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Cytotoxic correlation of some traditional medicinal plants using brine shrimp lethality test

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ABSTRACT

Ten Nigerian traditional medicinal plants, *C. nigricans* Vahl.; *P. biglobosa* Benth.; *C. alata* Linn; *J. curcas, C. paradisi* Macf., *S. angustifolia, A. cordifolia* Schum and Thonn., *N. latifolia* SM., *E. senegalensis* DC and *V. album* Linn belonging to a variety of plant families were extracted with various solvents. The extracts were screened using the brine shrimp lethality bioassay to determine candidate plants for isolation of cytotoxic principles. The calculated LC_{50} of the extracts ranged from 11-500μg/ml, except for *C. paradisi* and *S. angustifolia* with values > 1000μg/ml, indicating the presence of cytotoxic compounds which could be potent against various human diseases.

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INTRODUCTION

Interest in drugs of plant origin has grown considerably following resistance by several micro-organisms to certain drugs especially synthetic ones. The selection of plants species to be studied could be based on ethnomedical information, chemotaxonomic relation- ships [1] and use of plants in traditional medicine [2].

In order to choose a promising plant for activity–guided isolation, plant materials are extracted and evaluated biologically. The brine shrimp lethality bioassay presents a simple technique for identification of potential plants for isolation of cytotoxic compounds [3]. This procedure has proven to be useful, rapid and inexpensive. The activity of the extracts is manifested as toxicity to the brine shrimps and the median lethal concentration, LC_{50} (concentration of the extract that kills 50% of the shrimps), can be estimated. This calculation uses the method of linear regression analysis of the recorded percentage death of shrimps at 95% confidence level [4,5]. When the LC_{50} value is less than 1000 μg/ml, the extract is said to display toxicity in the Brine shrimp assay but values greater than 1000 μg/ml is said to indicate a level of toxicity that could induce probable side effects when administered for medicinal purpose.

EXPERIMENTAL

Plant material: *Cassia nigricans* Vahl.; *Parkia biglobosa* Benth.; *Cassia alata* Linn; *Jatropha curcas* Linn, *Citrus paradisi* Macf., *Stachytapheta angustifolia, Alchornea cordifolia* Schum and Thonn., *Nauclea latifolia* SM, *Erythrina senegalensis* DC and *Viscum album* Linn were collected from Sabon-Gari Local Government Area of Kaduna State, Nigeria. They were properly identified at the herbarium of Department of Biological Sciences, Ahmadu Bello University Zaria. A voucher specimen of each sample is available at the herbarium. The plant materials were air-dried and grounded.

Extraction: 80g of each plant material were successively extracted using the soxhlet extractor with petroleum spirit (60-80^{0}C) **(PS)**, chloroform **(C)**, ethyl acetate (**EA)**, and methanol **(M)**; and separately extracted with water **(W)**. The extracts were evaporated to dryness on a rotavapor at 40^{0}C and used for the Brine Shrimp lethality bioassay (BST).

Brine Shrimp Test: A solution of instant salt (Aquarium system, Ohio) was made by dissolving 10g of salt in distilled water (250ml) and transferred into the hatching chamber. 40mg of *Artemia salina* (leach) egg (Artemi, Inc California) was added. The chamber was kept under illumination using a florescent bulb for 48hours for the eggs to hatch into shrimp larvae. 30mg of each plant extracts were separately dissolved in 3ml of DMSO and from this 500, 250, 125, 62.5 and 31.25

µg/ml were prepared by serial dilution and tubes labeled accordingly. Each dosage was tested in triplicate giving a total of 15 test tubes for each sample. A control containing 50 µl of DMSO solvent was used for each test fraction. 3.5ml of the sea salt solution was added to each test tube, and 10 Larvae of *Artemia salina*, (taken 48-72 hours after the initiation of hatching) were added.

The final volume of solution in each test tube was adjusted to 5ml with sea salt solution immediately after adding the shrimp larvae. 24 hours after addition of the larvae, the number of surviving shrimps at each concentration were counted and recorded. The LC_{50} values for each extracts were calculated [4, 5].

Table 1: Plants General Characteristics and Calculated LC_{50} of Extracts

Plant	Family	Plant Part	LC_{50} (µg/ml) at 95% confidence interval				
			PS	C	EA	M	W
Stachytapheta angustifolia	Verbenaceae	Roots	nd	nd	nd	nd	> 1000
Alchornea cordifolia Schun & Thonn	Euphorbiacea e	Leaves	341.98	nd	269	181.13	nd
Nauclea latifolia SM	Rubiaceae	Roots	83.2	155.5	nd	nd	nd
Erythrina senegalensis DC	Papilioneceae	Leaves	500	nd	125	nd	nd
Viscum album Linn	Loranthaceae	Leaves	191	nd	nd	nd	250
Cassia nigricans Vahl.	Leguminoseae	Leaves	11.06	149.57	42.77	26.91	nd
Parkia biglobossa Benth	Leguminoseae	Stem	415.76	nd	478.63	107.15	nd
Citrus paradisi Macf.	Rutaceae	Leaves	> 1000	> 1000	nd	nd	nd
Jatropha curcas Linn	Euphorbiacea e	Leaves	nd	75.00	20.0	nd	133.8
Casia alata Linn.	Fabaceae	Stem	145.55	222.17	186.21	349.6	nd

nd = not determined

RESULTS AND DISCUSSION

Table 1 presents the general characteristics of the plants and the results of the brine shrimp lethality assay on the plants extracts as represented by the estimated LC_{50} values. The plants used for this study were selected based on ease of availability and their reported traditional medicinal uses. For example, *Cassia alata* Linn was selected because of its reported therapeutic efficacy [6, 7] and *Parkia biglobosa* Benth is reported to contain bioactive compound effective for the treatment of wounds [8,9].

Eight of the plants extracts have low to moderate LC_{50} values, indicating the presence of cytotoxic components at the concentrations employed. *S. angustifolia* water extract and *C. paradisi* petroleum spirit and chloroform extracts gave LC_{50} values greater than 1000µg/ml, which represents almost 100% mortality of the shrimps, an indication the extracts contain constituents that are toxic to the shrimps at the concentrations employed.

Based on the brine shrimp test, the order of potency of the petroleum spirit extracts is *C. nigricans* > *N. latifolia* > *C. alata* > *V. album* > *A. cordifolia* > *P. biglobosa* > *E. senegalensis*. For chloroform extracts it is *J. curcas* > *C. nigricans* > *N. latifolia* > *C. alata*. For ethyl acetate extracts it is *J. curcas* > *C. nigricans* > *E. senegalensis* > *C. alata* > *A. cordifolia* > *P. biglobosa*. For methanol extracts it is *C. nigricans* > *P. biglobosa* > *A. cordifolia* > *C. alata*. While for water extracts it is *J. curcas* > *V. album*. The LC_{50} values obtained for *C. nigricans* are the lowest when compared with the values of other plants. This plant is a possible candidate for study. Further work on the isolation of specific bioactive compounds from the plant is in progress.

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