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PHYTOCHEMICAL STUDIES AND COMPARATIVE EFFICACY OF THE CRUDE EXTRACTS OF SOME HAEMOSTATIC PLANTS IN EDO AND DELTA STATES OF NIGERIA

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ABSTRACT

Leaves of *Ageratum conyzoides* (L), *Alchornea cordifolia* (Schym and Thonn) Muel. Arg, *Aspilia africana* (Pers.) C. D. Adams, *Baphia nitida* (Lodd), *Chromolaena odorata* (L) K. R., *Landophia owariensis* (P. Beauv) and sap of *Jatropha curcas* (L) used traditionally to arrest bleeding in fresh cuts were comparatively investigated phytochemically and their ability to precipitate and coagulate blood plasma. Saponins and tannins were the most abundant compounds in these plants while flavoids were the least. Crude aqueous extracts of alkaloids, flavonoids, tannins and saponins from these plants precipitated and coagulated blood plasma within time limits of 4 to 120 seconds (for precipitation) and 15 to 1500 seconds (for coagulation). Results from prothrombin timing showed that *A. afriana* was the most efficacious haemostatic plant followed by *L. owariensis,* and *J. curcas* the least. Some similarities in their chemical composition established a scientific basis for common usage in traditional medicine.

Key Words: Phytochemical, crude extracts, haemostatic plants

INTRODUCTION

Since creation, man has used many plant materials by instinct, intuition or trial and error to combat various ailments. In recent times, over 25 percent of prescribed medicines have ingredients originating from plants (Sasson, 1992).

Phytochemistry tries to utilize chemical information to improve our knowledge about the components of modern day medicinal plants (Stace, 1980). Many individuals have made valuable contributions to the phytochemical studies of medicinal plants in Nigeria. A useful phytochemical review of *Justicia flava* was published by Sofowara and Odebiyi (1978) while Adesogan (1983) reported African *Khaya* species which are known to have antimalarial effects. Usmani *et al* (1993) extracted from the bark of *Holarrhena pubescens* some steroidal alkaloids; while Maxwell *et al.* (1995) also isolated another steroidal alkaloid from *Solanum triste* as 3-beta-amino-5-spirosolene and its previously synthesized dihydro derivative, 3-beta-amino-5-spirosalane which are useful in the manufacture of steroidal drugs.

Abdallas (1995) using *Ononix natrix* extracted a new natural product – N – arachidylanthrinilic acid and known compounds like gardenin B, xanthomicrol, medicarpin and beta-D-glucoside which have antidiuretic and antirheumatic properties. Peng *et al* (1995) isolated from the bulb of *Allium macrostemon* two new novel furostanol G and 1 and their chemical structures were established as 26 – O – beta glucopyranosyl, 2, 6 – diol and 3 – O – beta – D – glucopyranosyl, which have potentials for curing thoracic pains. *Achyrantes aspera* and *J. curcas* were found to possess haemostatic abilities and are applied as such in traditional medicine (Elewude, 1986). *Kigelia africana* was reported by Gbile (1986) to be effective in post partum haemorrhage while the leaves of *Newbouldia laevis* stops vaginal bleeding in cases of threatened abortion (Kargbo, 1982). Kone – Bamba *et al* (1987) studies the haemostatic potentials of fifteen medicinal plants in Cote d'voire, five of which namely, *J. curcas, Vernonia colorata, Hyptis pectinata, Piliostigma thonningii* and *Spondia mobin* showed ability to coagulate blood plasma. Penders and Delaude (1994) isolated six saponnins from *Melanthera scadens* and reported them to have haemostatic properties.

The seven species under investigation are the most commonly used plants in Edo and Delta States of Nigeria for arresting bleeding in fresh cuts. This work is aimed at achieving two purposes, first, to compare the phytochemical constituents of these species and second, to compare the efficacy of the extracts on their ability to coagulate blood plasma in order to

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TABLE 1. CHEMICAL CONSTITUENTS OF THE SEVEN HAEMOSTATIC PLANTS INVESTIGATED

Name of plant species	Tannins	Saponins	Alkaloids	Flavonoids
Ageratum conyzoides	++	++	+	+
Alchornea cordifelia	+++	+	+++	+
Aspilia africana	+++	+++	++	+
Baphia nitida	++	+++	++	+
Chromolaena odorata	+++	++	+	+++
Jathropha curcas	+++	+++	+++	+
Landolphia owariensis	+++	+++	+	+

KEY + to +++ indicate visually observed increasing concentrations from the lowest (+) to the highest (+++).

TABLE 2. PROTHROMBIN TIME FOR ALKALOID EXTRACTS FROM SEVEN COMMONLY USED HAEMOSTATIC PLANTS

Plant species	Precipitation time	Coagulation time
Control	51.2 secs.	7 mins.
A. Conyzoides	Instant	2.03 mins
A. cordifolia	Instant	2.44 mins
A. africana	Instant	4.35 mins
B. nitida	Instant	3.21 mins
C. odorata	1.30 min	7.35 mins
J. curcas	Instant	1.33 mins
L. owariensis	Instant	5.47 mins

elucidate the basis for their use in traditional medicine.

MATERIAL AND METHODS

Collection and processing of Samples

Seven plant species were used in this study, namely, *Ageratum conygoides* (L), *Alchornea cordifolia* (Schym and Thonn), *Aspilia africana* (Pers.) C.D. Adams, *Baphia nitida* (Lodd.), *Chromolaena odorata* (L) K.R., *Jathropha curcas* (L) and *Landophia owariensis* (P. Beauv) and they were obtained from Ekpoma and its environs. With the exception of *J. curcas* (L) in which the sap obtained from the stem was used, leaves were used for other species and these were dried at room temperature (30 ± 2°C) in the laboratory for 4 days.

Phytochemical Extraction Techniques

Alkaloid

This was extracted using a slightly modified method of (Maxwell *et al*, 1995). Dried leaves were blended and alkaloids were extracted from 20g of each sample using 50 ml of 10% acetic acid and left to stand for 4 hrs. The extract was filtered to remove cellular debris and then concentrated to about one-quarter of the original volume. One percent NH_4OH was added drop-wise until a precipate occurred. Crude alkaloid thus obtained was collected by centrifugation. The sap of *J. curcas* (L) (20ml) was treated with 30ml of 10% acetic acid, concentrated and precipitated with 10% N_4OH.

Flavonoids

Equal portions (20g) of the blended leaves of the various samples were extracted repeatedly and separately with 100ml of 80% aqueous methanol at room temperature. J. curcas (L) was subjected to the same treatment using the sap. All the extracts were evaporated to dryness (Bohm and Kocipai – Abyazan, 1994)

TABLE 3. PROTHROMBIN TIME FOR ELAVONOID EXTRACTS FROM SEVEN COMMONLY USED HAEMOSTATIC PLANTS

Plant species	Precipitation time	Coagulation
Control	51.2 secs.	7 mins.
A. Conyzoides	1.2 min	8.2 mins
A. cordifolia	Instant	25.01 mins
A. africana	Instant	0.50 mins
B. nitida	Instant	2.68 mins
C. odorata	1.30 min	0.55 mins
J. curcas	Instant	1.40 mins
L. owariensis	Instant	2.35 mins

TABLE 4. PROTHROMBIN TIME FOR TANNIN EXTRACTS FROM SEVEN COMMONLY USED HAEMOSTATIC PLANTS

Plant species	Precipitation time	Coagulation time
Control	51.2 secs.	7 mins.
A. conyzoides	1.52 min.	6.35 mins. *
A. cordifolia	Instant	12.86 mins *
A. africana	Instant	2.10 mins
B. nitida	Instant	1.50 mins
C. odorata	2.50 mins	4.34 mins
J. curcas	Instant	0.95 mins
L. owariensis	Instant	1.43 mins

and then diluted with 20ml of distilled water each to form a flavonoid solution.

Tannins

Tannins were extracted from 30g of blended dried leaves using 100ml of 75% aqueous acetone containing 0.10% ascorbic acid, and left for 3 hrs before the mixture was filtered. Acetone was removed under reduced pressure (Bohm and Kocipai – Abyazan, 1994). *J. curcas* (L) was similarly treated using 30 ml of sap.

Saponins

Leaves dried at room temperature were blended; 3.60g was weighed out from which saponnins were extracted using 30 ml of 75% ethanol and the extract was re-extracted with 100 ml of ethyl acetate (Peng *et al*, 1995). The organic solvent was then evaporated. The same treatment was applied to *J. curcas* using its sap.

Preparation of Tissue Thromplastin

Fresh brain tissues (from cow) was obtained from the Ekpoma abattoir. The mennings were removed and the brain was rinsed under tap to wash off accumulated blood. One hundred grammes (100g) of brain tissue were placed in a large mortar and covered with acetone. Using a pestle, the brain tissue was repeatedly turned and washed with acetone decanting the ensuing solution when it became cloudy. This procedure was continued until that tissue became dry and flaky and the acetone remained clear.

The acetone-dehydrated tissue was then placed on filter paper in an evaporating dish and air dried; 100 mg of this tissue was put in a test tube and 10 ml of isotonic solution (0.9% NaCl made up in de-ionised water) was added and mixed thoroughly. The tube and the content were then put in a water bath (56°C) for 10 mins. and mixing the contents at frequent intervals. The resulting cloudly supernatant was then aspirated (Sood, 1980). The suspension thus obtained was used as the thromboplastin reagent in the prothrormbin timing – time taken for blood plasma to coagulate; while tissue thromboplastin was used as a standard.

Preparation of oxalate plasma

4.5 ml of human blood was obtained by a clean vein puncture into a tube containing 0.5ml of 0.1M sodium oxalate. These were thoroughly mixed and centrifuged at 3,000 rpm for 10 mins. to separate the plasma.

Prothrombin Timing

0.1 ml of saline thromboplastin suspension and 0.1 ml of 0.15 M $CaCl_2$ were placed into a 10 x

TABLE 5.
PROTHROMBIN TIME FOR SAPONIN EXTRACTS FROM SEVEN COMMONLY USED HAEMOSTATIC PLANTS

Plant species	Precipitation time	Coagulation time
Control	51.2 secs.	7 mins.
A. Conyzoides	Instant	13.14 mins
A. cordifolia	Instant	21.83 mins
A. africana	Instant	0.26 mins
B. nitida	Instant	3.42 mins
C. odorata	1.30 min	2.24 mins
J. curcas	Instant	18.29 mins
L. owariensis	Instant	1.46 min

75 mm test tube. The tube was slightly agitated for proper mixing of the solution. After 1 min., 0.1ml of oxalate plasma was added and the stopwatch was started simulataneously. The solution was constantly observed with slight shaking at frequent intervals and the stopwatch was stopped immediately the appearance of a clot was observed nothing the time of clotting. This procedure was repeated thrice and the mean time calculated. Prothrombin time was then determined for the various phytochemical extracts of each plant sample, using the crude extracts in place of the saline thromboplastin suspension; further precipitation led to coagulation. Coagulation was assumed to be completed as soon as the tube can be inverted without displacing the clot, this was an average of 7 mins.

Results and Discussion

Tannins were abundant in *A cordifolia, A. africana, C. odorata, J. curcas* and *L. owariensis* but moderately present in *A. conyzoides* and *B. nitida*. Saponins were observed in large amounts in *A. africana, B. nitida, J. curcas* and *L. owariensis* whereas they were moderate quantities in *A. conyzoides* and *C. odorata* but slight quantity in *A. cordifolia*. Flavonoids were slightly present in all except *C. odorata*; alkaloids on the other hand were abundantly present in two species, moderate quantities in another two and slight in three (Table 1).

For tissue thromboplastin(control), precipitation occurred at 51.2 seconds while coagulation time was 7 mins. (table 2). It was observed in all the extracts investigated that precipitation of blood plasma occurred before there was complete coagulation of whole blood (see Tables 2 – 5). The findings in this work showed that all the seven plants were thrombogenic as applied in traditional medicine. The four different crude aqueous extracts of each of the samples were active on blood plasma coagulation though the time varied. This suggested that these substances could be substituted for clotting factor III in haemorrhagic conditions.

Alkaloid extract from *A. conyzoides* precipitated and coagulated blood plasma instantly and at 2.03 mins. respectively compared to the control where precipitation occurred at 51.2 secs. and coagulation took an average of 7 mins. Except in *C. odorata* where its alkaloid precipitation time was 1.30 mins., alkaloid from other species precipitated instantly. The longest congulation time of 7.35 mins. was observed in the alkaloid of *C. odorata* – 0.35 min above the average for the control. Introduction of the alkaloids drastically reduced coagulation time (Table 2).

With alkaiod extracts, best results were obtained in *J. curcas* which precipitated plasma instantly and coagulated it at 1.33 mins. This compares very well with protrombin time for tissues thomboplastin which was determined to

be 51.2 secs; normal plasma will coagulate between 60 and 70 seconds (Sood, 1989). The delay in coagulation observed in the control might be due to the diameter of the tube used, the larger the diameter, the longer the coagulation time. A possible explanation for this near normal result in *J. curcas* could be the relatively larger quantity of alkaloid (about 3 times more) obtained from the sap of this plant in comparism with the quantities extracted from the leaves of other species.

Flavonoid extracts precipitated plasma instantly in *A. africana* but took 1.3 min in *C. odorata;* extracts from both species had coagulation times of 0.50 and 0.55 mins. respectively (Table 3). These results deviated from the standard prothrombin time using tissue thromboplastin; observations of this range had been reported by Sood (1989). The most delayed coagulation time (8.25 mins) was observed in the extract obtained from A. conyzoides and this was 1.25 min longer than the control.

Tannins precipitated plasma instantly in five out of the seven species investigated; except in *A. conyzoides* and *C. odorata* where it took 1.52 and 2.50 mins respectively. (Table 4). Tannins were active on plasma coagulation in *J. curcas, L. owariensis* and *B. nitida*, and these occurred at 0.95, 1.43 and 1.53 mins. respectively. It might be inferred that addition of the tannin fractions reduced the coagulation time.

Saponin extracts are well know for their haemostatic activities because of their characteristic soapy nature (Trease and Evan, 1983; Evans, 1989). One would expect that all the saponin extracts should produce the best results. This was true for precipitation but for coagulation, prothrombin time using saponion extracts was relatively delayed except in *A. africana* which coagulated whole blood immediately after precipitation was observed (Table 4)

For other species, coagulation time ranged from 1.46 min. in *L. owariensis* to 21.83 mins. in *A. cordifolia* (Table 5). These results might reflect fairly the amounts, concentrations and possibly composition of saponins that were extracted from the species. Saponin extracts whose mean times were less than 7 mins. (coagulation time for the control) were very effective, and four species fell within this group as seen in Table 5.

Based on the results obtained in this study, these plants have nearly similar chemical composition, varying only in the quantity of each fraction present. These differences in the concentration of the chemical compounds account for the variations in their haemostatic abilities; having almost the same chemical make up established a basis for their common usage in traditional medicine. It could also be concluded from this study that *A. africana, L. owariensis* and *J. curcas* were more efficacious as haemostatic plants than other species investigated.

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