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Screening of Medicinal Plants Native To Kano and Jigawa States of Northern Nigeria, Using *Artemia Cysts* (Brine Shrimp Test)

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Abstract Eleven plant species belonging to 9 families were selected in this study on the basis of their uses in Hausa folk medicine. Extracts prepared from the plans were solvent partitioned and screened for activity in the brine shrimp (*Artemia cysts*) lethality test (BST). All the leaves extracts of *Cassia singueana* exhibited very high toxicity in brine shrimp test (BST) at LC_{50} values less than 11µg/ml. Some extracts of *Commiphra kerstingi*, *Jatropha curcas*, *Erythrina senegalensis* and *Securidaca longepedunculata* have showed remarkable toxicity in BST at LC_{50} values range between 4.5 - 367 µ*g/ml.* Only *Diospyros mespiliformis* (Ebenaceae) showed very low brine shrimp lethality at LC_{50} > 1000 µg/ml. The lethal concentration (LC_{50}) were determined at 95% confidence intervals by analyzing the data on a computer loaded with "Finney Programme."

Keywords: *artemia cysts, Brine shrimp test, toxicity, Jigawa, Kano*

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1. Introduction

In Africa, up to 80% of the population uses traditional medicine, while in industrialized countries the market for herbal medicine is growing steadily [1]. Medicinal plants have been used in the treatment and prevention of some infectious diseases such as cancer, malaria, and HIV/AIDS in various parts of Nigeria. Malaria, for instance is endemic throughout the country. World Health Organization (WHO) estimated malaria mortality rate for children under five in Nigeria at 729 per 100,000 [2]. The Ministry of Health reported that malaria is responsible for one out of ten deaths in pregnant women and has caused the Federal Government millions of US Dollars annually [3].

In Kano and Jigawa States of northern Nigeria, the majority of population relies heavily on traditional practitioners and medicinal plants to meet primary health care obligations. Although orthodox medicine may be available in these areas, herbal medicines are affordable and have often maintained popularity for historical and cultural reasons. Investigations into the chemical and biological activities of plants during the past two centuries have led to the discovery of novel and more effective therapecitic agents [4].

Fatope, 1995 [5] described a number of reliable and very sensitive bioassay techniques which are indicative of toxicity. These are the one-day duckling bioassay, the chick embryo test, the zebra fish test , test on insect or insect Larvae , the rat multipurpose screen and others. Most of these bioassay techniques cannot be used as rapid, general screening procedure for the detection of toxic secondary metabolites because of costs, specifity, sophistication or objections by animal right actives. As such McLaughlin and co-workers, 1991 [6] reported three simple bioassay procedures by his laboratory and successfully used them to direct the isolation of bioactive compounds from plant sources. Brine shrimp test (BST) is the simplest bioassay among the three. The assay was initiated by Meyer *et al*., 1982 and performed with minor modification in 1991 as a simple, rapid, in-house, bench-top and low cost prescreen for cytotoxicity, insecticidal and anti-malaria activities [6].

A positive correlation was successfully established between brine shrimp toxicity and 9KB (human epidermoid carcinoma of nasopharynx) cytotoxicity (p=0.036 and Kappa=0.56) respectively. Furthermore, BST was confirmed to be useful as a prescreen test for antitumour activity in a blind comparison with *in vitro* cytotoxicity and 3PS (*in vitro* P388 murine leukemia) activity (p=0.033-0.0331) [8,9,10,11]. In recent years scientists have been discovering various bioactive compounds with diverse chemical structures, guided by BST a simple and quick bioassay which can be carried out in a chemical laboratory.

In this study the brine shrimp, *Artemia cyst* was used, a simple zoologic organism (an arthropod). The assay was carried out to investigate the cyclotoxicity of extracts of some medicinal plants useful for malaria therapy in Kano and Jigawa States, northern Nigeria.

2. Materials and Methods

2.1. Plants Materials

The plant materials (Table 1) for this research work were collected between 7th and 23rd June, 2008 from Kano and Jigawa states of Nigeria. They were authenticated by Prof. Bala Sidi and Baba Ali Garko of Bayero University Herbarium. The voucher numbers of the plants were compared with the ones that were already available at the Herbarium. The plant materials were air - dried and milled.

Table 1. Brine Shrimp Test Activity of Targeted Plant Extracts

Plant Name and Family	Part used	Traditional Use	Fraction		BST [a]LC_{50}µg/ml
			Code	Yield (g)	
Anacardiaceae *Anacardium occidentale* Linn.	Bark	Anti-diabetic	F1	10.80	93.5([b]144.4-60[c])
			F3	0.80	>1000
			F4	1.10	>1000
			F5	1.20	>1000
Bignoniaceae *Stereospermum kunthianum* Cham.	Root	Anti-Malaria	F1	9.60	183(288-124)
			F2	0.50	102(216.5-48.6)
			F3	0.60	>1000
			F4	1.10	385(1045-193.3)
			F5	0.90	>1000
Burseraceae *Commiphora kerstingi* Engl	Leaves	Anti-Malaria	F1	11.60	5.8(14.7-0.5)
			F4	2.10	5(15.2-0.2)
			F5	1.91	23(41.7-10.5)
Curcurbitaceae *Momordica balsamina* Linn.	Whole plant	Anti-malaria	F1	9.70	246(491-136)
			F2	0.50	161(277-96)
			F3	0.21	>1000
			F4	0.91	>1000
			F5	0.60	169(290-96)
			F6	0.62	4.3(220-98)
Ebenacea *Diospyros mespiliformis* Hochst	Stem	Dysentery and skin eruption	F1	7.80	>1000
			F2	1.30	>1000
			F3	1.50	>1000
			F4	1.20	>1000
Euphorbiaceae *Jatropha curcas* Linn.	Leaves	Anti-malaria	F1	10.60	4.5(12.8-0.2)
			F2	0.80	367(900-190)
			F4	0.98	158(272-93)
			F5	0.63	5.8(14.6-0.5)
			F6	0.73	7.8(234-105)
Fabaceae *Acacia seyal* Del.	Bark	Anti-bacterial	F1	9.60	>1000
			F2	0.98	>1000
			F4	1.30	790(154.5-4927)
			F5	1.20	>1000
Cassia singueana Del.	Leaves	Anti-malaria	F1	8.90	3.5(12.5-0.05)
			F3	1.30	5(19.5-0.05)
			F4	0.90	3.4(11.6-0.06)
			F5	0.80	4.3(86-17.8)
			F6	0.50	11(30-1.07)
Erythrina senegalensis A. DC.	Bark	Anti-malaria Anti-ulcer	F1	7.30	179(312-103.3)
			F2	1.10	97(162.6-58)
			F4	0.98	118(254-56.7)
			F5	0.62	234(424.5-135)
			F6	0.82	93.5(154-56.8)
Poaceae *Panicum stagninum* Retz.	Whole plant	Anti-malaria	F1	8.40	236(450-173)
			F2	1.30	89.2(177.7-43.7)
			F4	0.90	53(100-26)
			F5	0.70	26(532-100)
			F6	0.73	179(312-103)
Polygalacea *Securidaca longepedunculata* Fresen.	Root	Anti-malaria	F1	6.70	131(209-85)
			F2	1.96	87(134.7-56)
			F4	1.22	7.1(13.2-1.6)
			F5	0.96	23(41.7-10.5

[a]LC_{50} µg/ml (95% confidence interval)
[b]Upper limit confidence
[c]Lower limit confidence.

2.2. Preparation of Plant Extracts

Two hundred grams (200g) of each plant (Table 1) were separately extracted by percolation at room temperature with 1 litre of ethanol for 2 weeks. The percolates were then filtered and the solvent evaporated using the rotary evaporator at 40°C [12] to give a residue, Fl. Fl (4 – 4.5g), was partitioned between water and chloroform (300ml, 1:1) in a separatory funnel. After removal of the chloroform layer, the water layer was further washed 3 times 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 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 reside (F6). The residues were weighed and screened for activity in the brine shrimp lethality test (BST) (Table 1).

2.3. Brine Shrimp Lethality Test

The eggs (Premium Grade) of *Artemia cysts* were purchased from M & M suppliers. Bothell USA. 50mg of eggs were added to a hatching chamber containing Ocean/Sea Water (75ml). The hatching chamber was kept under inflorescent bulb for 48h for the eggs to hatch into shrimp larvae. 20mg of test fractions Fl, F2, F3, F4, F5 and F6 of the various plant species were separately dissolved in 2ml of methanol, from this, 500, 50, and 5µl of each solution was transferred using micro liter syringe (Hamilton Bonaduz AG – Switzerland), into vials corresponding to 1000, 100, and 10µg/ml, respectively. Each dosage was tested in triplicate. The vials (9 per test fraction) and one control containing 500µl of solvent only were allowed to evaporate to dryness in about 48h at room temperature. 4.5ml of Ocean/Sea Water was added to each vial, and 10 larvae of *A. cysts* (taken 48 - 72h after the initiation of hatching) were added using a Pasteur pipette 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 [13]. The LC_{50} values of the brine shrimps obtained for extracts of the plants studied were recorded (Table 1).

3. Results and Discussion

The cytotoxic effects of plant extracts is reported as LC_{50} values in µg/ml with 95% confidence intervals as determined by a Finney PC computer programme (Table 1). Plant extracts exhibited LC_{50} values > 100 µg/ml are considered inactive while those with < 200 µg/ml demonstrate very high cytotoxicity.

The strong toxic effect on *A. cysts* was observed for all ethanol crude (F1) extracts of the targeted plants except *A. seyal* and *D. mespiliformis* which were found inactive (LC_{50} > 100 µg/ml). Their LC_{50} values varied between 3.5 µg/ml to 246 µg/ml. This is in accordance with the popular use of the plants in the treatment of malaria fever in northern Nigeria.

Highly significant cytotoxicity was recorded on all the fractions of *S.kunthiamum, C. kerstingi, J. curcas, C. singueana, E. senegalensis, P. stagninum and S. longepedunculata.* The LC_{50} values in case of *C. singueana* varied between 3.4 µg/ml to 11 µg/ml. Whereas LC_{50} of *C. kerstingi* varied between 5.8 µg/ml to 23 µg/ml. Therefore, it seems that the data on *C. singueana* agrees with the positive findings of the studies carried out against rodent plasmodia infection, nociception, pyroxia and inflammation in mice and rats [14]. Less than 5 µg/ml of lethal concentrations was recorded by the ethanol (F1) leaf extract of *J. curcas*, ethanol (F1) and ethyl acetate (F4) extracts of *C. singueana*, methanol fraction (F6) of *M. balsamina* and petroleum ether (F5) fraction of *E. senegalensis* bark. All the fractions of *D. mespiliformis* and *A. seyal* did not show any activity in brine shrimp test (LC_{50} > 1000 µg/ml).

In conclusion, over 90% of the plant species used in this study showed significant cytotoxicity in brine shrimp test (BST) at very low LC_{50} values. The results could lend very strong scientific backing to the claims of the traditional medical practitioners in northern Nigeria, who use the plants to treat malaria and other pathogenic diseases. The results could serve also as a basis for pharmacological and phytochemical research, towards discovering novel bioactive compounds [15]. Furthermore, specific bioassay techniques are necessary in order to confirm these findings.

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