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DETERMINATION OF TOXICITY LEVELS OF SOME SAVANNAH PLANTS USING BRINE SHRIMP TEST (BST)

Adoum, O. A.
Department of Pure and Industrial Chemistry, Bayero University, P.M.B. 3011, Kano – Nigeria.
Email: Adoum01@yahoo.com

ABSTRACT
Twenty plant species belonging to 15 families were selected in this study on the bases of their uses in Hausa and Kanuri folk medicine to cure malaria and cancer diseases. Extracts prepared form the plants were solvent partitioned and screened for activity in the brine shrimp (Artemia salina Leach) lethality test (BST). Aqueous and ethyl acetate extracts of the roots of Cochlospermum tinctorium A. Rich and the chloroform soluble fraction (F_2) of stem bark of Entada sudanica Schweinf exhibited very high lethality on brine shrimp larvae at LC_{50} values 8 (26 – 3), 10(32 – 6), and 6(15 – 0) µg/ml respectively. Sclerocaria birrea, Momordica charantia, Borehaavia diffusa and Nauclea aculeata extracts also exhibited potent activity at LC_{50} values <60 µg/ml. The lethal concentrations (LC_{50}) were determined at 95% confidence intervals by analyzing the data on a computer loaded with "Finney Programme."

Keywords: Artemia salina, Brine shrimp test, Folk medicine, Savanna plants.

INTRODUCTION

The use of medicinal plants to treat malaria fever and cancer is well known throughout history. However, their use in northern Nigeria is well documented (Dalziel, 1916; Akinniyi and Sultanbawa, 1983; Maydell, 1990; Muhammadu, 1990; Aliyu, 2006). The investigation of plants for bioactive secondary metabolites has become inevitable due to significant correlation between their uses in traditional medicine and the observed bioeffects of their extractive (Fatope, 1995). Screening of African Savanna plants for toxicity effects have been carried out but never exhausted (Fatope, 1993; Adoum *et al.,* 1997).

In our effort to identify bioactive natural products, the present study is aimed at investigating the cytotoxic effects of plants selected form 15 families. The selection was based on their uses in the Hausa and Kanuri folk medicines to treat malaria fever and cancer. Brine shrimp lethality test (BST) has been employed as an alternative bioassay technique to screen the plant extracts (Meyer *et al.,* 1982; Mitscher, 1972).

MATERIALS AND METHODS

Plant Materials

The targeted plants (Table 1) used in this research work were collected between May and November 2005 from Dala, Kumbotso, and Madobi Local Governments of Kano State of Nigeria. The plants were identified by taxonomists of Bayero University Herbarium, Kano and their voucher numbers were compared with the ones that were already available at the Herbarium. The plant materials were air – dried and milled.

Preparation of plant extracts

Two hundred grams (200g) of each plant (Table 1) was separately extracted by percolation with 2 litres of ethanol for 2 weeks. The percolates were then filtered and the solvent evaporated using the rotary evaporator at 40°C (Fatope *et al.,* 1993) to give a residue, F1. F1 was partitioned between water and chloroform (400ml, 1:1) in a separating funnel. After removal of the chloroform layer, the water layer was further washed with ethyl acetate (200ml) and then separated. The chloroform and ethyl acetate layers were concentrated under vacuum below 40°C to give the chloroform F2 and ethyl acetate, F4 soluble residues. The water soluble layer was concentrated using waterbath to give F3 residue. F2 was further partitioned between 90% aqueous methanol (200ml) and petroleum ether (200ml). The two layers were separately evaporated under vacuum to dryness to give the petroleum ether soluble residue (F5) and the aqueous methanol soluble residue (F6). The residues were weighed and screened for toxicity in the brine shrimp lethality test (BST).

Brine Shrimp Lethality Test

Brine shrimp lethality bioassay, was carried out to investigate the cytoxicity of plant extracts. 50mg of *Artemia salina* (Leach) eggs were added to a hatching chamber containing Ocean/Sea water (75ml). The hatching chamber was kept under an inflorescent bulb for 48h for the eggs to hatch into shrimp larvae. 20mg of test fractions F1, F2, F3, F4, F5 and F6 of the various plant species were separately dissolved in 2ml of methanol, form this, 500, 50, and 5µl of each solution was transferred into vials corresponding to 1000, 100, and 10 µg/ml, respectively. Each dosage was tested in triplicate. The vials (9 per test fraction) and one control containing 500µl of solvent were allowed to evaporate to dryness in about 48h at room temperature.

Fourty five millilitre (45ml) of Ocean/Sea water was added to each vial, and 10 larvae of *A. salina* Leach (taken 48 – 72h after the initiation of hatching) were added to each vial. The final volume of solution in each vial was adjusted to 5ml with Ocean/Sea water immediately after adding the shrimps. One drop of dimethyl sulphoxide (DMSO) was added to the test and control vials before adding the shrimps to enhance the solubility of test materials. LC_{50} values were determined at 95% confidence intervals by analyzing the data on a computer loaded with a "Finney Programme." The LC_{50} values of the brine shrimps obtained for extracts of the plants studied were recorded.

RESULTS AND DISCUSSION

From the results (Table 1) the most cytotoxic extracts were the aqueous and ethyl acetate extracts of the roots of *Cochlospermum tinctorium* and the chloroform soluble fraction (F2) of stem bark of *Entada sudanica* which have exhibited very high lethality on brine shrimps at LC_{50} values, 8 (26 – 3), 10 (32 – 6) and 6(15 – 0) µg/ml, respectively. This result properly explains the popular uses of the two plants in the treatment of cancer, ulcer and malaria diseases by the Hausawa and Kanuris of northern Nigeria and in the Republic of Mali as well (Adiaratou *et al.,* 2005; Nergard *et al.,* 2005). The extracts of *Sclerocaria birrea, Ipomae repens, Momordica charantia, Borahaavia diffusa* and *Nauclea uculeata* showed remarkable toxicity on the brine shrimp larvae at LC_{50} values less than 60 µg/ml (Table 1). Some extracts from *Kigelia africana, M. charantia, Acacia nilotica* and *N. aculeata* demonstrated moderate toxicity on brine shrimps. The activity of *A. nilotica* and *K. africana* on BST is found to be consistent with the larvacidal effects of the two plants on culex mosquito. At 400 µg/ml *A. nilotica* killed 50%, 200 µg/ml killed 20% and 40 µg/ml killed 7%, whereas *K. africana* killed about 70%, 40% and 10% at the test concentrations, respectively (Taura *et al.,* 2004). Other plant species, however, showed very low lethal toxicity on brine shrimp larvae.

In conclusion eleven out of 20 plant species used in this study showed significant toxicity against brine shrimp larvae of *Artemia salina* at lower LC_{50} values. The results could serve for further pharmacological and phytochemical research.

Table 1: Brine Shrimp test toxicity of plant extracts under study

Plant name and family	Part used	Traditional use	Fraction	BST LC_{50} (µg/ml) [a]
Anacardiaceae				
Lannea barteri (K & Gillet)	Bark	Anti-malaria	F1	>1000
Sclerocaria birrea A. Rich	Stem bark	Anti-cancer &	F2	326.5 (549 – 203) [b]
	Stem bark	Anti-malaria	F4	37.1 (155 – 24) [c]
Annonaceae				
Annona senegalensis Pers.	Roots	Anti-malaria	F1	>1000
	Stem bark	Anti-malaria & Anti – cancer	F1	>1000
Bignoniaceae				
Kigelia africana (Lam). Benth	Stem	Anti-cancer	F1	>1000
	Leaves	Anti-cancer	F1	>1000
	Roots	Anti-cancer	F1	593 (1300 – 331)
	Fruits	Anti-cancer	F1	124 (199 – 78)
	Fruits		F2	>1000
	Fruits		F4	495 (858 – 306)
	Fruits		F5	>1000
	Fruits		F6	>1000
Bromeliaceae				
Ananas comosus L. Merr.	Fruit peel	Anti-cancer	F1	>1000
	Fruit peel		F2	>1000
	Fruit peel		F3	>1000
	Fruit peel		F4	>1000
Burseraceae				
Boswellia dalziellii Hutch.	Stem bark	Antimalaria	F1	>1000
Commiphora kerstingii Engl.	Bark	Anti-cancer	F1	209 (354 – 125)
Cochlospermaceae				
Chochlospermum tinctorium Rich.	Roots	Anti-malaria	F1	29 (37 – 9)
	Roots		F2	231 (21378 – 0.5
	Roots		F3	8 (26 – 3)
	Roots		F4	10 (32 – 6)
	Roots		F5	580 (1768 – 260)
Convolvulacea				
Ipomea repens Lam	Leaves	Anti-malaria	F1	50 (120 – 151)
	Stem	Anti-malaria	F1	> 1000

Table continue

Plant name and family	Part used	Traditional use	Fraction	BST
				a LC_{50} (µg/ml)
Curcubitacea				
Momordica charantia Linn	Leaves	Anti-cancer	F1	12 (389 – 0.08)
	Leaves		F2	310 (619 – 166)
	Leaves		F3	> 1000
	Leaves		F4	> 1000
	Leaves		F5	220 (456 – 126)
Fabaceae				
Cassia goratensis Martha (Stewart)	Roots	Anti-cancer	F1	> 1000
Entada sudanica Schweinf	Stem bark	Anti-malaria	F1	473 (1818 - 212)
	Stem bark	& anti-cancer	F2	6 (15 – 0)
	Stem bark		F3	205 (673 – 86)
	Stem bark		F4	> 1000
Leguminosae				
Acacia nilotica (Linn) Willd	Stem bark	Anti-malaria	F1	565 (2955 – 335)
	Stem bark	& anti-cancer	F2	> 1000
	Stem bark		F3	209 (354 – 1250)
	Stem bark		F4	> 1000
	Stem bark		F5	> 1000
Cassia occidentalis Linn				
Detarium microscarpum (Guiill & Perr	Leaves	Anti-malaria	F1	> 1000
	Leaves	Anti-malaria	F1	> 1000
Anthraceae				
Lawsonia inermis Linn	Roots	Anti-cancer	F1	160 (250 – 112)
	Roots		F2	> 1000
	Roots		F3	> 1000
	Roots		F4	> 1000
	Roots		F5	> 1000
	Roots		F6	> 1000
Moraceae				
Ficus platyphylla Delile	Leaves		F1	> 1000
Nyctaginaceae				
Boerhaavia diffusa Linn	Roots	Anti-malaria & anti-cancer	F1	39 (87 – 12)
Rhamnaceae				
Zizyphus jujube (Linn) Lam	Stem	Anti-cancer	F1	> 1000
	Stem		F2	> 1000
	Stem		F3	> 1000
	Stem		F4	> 1000
Rubiaceae				
Nauclea aculeate (SM) Bruce	Leaves		F1	> 1000
	Leaves		F2	237 (1458 – 86)
	Leaves		F4	> 1000
	Leaves		F5	60 (136 – 25)
	Leaves		F6	17 (1.25 – 0.00)

Key:
a LC_{50} µg/ml (95% confidence interval)
b upper limit confidence
c lower limit confidence
F1 = ethanol fraction
F2 = chloroform fraction
F3 = water fraction
F4 = ethyl acetone fraction
F5 = petroleum ether fraction
F6 = methanol fraction

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