



Preliminary phytochemical investigation and diuretic studies of *Alstonia boonei* stem bark in male Wistar rats

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Abstract

Objective: Stem bark of *Alstonia boonei* was screened phytochemically for the presence of various plant secondary metabolites and for diuretic properties. **Methods:** The diuretic properties of 3 extracts of *A. boonei* stem bark were evaluated by determination of urine volume, electrolyte concentration and diuretic potency in male Wistar rats. Extracts were administered orally at doses of 250 and 500mg/kg respectively. In the assay frusemide, was used as reference drug while 0.9% saline was used as control. **Results:** Preliminary phytochemical examination of this drug indicated the presence of indole alkaloids and saponins but absence of anthraquinones and cardenolides. All the extracts exhibited a dose dependent diuretic property with the methanol extract displaying the highest activity with a potency value of 2.0 at a dose of 500mg/kg/hour while the same dose of hexane and chloroform gave a value of 1.9 and 1.0 respectively. **Conclusion:** The result of this experiment suggest that *Alstonia boonei* stem bark extract possessed significant diuretic activity and may be useful in the treatment of urinary oedema and hypertension.

Keywords: *Alstonia boonei*, diuretic activity, plant extracts.

1. Introduction

Alstonia boonei De Wild (Apocynaceae) is a large tree distributed throughout West Africa and grows widely in Senegal, Ethiopia, Zaire and Nigeria [1]. The stem bark is reported to be useful in the treatment of malaria [2, 3], snakebite [4, 5], rheumatic pain [6] and oedema [7]. Phytochemical examination of *Alstonia boonei* has provided a large variety of

compounds, which include the alkaloids [8 - 10] and terpenoids [11]. Some of the reported biological activities of the genus include antirheumatic [12], cytotoxic [13], and antiparasmodial properties [14]. The present investigation is an assessment of the diuretic activity of three extracts of *Alstonia boonei* stem bark.

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2. Materials and methods

2.1 Plant material

A. boonei stem bark were collected in February 1998, from the railway quarters, Dugbe, Ibadan. A voucher specimen, authenticated by Mr. G. Ighanesebhor of the Forestry Research Institute Herbarium, Ibadan, Nigeria was deposited in the herbarium of the institute.

2.2 Preparation and extraction of plant materials

Sun-dried powdered stem bark (250 g) gave 6.34 g of hexane, 16.81 g of chloroform and 2.35 g of methanol extracts by stepwise maceration at room temperature for 72 h respectively. The extracts were air-dried and stored in universal bottles at room temperature (27-30°C) prior to use.

2.3 Animals

Male wistar rats (120 - 180 g) were obtained from the clinical animal house, College of Medicine, University of Ibadan. They were divided into eight different groups of 5 per group and kept in standardized environmental conditions and maintained on a standard rodent diet (Ladokun feeds Ltd., Ibadan, Nigeria) and water *ad libitum*.

2.4 Phytochemical studies

The powdered drug was screened preliminarily to confirm the presence of alkaloids and the other secondary metabolites, using our previous procedures [15].

2.5 Diuretic activity

Rats housed in eight groups of five each were hydrated with 0.2 ml/kg of water orally before drug/extract administration. Saline (0.9 %) and 10 mg/kg Frusemide served as control and standard drug respectively. Extracts were stabilized with 2.5 % Tween 80 and saline, thereafter 250 mg/kg and 500 mg/kg of hexane, chloroform, and methanol extract of *A. boonei*

in addition to saline and 10 mg/kg of frusemide were administered orally to animals in each group. Urine collection was made at 1 h and 24 h after drug administration and the urine volume was measured with graduated syringes. Na⁺ and K⁺ concentration were determined with flame photometer (Jenway) while Cl⁻ concentration was estimated by standard mercuric nitrate titration method [16]

The mean urine volumes were determined and diuretic potency was assessed by comparison of the urinary excretion due to the extracts with respect to the standard drug. The results of urine volume and electrolyte analysis were expressed as mean \pm S.E.M Student's *t* - test was used to evaluate the significance of differences between the treated and control groups.

3. Results

3.1 Preliminary phytochemical studies

The result of preliminary phytochemical screening (Table 1) of the powdered stem bark of *A. boonei* indicated the presence of alkaloids and saponins and absence of anthraquinones and cardenolides. The result of diuretic activity presented in Table 2 shows that all the three extracts were not active after 1 h. At 24 h, hexane, chloroform and methanol extracts displayed moderate to high activity respectively confirming that activity improved with time.

Table 1

Phytochemical screening of *Alstonia boonei* stem bark.

Secondary metabolites	<i>A. boonei</i> stem bark
Anthraquinones	-
Cardenolides	-
Saponins	++
Indole Alkaloids	+++

- not detectable; ++ medium concentration; +++ high concentration.

Table 2

Diuretic activity of *Alstonia boonei* stem extract in male wistar rats.

Extract/ Drug	Dose mg/kg	Mean Urine Volume(ml)		Diuretic Potency		Electrolyte Concentration					
		1h	24h	1h	24h	1h			24h		
						Na+	K+	Cl-	Na+	K+	Cl-
Hexane	250	0.15±	2.04±	0.94	0.33	78.2±	124.6±	58.6±	73.4±	124.3±	48.5±
		0.05	0.45			16.9	6.4	8.9	13.21	6.18	3.39
	500	0.19±	2.06±	1.19	0.33	85.7±	130.9±	63.2±	86.7±	126.8±	64.9±
		0.10	0.57			9.5	7.1	14.4	6.54	15.7	11.88
Chloroform	250	0.15±	3.94±	0.94	0.63	76.9±	135.4±	49.2±	102.1±	161.1±	80.3±
		0.02	0.79			10.88	18.35	20.3	7.09	12.70	16.27
	500	0.16±	4.52±	1.00	0.72	79.1±	134.0±	59.3±	111.2±	182.1±	82.8±
		0.07	1.24			8.87	10.27	11.87	15.7	18.05	15.46
Methanol	250	0.14±	5.45±	0.88	0.80	79.8±	134.1±	50.1±	128.8±	186.7±	87.1±
		0.02	0.49			12.97	21.13	5.08	13.50	14.48	10.03
	500	0.32±	5.20±	2.00	0.83	78.9±	130.5±	58.1±	131.9±	194.4±	94.4±
		0.24	1.12			12.42	9.28	11.33	9.34	16.63	12.49
Saline (0.9%)		0.12±	1.68±	0.75	0.27	83.8±	133.9±	49.5±	83.7±	132.5±	48.9±
		0.05	0.54			16.6	6.0	8.6	8.28	7.8	8.13
Frusemide 10mg/kg		0.16±	6.3±	1.00	1.00	76.8±	130.1±	46.9±	128.1±	192.2±	79.5±
		0.03	0.70			10.69	13.4	4.26	8.89	16.02	10.41

a Values (except diuretic potency) are mean S.E.M (n=8); **p<0.01; Vs control; Student's *t* - test. Diuretic potency is a ratio of urine volume due to tested drug to that of standard drug.

4. Discussion and conclusion

All the extracts showed a significant dose-dependent diuretic activity at 1 h and 24 h respectively. At a concentration of 250 mg/kg, the hexane extract gave a mean urine volume of 0.15 ± 0.05 , chloroform gave 0.15 ± 0.02 while methanol extract gave 0.1 ± 0.02 after 1 h. This dose of each of the three extracts gave a mean urine volume of 2.04 ± 0.45 , 3.94 ± 0.74 and 5.45 ± 0.49 at 24 h respectively. The methanol extract (500 mg/kg) produced urine with Na^+ , K^+ , Cl^- content of 18.9 ± 12.42 , 130.5 ± 9.28 , 58.1 ± 11.33 after 1 h and 131.9 ± 9.34 , 194.4 ± 16.63 , 94.4 ± 12.49 at 24 h respectively (Table 2). The diuretic potency of *A. boonei* stem bark extracts was dose dependent (Table 2).

After 1 h all the extracts were slightly potent at 250 mg/kg. However, at 500 mg/kg hexane and chloroform extracts were moderately potent while methanol extract was highly potent. A potency value of 0.94 for hexane, and 0.88 were obtained for both chloroform and methanol extract respectively at a dosage of 250 mg/kg after 1 h while 500 mg/kg of hexane chloroform, and methanol extracts gave a potency value of 1.19, 1.00, and 2.00 respectively at same period. Thus hexane and chloroform extracts were moderately potent while methanol extracts was highly potent at this dosage and period.

At 24 h there was a decrease in potency in all the extracts when compared to the standard drug. 250 mg/kg of hexane, chloroform and methanol

gave a value of 0.33, 0.63 and 0.80 respectively (Table 2).

Earlier studies have indicated that the presence of indole alkaloids in *A. macrophylla* was responsible for antiplasmodial property [14] as well as hypotensive properties of *A. boonei* stem bark [17]. The presence of indole alkaloids in the powdered stem bark, chloroform and

methanol extracts of *A. boonei* could be the reason for the observed diuretic activity in *A. boonei*.

The results of this preliminary study support the presence of effective diuretic agents in the stem bark of *A. boonei*. This drug may be suitable in the treatment of urinary oedema and hypertension.

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