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Short Communication

Suitability of the leaf extract of *Jatropha gossypifolia* as an anticoagulant for biochemical and haematological analyses

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The extract of *Jatropha gossypifolia* leaf was obtained by crushing the leaf in a mortal and the fluid expressed out. The suitability of the leaf extract as an anticoagulant for biochemical and haematological analyses was determined. The anticoagulant effect of the extract was found to be highest at a concentration of 0.1 ml per ml of blood. Blood tubes with dried leaf extract at the stated concentration were used for obtaining plasma. Mean plasma glucose values obtained from fluoride oxalate plasma were compared with values obtained from dried leaf extract plasma. The values obtained for biochemical parameters with the exception of bicarbonate from the leaf extract plasma samples were significantly higher (P<0.05) than values obtained from conventional anticoagulants. The leaf extract was later found to contain each of these parameters in high concentrations. The results of heamatological parameters obtained from the leaf extract and those of the dipotassium ethylenediamine tetraacetic acid were comparable. The leaf extract is suitable as an anticoagulant for heamatological analysis but must be purified to remove interfering substances for it to be suitable for biochemical analysis.

Key words: *Jatropha gossypifolia*, anticoagulants, analysis.

INTRODUCTION

Jatropha gossypifolia belongs to the family eurphorbiaceae and the order, “Geraniale”. Other members of the family include *Jatropha curcas*, *Jatropha multifida*, *Jatropha podagrica* and *Bridelia ferriginea*. The common name for *J. gossypifolia* is pignut or fignut, and in Yoruba land it is commonly known as “Lapalapa” (Odebiyi and Sofowora 1998). The fruits are three-celled with one seed per cell. *J. gossypifolia* is the common red species planted around houses, and is used as a therapeutic agent in different ways. The leaf decoction of *J. gossypifolia* is used for bathing wounds (Morton, 1968). Morton (1981) and Omoregbe et al. (1996) reported that the leaf bath is used for sores, sprains, rash and bewitchment in Latin America and the Caribbean; the poultices are used for sores and pain in Trinidad (Morton, 1981).

The stem sap stops bleeding and itching of cuts and scratches (Morton, 1981; Hasten et al., 1996) . In Southern Nigeria, the extract from fresh leaf applied with crushed leaf is routinely used by herbalists and local people to stop bleeding from the skin and nose.

The anticoagulant activity of the leaf extract of *J. gossypifolia* was detected while trying to examine its coagulant properties, hence the aim of our present study was to determine the suitability of the anticoagulating agent in the leaf extract of *J. gossypifolia* for collection of blood for biochemical and haematological analyses.

MATERIALS AND METHODS

Materials

100 g of fresh leaves of *J. gossypifolia* was crushed in a mortal for 10 min and the raw juice collected by straining the crushed leaves. The raw juice was centrifuged at 3,500 rpm for 10 min, and the clear supernatant collected. The minimum volume of the extract that achieved anticoagulation was determined by the method of Lee and White (1968).

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Freshly collected whole blood was added to different Khan tubes containing 0.00, 0.02, 0.04, 0.06, 0.08, 0.10, 0.12, 0.14, 0.16, 0.18 and 0.2 ml of leaf extract. The time taken for clot formation in each tube was recorded. The anticoagulant effect of the extract was achieved at 0.1 ml of the leaf extract, which was then pipetted into plain specimen bottles and allowed to dry for 72 h at room temperature.

Biochemical analysis

The analysis was categorized into two. In the first category, 10 ml of blood was obtained by clean venepuncture, 5 ml dispensed into lithium heparin bottles (Control) and 5 ml into dried leaf extract bottles (Test). Sodium (Na^+), Potassium (K^+), bicarbonate (HCO_3^-), Chloride (Cl^-), urea, total protein (TP), albumin (Alb), and globulin (Glob) were determined from these blood samples by the method of Norbert (1986).

In the second category, 5 ml of blood was obtained by clean venepuncture, with 2.5 ml dispensed into fluoride oxalate bottles (controls) and the remaining 2.5 ml into dried leaf extract bottles (test). Blood glucose was determined from these blood samples by Norbert (1986) method.

Haematological analysis

10 ml of blood was obtained by clean venepuncture, with 5 ml dispensed into bottles containing dipotassium EDTA (control) and 5ml dispensed into dried leaf extract bottles (test). Packed cell volume (PCV), total and differential white cell count (WBC), platelets, erythrocyte sedimentation rate (ESR) were determined from these bottles as described by Dacie and Lewis (2001).

Statistics

The mean and standard deviation and the level of significance for the differences between means were computed by students test SPSS 6.

Table 1. Biochemical parameters in blood treated with leaf extracts and standard controls.

Parameter	Fluoride oxalate	Lithium heparin	Leaf extract
Glucose (mmol/L)	3.69 ± 0.31		7.46 ± 0.63
Sodium (mmol/L)		134.7 ± 13.07	199.01 ± 31.13
Potassium (mmol/L)		3.93 ± 0.25	2.37 ± 3.41
Bicarbonate (mmol/L)		24.23 ± 3.11	19.41 ± 4.93
Chloride (mmol/L)		98.53 ± 6.12	111.02 ± 5.01
Urea (mol/L)		2.85 ± 0.53	5.13 ± 1.17
Total Protein (g/L)		59.00 ± 12.13	73.41 ± 6.13
Albumin (g/L)		32.33 ± 6.71	37.35 ± 7.93
Globulin (g/L)		24.91 ± 8.01	30.66 ± 6.91

n = 30 for each parameter.

Table 2. Concentrations of different biochemical parameters in leaf extracts.

Parameter	Concentration in the leaf extract
Glucose (mmol/L)	10.73 ± 0.79
Sodium (mmol/L)	30.0 ± 4.12
Potassium (mmol/L)	11.8 ± 2.11
Bicarbonate (mmol/L	9.1 ± 0.33
Chloride (mmol/L)	19.1 ± 3.43
Urea (mmol/L)	2.76 ± 0.79
Total Protein (g/L)	12.00 ± 1.30
Albumin (g/L	7.85 ± 0.76
Globulin (g/L	3.30 ± 0.31

n = 30 for each parameter.

Table 3. Haematological parameters in blood treated with leaf extracts and standard K_2EDTA.

Parameters	K_2EDTA	Leaf extract
PCV (%)	44.37 ± 4.10	44.29 ± 3.39
WBC (mm^3)	5,150.40 ± 1,925.15	5,105.35 ± 1,879.17
Neutrophils (%)	54.95 ± 9.15	54.83 ± 9.00
Lymphocytes (%)	40.37 ± 9.37	40.75 ± 8.95
Eosinophils (%)	1.63 ± 0.71	1.59 ± 0.91
Monocytes (%)	2.71 ± 1.83	2.59 ± 1.73
Platelets (mm^3)	136,941.72 ± 35,160.16	136,857.39 ± 35,201.25
ESR (mm/hr)	1.10 ± 0.93	1.07 ± 0.85

n = 30 for each parameter.

RESULTS AND DISCUSSION

The concentrations of blood glucose, Na^+, K^+, HCO_3^-, Cl^-, Urea, TP, Alb, and globulin are shown in Table 1. The amount of glucose in blood treated with leaf extract was significantly higher ($P<0.05$) than that treated with fluoride oxalate (Table I). The amounts of Na^+ , K^+, HCO_3^-, Cl^-, Urea, TP, Alb and globulin from blood treated with the leaf extract were significantly higher ($P<0.05$) than the lithium heparin treated blood with the exception of HCO_3^-. Bicarbonate was the only parameter that was significantly lower ($P<0.05$) in the leaf extract plasma than the lithium heparin sample in spite of its high concentration in the extract (Table 2).

The concentrations of Na^+ , K^+, HCO_3^-, Cl^-, Urea, TP, Alb, Glob, and glucose in the leaf extract itself are shown in Table 2. Values obtained for the haematological parameters using leaf extract are comparable to those obtained using K_2.EDTA (Table 3).

From our findings, coagulant property of *J. gossypifolia leaf* could not be demonstrated. Hence the use of the leaf extract of *J. gossypifolia* by herbalists to stop bleeding

requires further scientific exploration. The pseudo-haemostatic activities may be due to pressure applied by the crushed leaf on the blood vessels, which is sufficient to trigger normal homeostasis.

The present study showed that the leaf extract of *J. gossypifolia* has anticoagulant properties. The pH of the leaf extract was found to be 7.0 whereas the pH of the blood plasma is between 7.35 to 7.45, therefore the increased hydrogen ion concentration (H^+) in the leaf extract was responsible for the reduced HCO^-_3 observed in the leaf extract plasma.

One of the criteria for selecting a good anticoagulant is that it should not interfere by adding to or subtracting from the components under study. Since the leaf extract added to the true value of these components, it is therefore not a suitable anticoagulant when these biochemical parameters are to be estimated.

However, the leaf extract did not interfere with red cell count as revealed by the PCV and their morphology as seen under the microscope by examination of stained thin blood film. It also did not interfere with the total white cells, differential, platelets and ESR, hence it can be used as a substitute anticoagulant for K_2EDTA in routine hematological investigations. We do not know yet if the inclusions in the leucocytes are interfered with by metabolites in the leaf extract. To avoid this possibility the purified leaf extract may be more suitable for haematological analysis. The pure anticoagulant in the extract has to be extracted and characterized. This could be a substitute for K_2 EDTA, and may also be useful for assaying biochemical parameters.

Anticoagulants such as heparin and warfarin are used therapeutically to control thrombosis. If the leaf is experimented on laboratory animals and the results are satisfactory, it could also be used therapeutically. In conclusion, *J. gossypifolia* extract can be used for haematological investigations now, but its active chemical must be isolated and purified before it is used for biochemical analysis.

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