetanus (Iyoriobhe, pers. comm.).

A recent report ascribing antisickling properties to the crude aqueous extract of root wood (Sofowora & Isaacs, 1971) and some active principle (Sofowora & Isaacs, 1975) has restimulated interest in the biological implications of some of the extracts and extractives. We had earlier reported procoagulant properties of the crude aqueous root extract (Essien & Okogun, 1976), and platelet aggregating activity of Fagarol (Essien & Ebhota, 1978). We now present confirmatory evidence of the

procoagulant activities of the crude extract which was more potent if derived from root bark than root wood.

Materials and methods

Preparation of extracts

The roots of the plant were obtained from Olokomeji, a forest near Ibadan. Root bark was separated from the root wood before 80.2 g of the bark and 75.0 g of the root wood were separately crushed and separately soaked in 4 l water for 5 days. Twenty-four ml fractions of each extract were lyophilized and reconstituted in tris buffer pH 7.4 at a concentration of 2.5 mg/ml of extract shortly before use. Following preliminary results which had shown less potency of the root wood extract, additional extract of root wood at 5mg/ml was also prepared in the tris buffer shortly before use.

Methanol extract

The lyophilized aqueous extract of root bark or root wood was further extracted by mixing each fraction with an equivalent volume of methanol. It was corked and left for 24 h at room temperature before centrifugation and relyophilization. This fraction was reconstituted with tris buffer to 1/10 the original volume from which the aqueous extract was derived.

Hexane extract

Chopped root wood (3 kg) or root bark (2 kg) was continuously extracted with 20 ml fraction of hexane for 24 h, lyophilized and reconstituted to 1/10 the original volume with tris buffer pH 7.4 just before use.

The residue of aqueous extract

The lyophilized aqueous extract was successively extracted with hexane and methanol and the residue that was left was reconstituted with tris buffer to a concentration of 2.5 mg/ml or 5 mg/ml of extract just before use.

Fagaramide and xanthoxylol

Chemically pure substances were obtained after hexane and methanol extracts, crystallization (Eshiet & Taylor, 1966; Calderwood & Fish, 1966; Enyinihi, 1974; Ayafor, 1978) and purification by fractional crystallization (Ayafor, 1978).

Blood plasma samples

Blood samples were collected from a total of 69 blood donors in University College Hospital (UCH) blood bank. Each sample obtained by non-contact clean venepuncture, was mixed in a plastic container with 3.8% trisodium citrate (9:1, v/v). It was centrifuged at 3000 $g \times 5$ min at room temperature and the plasma was separated, kept on crushed ice and used in the tests usually within 1 h of collection. Plasma samples from Factor VIIIC (FVIIIC) or FIXC patients were obtained from our stocks which are usually collected as described above and stored in aliquots at −70°C. Each aliquot was thawed once only at 37°C and used immediately. All unused portions were discarded. To test for deterioration of stored plasma, an aliquot was usually thawed once and checked against a normal plasma by the prothrombin time (PT) test. Normal results of PT, expressed as clotting time and ratio showed that such stored plasma had not deteriorated.

Blood coagulation tests

Modification of prothrombin time (Quick, 1935) and partial thromboplastin time activated with kaolin (PTTK) (Hardisty & Ingram, 1965; Essien, 1974) were used in these studies.

For PT tests, a volume of test or control plasma/extract mixture was first incubated at 37°C for periods varying from 1 to 9 min (Tables 1–4). 0.1 ml of the incubated mixture was then mixed with an equal volume of human brain extract previously thawed at 37°C immediately before use and the PT test was then completed with addition of 0.1 ml of 0.025 M $CaCl_2$. Duplicate determinations on test and control plasma/extract samples wre carried out in a balanced order to minimize drift (Ingram, 1965) and mean values were taken.

The PTT(k) was similarly modified by

preincubating at 37°C, a similar volume of test or control plasma/extract mixture with kaolin. The test was completed by successively adding inosithin and prewarmed $CaCl_2$ to the samples and determining the clotting time.

Control experiments

The control plasma in each batch of tests consisted of normal or factor-deficient plasma diluted either with the extract or where appropriate, with tris buffer to the same extent as the plasma/extract mixture.

Results

The crude aqueous root bark extract at a final concentration of 2.1 μg/μl of plasma progressively shortened the PT of normal plasma on incubation. An identical concentration of root wood extract was less potent (Table 1, Fig. 1). Xanthoxylol and fagaramide did not affect the PT of normal or factor-deficient plasma samples (FVIIIc or FIXc).

The aqueous root bark also shortened the partial thromboplastin time test (PTT) of normal plasma at all incubation times of the plasma/extract mixture (Fig. 2). A less marked effect was observed when the residue, after successive extraction of lyophilized aqueous extract with methanol and hexane, was incubated with normal plasma (Table 2). Equi-

Table 1. Effect of aqueous extract of root bark or root wood on the PT of normal plasma. The final extract concentration in each case was 2.1 μg/μl of plasma for root bark and root wood respectively. The clotting time is expressed in seconds (sec)

Root bark			Root wood	
Control	Test	Incubation time (min)	Test	Control
22.6	23.0	1	22.2	22.5
23.0	21.7	2	17.8	21.3
23.8	15.3	4		
22.9	5.3	5	17.6	21.0
24.4	Clotted	6		
22.6	Clotted	7	16.9	20.6
23.2	Clotted	9		

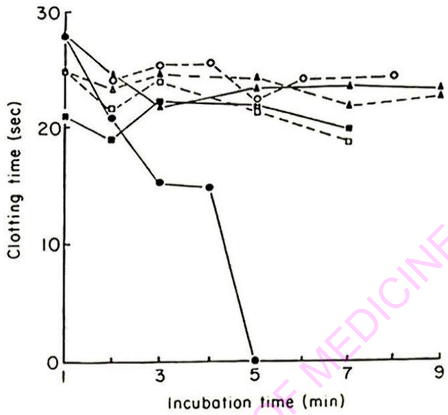

Fig. 1. Effect of xanthoxylol, fagaramide and the root bark extract on the PT of normal plasma. ●—● test mixture of plasma with aqueous extract of root bark; ○—○ test of plasma control for aqueous extract; ▲—▲ test on plasma/fagaramide mixture and △—△ control for fagaramide; ■—■ test on plasma/zanthoxylol mixture and □—□ control for zanthoxylol.

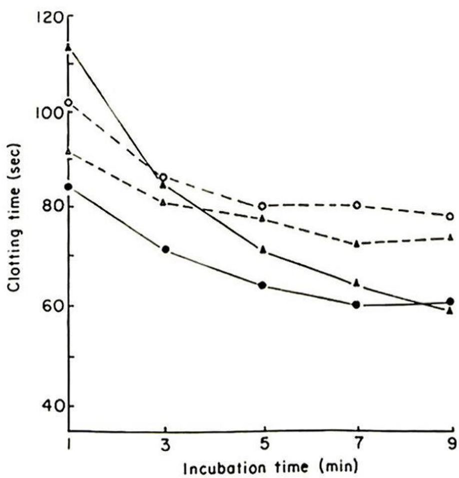

Fig. 2. Effect of root wood (4.2 μg/μl) and root bark (2.1 μg/μl) extracts on PTT(k) of normal plasma. ●—● test on plasma/extract mixture, ○—○ control for root bark; ▲—▲ root wood extract/plasma mixture, △—△ control for root wood.

Table 2. Effect of *residue on PTT(k) of normal plasma

Incubation time (min)	Clotting time (sec)	
	Control	Test
1	70.6	75.5
3	68.2	70.4
5	70.3	37.4
7	63.9	—
9	60.8	40.0

Values are mean of duplicate determinations of a typical result.

*Residue of root bark was obtained from lyophilized aqueous extract after successive extraction with methanol and hexane.

valent concentration of xanthoxylol and fagaramide showed no effect on PTT of normal plasma (Fig. 3).

The PTT of factor VIIIC deficient plasma (FVIIIC) was shortened following incubation with the root bark extract. No such effect was observed when the extract was incubated with FIX deficient plasma (FIXC) (Tables 3 and 4).

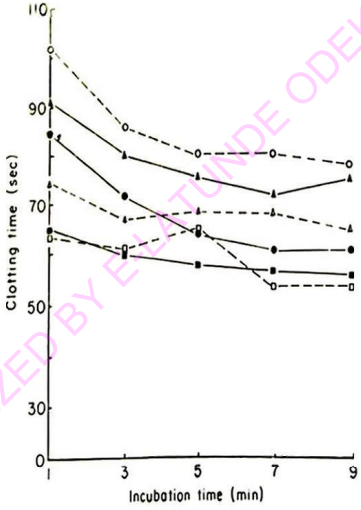

Fig. 3. Effect of xanthoxylol, fagaramide and the root bark extract on the PTT(k) of normal plasma. ●—● plasma/root bark extract mixture; O—O control for root bark extract; ▲—▲ plasma/fagaramide; △—△ fagaramide control; ■—■ xanthoxylol/plasma; □—□ xanthoxylol control.

Table 3. Effect of root bark on PTT(k) of FVIIIc deficient plasma samples

Incubation time (min)	VIIIC Clotting time (sec)		
	Test	Control	Difference
1	251.8	223.3	†28.5
3	212.7	202.1	†10.6
5	177.7	195.4	*17.7
7	145.4	194.6	*49.2
9	132.5	185.7	*53.2

Test and control plasma samples are as described in text.

The difference values refer to the difference between control and test plasma clotting times.

*Indicates a shorter test time relative to its corresponding control.

†Means that the test clotting times were longer than control.

Root wood extract shortened the PTT(k) of normal plasma only at higher concentrations (4.2 µg/µl) than root bark whose effect was observed with 2.1 µg/µl concentration. With lower root wood concentration (2.1 µg-µl), there was no difference between control and test values (Table 5).

Discussion

These results confirm an earlier report that the crude aqueous root extract of *Fagara xanthoxyloides* lam plant possesses procoagulant activities (Essien & Okogun, 1976). They have extended that finding to show that, on a weight basis (w/w), root bark activity was about twice that of root wood. When this finding is considered along with an earlier report which indicated that the same fraction had antisickling properties (Sofowora & Isaacs, 1971) the clinical implications and risk of treating patients with sickle cell anaemia or disease in crisis with the extract are clear. The painful crises of sickle cell diseases (anaemia and disease) are in part a manifestation of sludging of sickled erythrocytes in the tissue capillaries with resultant tissue anoxia or necrosis and often quite severe pain. In the original study, a higher concentration of xanthoxylol than that used in this study demonstrated inhibitory effect on the PTT of normal plasma. A similar concentration was not

Table 4. Effect of root bark extract on PTT(k) of Factor IXc deficient plasma

Clotting time				Clotting time		
Test	Control	Difference	Incubation time (min)	Test	Control	Difference
258.2	227.0	−31.2	1	292.4	267.0	−25.4
255.6	186.8	−31.2	3	283.3	266.2	−17.1
226.8	186.8	−40.0	5	—	261.0	—
233.2	191.2	−42.0	7	258.9	254.1	− 4.8
211.2	190.0	−21.2	9	259.2	250.0	− 9.2

Representative results on FIXc deficient plasma samples.

The extract did not shorten PTT(k); the suggestion of inhibitory effect in some instances was not a regular observation.

Table 5. Effect of low and high concentrations of root wood on the PTT of normal plasma

Low concentration (2.1 µg/µl)			High concentration (4.2 µg/µl)	
Control	PE	Incubation time (min)	Control	PE
101.8	95.8	1	108.2	111.0
86.0	83.7	3	93.0	80.7
80.4	80.5	5	96.8	65.7
80.8	76.6	7	95.8	—
78.8	75.2	9	90.2	65.8

Low concentration of root wood = 2.1 µg/µl of plasma/extract mixture. High concentration = 4.2 µg/µl. PE = plasma/extract mixture. Incubation was at 37°C for the duration of times stated.

used in this study since it was designed to investigate, among other problems, the effect of using concentrations of the extracts/extractives *in vitro* approximately similar to those estimated from discussion with patients to be taken by them in sickle cell crises or for prophylactic purposes.

The exact chemical agent(s) from *Fagara xanthoxyloides* lam plant which manifested procoagulant activities on normal and deficient plasma (FVIIIc) has not been established nor was the mechanism of action investigated further. However, the contrasting results on FVIIIc and FIXc plasmas which showed accelerated reactions in FVIIIc but not in FIXc plasma samples revealed effective substitution for FVIIIa functions in the intrinsic coagulation pathway. This has clinical implications also for classical haemophilic patients. The effect on the extrinsic pathway could suggest involvement of the extract at several points on that pathway excluding direct activation of fibrinogen earlier shown not to be affected (Essien & Okogun, 1976).

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