

Fibrinolytic Activity of Some Nigerian Medicinal Plants

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Abstract: The first line drugs in the management of thromboembolic diseases in Nigeria include Urokinase and Streptokinase. These drugs come with their attendant shortcomings. The need to source for cheap and accessible alternative therapy forms the basis of this study. The fibrinolytic potentials of 10 Nigerian medicinal plants: *Anona senegalensis*, *Buchholzia coriacea*, *Citrullus colocynthis*, *Cnidocolus aconitifolius*, *Curculigo pilosa*, *Nicotiana tabaccum*, *Parinari curatellifolia*, *Pepperomia pellucida*, *Sida acuta* and *Xylopiya aethiopic*a extracts were evaluated using water and streptokinase as negative and positive controls respectively. Trace metal (Cu, Fe, Ca and Zn) analysis of the plant extracts was carried out using atomic absorption spectrophotometer. Methodology involved *in vitro* clot lysis method, the crude methanolic extract of *Parinari curatellifolia* (56.122%), *Cnidocolus aconitifolius* (48.385%), *Anona senegalensis* (46.360%), *Xylopiya aethiopic*a (43.205%) and *Buchholzia coriacea* (27.062%) were found to have significant ($P > 0.001$) fibrinolytic activity. Maximum effect was exhibited by the positive standard: Streptokinase ($60.20 \pm 4.30\%$), followed by *Parinari curatellifolia* ($56.122 \pm 3.86\%$), our findings reveal that fibrinolytic activity is independent of elemental Copper or Iron content of the extracts. This study indicated that methanolic extracts of *Parinari curatellifolia*, *Cnidocolus aconitifolius*, *Anona senegalensis*, *Xylopiya aethiopic*a and *Buchholzia coriacea* could serve as cheap and accessible alternative.

Key words: Fibrinolytic, *Anona senegalensis*, *Xylopiya aethiopic*a, calcium, streptokinase.

1. Introduction

Nature has been a source of medicinal agents for thousands of years and continues to be an abundant source for novel chemo-types and pharmacophores, and provides novel chemical scaffolds for elaboration by combinatorial approaches [1].

The chemical constituents present in herbs are a part of the physiological functions of living flora and hence they are believed to have a better compatibility with human body [2]. There is an increased uprising interest in traditional herbal medicine since couple of decades due to their incredible pharmacological activities, economic viability and less side effects in different health care management system [3].

CVS (cardiovascular diseases) involving deep vein thrombosis, stroke, heart attack and hypertension are

the main causes of morbidity and mortality throughout the world every year [4]. The number of patient suffering from CVS is ever increasing yearly worldwide. This phenomenon may be attributed to unbalanced and inadequate diet. Fast food and processed foods have taken over healthy traditional dietary habit [5].

Thrombosis is one of the leading causes of thrombo-embolic disorder affecting millions of persons worldwide [4]. Thrombolytic agents activate the inactive plasma plasminogen to plasmin which in turn degrades fibrin to soluble products and thus establish normal blood flow (fibrinolysis) [6]. Two plasminogens which occur naturally in blood are the t-PA (tissue type) and the u-PA (Urokinase type). Both t-PA and u-PA are trypsin-like serine proteases, which activate plasminogen directly [7, 8].

Extensively investigated sources of fibrinolytic agents include snakes, earthworm, bacteria,

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actinomycetes, fungi [9] and plants [10, 11]. The plasminogen activators from these sources are mainly categorized into two types: non-fibrin specific plasminogen activators such as Streptokinase, Urokinase, and fibrin specific plasminogen activators such as tissue-type plasminogen activators [12].

Streptokinase is a life-saving clot-dissolving drug routinely prescribed universally in the management of heart attack and thrombo-embolic diseases. Streptokinase is a protein secreted to the growth medium by many β -haemolytic Streptococcus strains [7, 8].

Activated plasmin, a natural fibrinolytic agent lyses clot by conversion of fibrinogen and fibrin contained in a clot into monomer, which with platelets and other haemostatic element underlies the pathological process of the acute occlusive disorders [6, 13].

Anona senegalensis otherwise called wild custard apple belongs to the family of Anonaceae. The Yoruba tribe in southern Nigeria calls it "abo". This plant is indigenous to Africa with exception of the northern Africa. The leaves are simple, alternate, oblong, ovate or elliptical. The leaf is green to bluish green with entire margin, short petiole. The fruit is formed from many fused carpel, fleshy, lumpy and egg-shaped. The fruit is usually green but yellow when ripe. The phytochemical constituents include saponin, steroids, flavonoids and glycosides. It is used traditionally as antimicrobial agent and anti-arthritis for intestinal worm and guinea-worm. It can also be used for swollen eyelid and anti-venom [13, 14].

Buchholzia coriacea popularly called wonderful kola belongs to the family Cappariaceae. This plant is an evergreen shrub widely found in West Africa, the seed has sharp and pungent taste when chewed. The leaf is large, obovate, oblanceolate or elliptic, shortly acuminate or acute at the apex; glabrous with prominent midrib below. The flower of the plant is simple or lightly branched raceme. Tannin, flavonoids, saponin alkaloid and glycosides are the secondary metabolite present. Medicinal use includes antihypertensive, antimicrobial, anti-rheumatic,

antidiabetic, antimalarial and trypanosomiasis for snake bite and other uses [15].

Citrullus colocynthis belongs to the family of Cucurbitaceae, with the popular name Bitter Apple. It is native to dry areas of the northern Africa, even though it is widely distributed in the Sahara region of Asia. This is an annual or perennial (wild) herbaceous plant. The stems are angular and rough. The leaves are rough 3-7 lobed, 5-10 cm long. The flower is monoecious, solitary peduncle with ancillary corolla. The fruit is globular, 4-10 cm in diameter. The phytochemicals present are tannin, saponin, alkaloids, flavonoids and glycosides. Traditionally this plant is used to treat stomach troubles and sexually transmitted diseases. Mixture of the powdered leaf and palm oil is used for the treatment of fungal infection. The seed decoction is used as a purgative [16].

Cnidioscolus aconitifolius (Spinach) belongs to the family Euphorbiaceae, which the Niger-Delta indigenous people nick named "Hospital-too-far" as result of its numerous medicinal importance. It is called "efo Iyana-Ipaja" by the inhabitant of the south-western part of Nigeria. This plant is believed to have originated from Yucatan Peninsula of Mexico. It is a shrub, with succulent stem and leaf, which exude milky sap when cut. It produced intense itching when the leaves are harvested with bare hand. The plant can grow to a height of 6 m. The leaves (32 × 30 cm) are pinnately lobed with succulent petiole. The flower is small and dichotomous. The phytochemical constituents are saponin, flavonoids, alkaloids and cardiac glycosides. It is used to cure alcoholism, insomnia and scorpion sting and to darken grey hair and brain improvement [17].

Curculigo pilosa is of Hypoxidaceae family, known as "epakun" by Yoruba tribe in Nigeria. A savannah and herbaceous plant with stout, erect rhizome bearing a cluster of grass like leaves up to 60 cm long with flowers which shoot up to 20 cm at the end of dry season. The main phytochemical presents are alkaloids, saponin, flavonoids, phenol, tannin, cardenolides and

traces of anthraquinones. The plant is mainly used to treat hernia, infertility and to be as antimicrobial agent [18].

Large leaved tobacco named *Nicotiana tobaccum* (Solanaceae) is an annual shrub or small tree which is native to tropical and subtropical America. The plant is about 0.90-1.5 m tall depending on the variety. The leaves are elliptical or oblanceolate, with clustered flowers. Tobacco leaves contain 2%-8% nicotine either as maleate or citrate. 64% of the total nicotine exists in the leaves, 18% in the stem, 13% in the root and 5% in the flower. The secondary metabolites include tannin, flavonoids, phytosterols and triterpenoids. Traditionally, powdered leaf mixed with palm oil is applied to the chest for respiratory complaint. The stem and the leaf can be grounded, dropped on hot coal and inhaled as stimulant. It can be instilled as an enema for the treatment of intestinal worms [19].

Parinari curatellifolia is also known as rough skinned plum which belongs to Chrysobalanaceae family. The leaf of this plant is entire, hard and alternate, distichously with stipules. The flower is greenish-white cyclic zygomorphic, the sepal and the petals are free. The seed is covered with a papery coat which turns brownish after drying. It contains tannin, saponin, carotenoids alkaloids, sterol and flavonoids as phytochemical constituent. The plant is used in dressing of wounds, fracture and dislocation for stomach ache as an antihypertensive and antidiabetic [20, 21].

Pepperomia pellucida otherwise called Silver Bush belongs to Pepperomia family. This plant is indigenous to tropical India. It is an annual herb with pale green translucent erect stem. The leaves are glabrous, medium green on upper surface, while the lower surface is whitish green, thinly fleshy, broadly ovate 1.5-4.0 cm, palmately 3-5 nerved, the apex is acuminate and the base subcordate. The root is fibrous. The phytochemical constituents include sesquiterpenes, phytosterols, flavonoids and stigmasterol. The decoction of the root is used for the treatment of fever,

the mashed aerial parts is applied topically to dress wound, so also as antimicrobial agent, impotence, breast cancer, rheumatic pain and renal disorder [22, 23].

Sida acuta belongs to the family called Malvaceae, it is also called Wire weed. It is indigenous to Mexico and central America. The plant is a tough, erect perennial shrub, branching from the base, up to 1 m. It has a strong branching taproot. The leaves are lanceolate, 2-9 cm long, toothed margin, hairless or with simple hairs. The fruit is wide separating into one seeded mericarp. The flower is usually solitary or 2-3 together on stalks 2-5 mm long. The phytochemical constituents include alkaloids, saponin, tannin, flavonoids and phytosterols. It is used for the treatment of fever, headache, diarrhea/dysentery, malarial and worm infestation, also used as an antioxidant [24, 25].

Xylopia aethiopica otherwise known as West African pepper tree belongs to Anonaceae. It is largely found in low land rainforest of west, central and southern Africa. The plant is a tall, slim, evergreen and aromatic tree 15-30 cm high and about 60-70 cm diameter. The leaves are simple, alternate, oblong, and elliptic to ovate. The fruit is small, 7-24, forming dense cluster, twisted bean like pods, dark brown, cylindrical 1.5-6 cm long and 4-7 mm thick and the contours of the seeds are visible from outside. The seed is black, 5-8 per pod. Phytochemical screening reveals saponin, tannins, flavonoids, cardiac glycosides and steroids. The fruit is used as Flavourant in local delicacy "pepper soup". The fruit is used for cough (bronchitis), stomach ache, dizziness, amenorrhea, bulimia, neuralgia, boils and skin eruption [21, 26].

However, it is a fact (personal communication) that some of these plants apart from being accessible and very cheap are commonly consumed as vegetable, spices and plant of significant medicinal value in folk medicine. Thus, investigating their fibrinolytic potentials forms the basis of this study and the trace metal analyses to explain their *in vivo* activities.

2. Materials and Methods

Plant collection and identification: The plants materials were collected between the month of June-October 2012 in and around both Akure and Port-Harcourt, Nigeria. The plants were identified and authenticated by Mr. AA Ekundayo of Taxonomy department of FRIN (forest research institute of nigeria), Ibadan, Nigeria.

2.1 Plant Material

The collected plant material was air dried for 2 weeks before they were milled into coarse powder. The powdered crude plant materials were individually de-waxed with n-Hexane. The treated powders (200 g) were individually macerated with methanol (2 L) at room temperature for 72 h, after which they were filtered using Whatman filter paper No. 4. Thereafter, the filtrate obtained was further concentrated using rotary evaporator. The concentrates obtained were further dried using water bath set at 40 °C for 4 h.

2.2 Herbal Preparation

The methanol extracts were suspended in 10 mL of methanol to make a stock solution of 100 µg/mL. These suspensions were kept overnight, decanted and filtered using Whatman No. 1 filter paper. These solutions were ready for *in vitro* evaluation of clot lysing activity.

2.3 Chemicals

Lyophilized streptokinase (CSL Behring GmbH, 35,041 Marburg, Germany), all other chemicals and reagents were analytical grade.

2.4 Ethical Approval

Informed consent from healthy volunteers were collected and approved by the institutional ethics committee, and therefore the study was performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments.

2.5 *In vitro* Thrombolysis Activity

Sterile water for injection (5 mL) was added to the commercially available lyophilized Streptokinase vial (1,500,000 IU) and mixed properly. This suspension was used as stock from which 100 µL (30,000 IU) were used for *in vitro* thrombolysis experiment.

Fibrinolytic experiment was carried out as reported by Prasad et al. [27]. In brief, 5 mL venous blood drawn from healthy volunteers was distributed in different pre-weighed sterile Eppendorf tubes (500 µL/tube) and incubated at 37 °C for 45 min. After clot formation, with the aid of Pasteur pipette, the serum was completely removed without disturbing the formed clot. Thereafter the Eppendorf tubes were re-weighed. The experiments were repeated five times for each of the samples.

All the *in vitro* experimental results were expressed as mean \pm SEM of a number of experiments ($n = 5$), and data were evaluated by using student's *t*-test. $P > 0.001$ was considered significant.

3. Results and Discussion

A number of plant sources especially several fruits and vegetables have been studied for their supplements having biological activities such as fibrinolytic activity and there is evidence that consuming such food leads to prevention of coronary events and consequently stroke [10, 11]. Trace elements are implicated in the process of fibrinolysis [6] and as a result of this, these trace elements: Cu, Zn, Fe and Ca were randomly analyzed using atomic absorption spectrophotometer for the 10 plants extracts.

Using Fig. 1 below as our guide, the concentrations of copper across all the extracts were practically constant with an average value of 0.048 ± 0.005 ppm. This implies that Cu probably has no effect on the fibrinolytic activity of the plants.

The level of Zn in the first eight samples seems constant with an average value of 0.048 ± 0.035 ppm, giving the impression that the fibrinolytic activity may not depend on the level of zinc, but *Buchholzia*

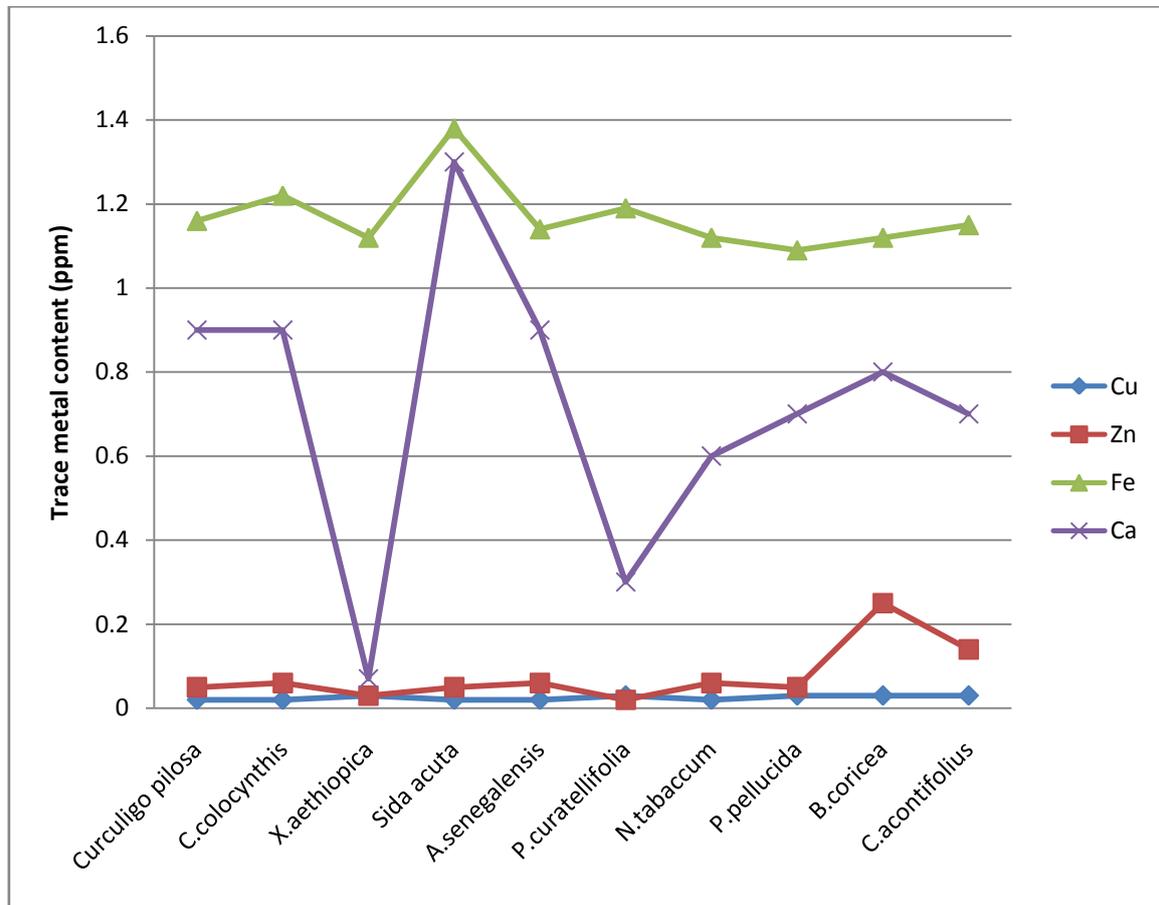


Fig. 1 Trace metal content (ppm) of the methanolic extracts of the plants.

coriaceae gave approximately fivefold increase in the levels of zinc of the plant extracts; meanwhile, *Cnidioscolus aconitifolius* shows a threefold decrease in zinc concentration of the extracts. This significant increase in zinc level may account for the insignificant fibrinolytic activity of *Buchholzia coriaceae* in the methanolic extract (no previous work on the fibrinolytic activity of this plant exist). *Parinari curatellifolia* has the least concentration of zinc (0.02 ppm) amongst the plant extracts; which partially account for its significant fibrinolytic activity in the methanolic extract. Zn is purported to influence haemostasis by impacting on platelet aggregation and coagulation [20].

Calcium had been one of the essential element which serves as a cofactor in clot formation, therefore, calcium is expected to play a significant role in plant extract fibrinolytic activity. Thus it is expected that

high levels of calcium will inhibit fibrinolytic activity. In this work, *Sida acuta* has the highest level of calcium (1.30 ppm) accounting for its anti-fibrinolytic activity thereby justifying its use in arresting bleeding and consequently its wound healing effect [25]. *Xylopiya aethiopica* had the lowest calcium concentration, thus this may account for the significant fibrinolytic activity of its methanolic extract.

This study shows that both *Sida acuta* (1.38 ppm) and *Peperomia pellucida* (1.09 ppm) have a very high level of iron when compared with other plant extracts. *In vitro* fibrinolytic activity of both plant extracts also shows that they possessed insignificant fibrinolytic activity thus implying that fibrinolytic activity of the extract is inversely proportional to the concentration of the iron contain in the extract.

A critical appraisal of Fig. 2 reveals that on addition of 100 μ L SK (streptokinase), a positive control

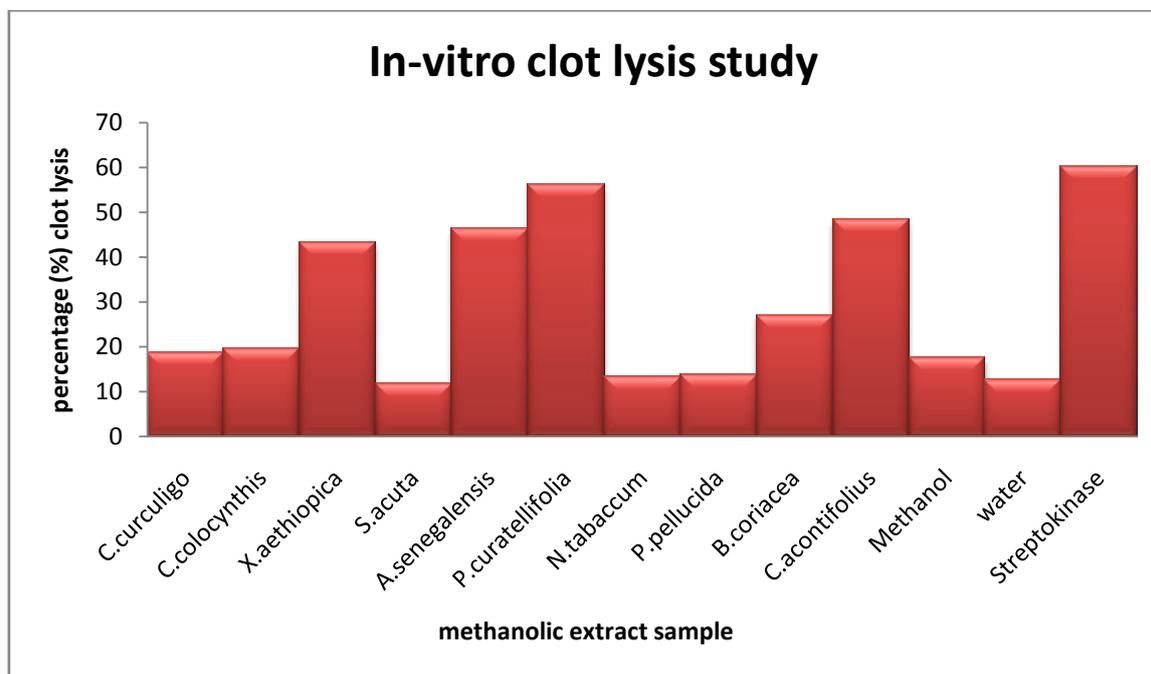


Fig. 2 Percentage clot lysis by the methanolic extracts of the various plant and controls.

(30,000 IU), to the clots, the percentage of the clot that lysed was $60.200\% \pm 4.303\%$. On the other hand, distilled water which serves as the negative control exhibited an insignificant clot lysis activity ($12.608\% \pm 2.230\%$). The mean difference in clot lysis percentage between positive and negative control was found to be statistically significant ($P < 0.005$). The *in vitro* thrombolytic activity study revealed that methanol extract of *Parinari curatellifolia* exhibited highest thrombolytic activity (56.122%) after streptokinase. However, significant thrombolytic activity was also demonstrated by the methanol extracts of *Cnidoscolus aconitifolius* (48.385%), *Anona senegalensis* (46.360%), *Xylopiya aethiopic* (43.205%) and *Buchholzia coriacea* (27.062%) at a dose of 100 μL (100 $\mu\text{g/mL}$). Furthermore, the percentage clot lysis for *Parinari curatellifolia* (56.122%) is not significantly different from that of the positive control; streptokinase ($60.2\% \pm 4.303\%$), $P = 0.005$. Streptococcus is a major ingredient in the production of streptokinase [11]. Moreover, with exception of *Buchholzia coriacea* (with lowest activity) the other medicinal plants with significant

fibrinolytic activity in this study are active against streptococcus, suggesting that they may have similar mechanism of fibrinolytic activity.

4. Conclusions

Thus fibrinolytic activity of the extract may be due to combined effect of the trace metals or other bioactive constituents present in the plant. The result of the present study showed that *Parinari curatellifolia*, *Cnidoscolus aconitifolius*, *Anona senegalensis*, *Xylopiya aethiopic* and *Buchholzia coriacea* exhibits an interesting and significant fibrinolytic activity. To the best of our knowledge, this work is a pioneer fibrinolytic work on the plants under investigation from the review of previous work on these plants. However, *in vivo* clot lysis property and active components of the plants with significant fibrinolytic activity in this work are yet to be investigated. Further work is in progress in our laboratory to compare the fibrinolytic activity of both methanolic and ethyl acetate extract of the same plants, furthermore, we intend to isolate and characterize the actives of the plants extracts.

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