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Short Communication

Blood Clotting Effect of Leaf Extracts of *Bryophyllum pinnatum*

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Abstract. Clotting time of blood samples drawn from eight patients was determined using three variants of plant extracts (crude, aqueous and chloroform) of the leaf of *Bryophyllum pinnatum*. These extracts clotted the test blood samples faster than the untreated blood samples, which ranged between 0.34-3.27 min. Blood samples pretreated with heparin (anticoagulant) did not clot, to any extent, when treated with the *B. pinnatum* extracts. All the test blood samples showed different clotting times. The clotting efficiency of the different extracts was crude > aqueous > chloroform > control. The clotting time for different test blood samples, not treated with any variant of the plant extracts (control samples), ranged between 10.363±0.012 and 14.483±0.008 (min). Normal blood clotting time is between 6-10 min, which may indicate deficiencies in one or more clotting factors in the untreated test blood samples drawn from the study subjects.

Keywords: blood clotting, *Bryophyllum pinnatum*, anticoagulant

Blood is a very vital medium of life in the humans. Plasma is the fluid portion of the blood, which constitutes 55% of its volume (Guyton, 1986). Plasma clots on standing, but remains in liquid form if an anticoagulant is added. It is important that the fluid nature of blood is maintained in normal living systems. In certain situations, blood is lost or spilled from open injuries or internal haemorrhage (Guyton, 1996). Bleeding is arrested when clots are formed in the blood (hemostasis). Excessive bleeding can be caused by deficiency of any one of the many blood-clotting factors. However, three major types of bleeding tendencies, namely, vitamin K deficiency, thrombocytopeonia (platelet deficiency), and haemophilia have been studied (Harker, 1987). Excessive bleeding time is desirable to be reduced. The aim of this study was to find out natural remedies based on a plant material for controlling excessive bleeding. This study is expected to be further useful for finding natural agents that may be applied to solve the bleeding problems of haemophiliacs. These natural agents will replace the very expensive clotting factor VIII (antihaemophilic factor A, antihaemophilic globulin), or factor IX (plasma thromboplastin component, antihaemophilic factor B), and become widely available, if discovered (Charin, 1984). Anti-coagulants prevent clot formation, for example, heparin. These act by preventing the activation of factor XI (plasma thromboplastin) and by inhibiting the action of thrombin (Cleavon, 1993), histamine, oxalates and coumarin (dicoumarol derivatives, which inhibit the action of vitamin K). Coagulants, on the other hand, are chemical principles that activate the formation of thrombin and encourage clot formation.

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Chemical agents present in some plant extracts are known to have coagulant properties. The leaf sap of *Bryophyllum pinnatum* is used in Ivory Coast to stop bleeding of cuts and in Mexico for the treatment of failure of menstruation. The leaf mash compounded with palm oil or butter is applied to treat wounds, burns, abscesses, ulcers, sores, swellings and pains. The plant is an ingredient of a prescription used in Congo to hasten the expulsion of the after-birth fluids. Among the numerous medicinal attributes, the plant is reported to have antidiarrhoea, antiulcer, antiinflamatory, antidiabetic, antipyretic, antibacterial, antifungal, and spasmogenic effects. It is used in the Philippines as analgesic to treat headache and rheumatism, in the Ivory Coast for earache and ophthalmic emergencies, in Gabon for itching, in Congo against allergic inflammation, fungal and eczematous infections, and in Nigeria as a diuretic. On account of the wide spectrum of medicinal uses, including those related with blood problems, the leaf extract of *B. pinnatum* was investigated for its blood clotting ability.

Prepration of the extracts. Fresh leaves of *B. pinnatum* were collected (September) from around the Pharmacognosy Department, University of Benin and from the adjacent Ekosodin Village. About 3.5 kg of the leaves were chopped and immersed in distilled water (6 litres) and boiled (1 h). The supernatant was decanted, cooled and collected as the aqueous extract, which was concentrated in a rotatory evaporator and stored in amber coloured bottles as the crude extract. To a part of this crude extract was added 100 ml distilled water and twice its volume of chloroform in a separatory funnel. Both the chloroform and aqueous extracts were separated and further concentrated. Three different extracts were thus obtained

Table 1. Coagulation time (min) of human blood samples treated with crude, aqueous and chloroform extracts of *Bryophyllum pinnatum* leaves

Blood sample code	Crude	Aqueous extract	Chloroform extract	No extract**
A	11.21±0.01	11.34±0.01	14.41±0.18	14.48±0.01
B	10.47±0.01	11.02±0.02	12.18±0.03	12.23±0.02
C	10.06±0.02	10.33±0.02	11.25±0.83	11.11±0.01
D	10.02±0.02	10.07±0.06	10.39±0.01	10.36±0.01
E	10.50±0.02	11.02±0.01	11.14±0.02	11.19±0.01
F	12.10±0.01	12.20±0.01	12.55±0.03	13.20±0.01
G	11.58±0.01	12.11±0.01	12.54±0.01	13.03±0.02
H (control-1)*	no coagulation	no coagulation	no coagulation	no coagulation

*blood samples pretreated with the anticoagulant, heparin (control-1); **blood samples not treated with *B. pinnatum* extracts (control-2).

by this process, namely, the crude extract (obtained on boiling the leaves in distilled water), the chloroform extract, and the aqueous extract. These three extracts were used in the blood clotting tests.

Blood clotting tests. Eight blood samples were tested for clotting; seven with the three variants of the *B. pinnatum* extracts and one with the anticoagulant heparin as the control-1. To 0.2 ml each of the three extracts were separately added 0.5 ml of the test blood samples. Control-2 comprised of blood samples to which neither heparin (as anticoagulant), nor *B. pinnatum* extracts (as coagulants), were added. Runs were done in triplicate. Observations for blood clotting were recorded every 30 sec and the time taken for clotting was recorded in min (Table 1).

The seven blood samples showed different clotting time. The crude extract induced clotting at relatively faster rate than the aqueous or the chloroform extracts. The order of clotting was observed to be: crude > aqueous > chloroform > control-2 (untreated test blood). The rate of clotting was fastest in the presence of crude extract, which in comparison with control-2 was faster between 0.34-3.27 min. No clotting in control-1 containing heparin was observed. It is significant to note that the normal clotting time of blood samples to which no *B. pinnatum* extracts were added (control-2) was quite high (10.36-14.48 min) than the usually expected 6-10 min, which may indicate a deficiency of one or more clotting factors in the population from which the blood samples were taken. Further studies on the chemical constituents in the leaves of *B. pinnatum* responsible for blood clotting are indicated.

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References

Chavin, S.I. 1984. Factor III: structure and function in blood clotting. *Am. J. Hematol.* **16:** 279-297.

Cleavon, G. 1993. *The Design of Synthetic Inhibitors to Thrombin,* pp. 81-143, Plenum Publishing Corp., New York, USA.

Guyton, A.C. 1996. *Medical Physiology,* pp. 469-472, 9th edition, W.B. Saunders and Co., New York, USA.

Guyton, A.C. 1986. *Medical Physiology,* pp. 146-148, 8th edition, W.B. Saunders and Co., New York, USA.

Harker, I.A. 1987. Acquired disorders of platelet function. *Am. N. Y. Acad. Sci.* **509:** 18.