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CYTOTOXICITY STUDIES ON SOME NIGERIAN MEDICINAL PLANTS

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ABSTRACT:
Investigations into the cytotoxic activity of extracts of ten medicinal plants found in the Nigerian floral environment were carried out against the larvae (nauplii) of *Artemia salina* (Leach). The phytochemical screening revealed that both anthraquinones and cyanogenic glycosides were absent in all the plants except *A. indica* which tested positive for cyanogenic glycosides. However, alkaloids, saponins, tannins, cardiac glycosides, terpenes and flavonoids were either present or absent. The brine-shrimp lethality was analyzed using the Finney probit program and expressed in LD_{50} (ppm). The leaf extracts of *P. Angolensis*, *C. Papaya* and *A. Indica* were strongly active at LD_{50} values of 2.56, 2.73 and 76.56 ppm which compare favourably with literature values below 200 ppm which are considered as 'significant'. However, the LD_{50} values of leaf extracts of *A. Hispida* (555.92 ppm) and *A. Wilkesiana* (Lace variety) (384.45 ppm) and seed extract of *G. Kola* (567.76 ppm) which are above the 200 ppm are regarded as 'marginal' or weakly active. These findings have revealed potential plant templates which could further be studied for detailed anti-cancer or tumor activity.

Keywords: Brine-shrimp lethality, Anticancer, Extracts, *P. Angolensis, C. Papaya.*

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INTRODUCTION

Plants are great sources of medicines, especially in traditional healthcare where their use is of huge importance in the treatment of various diseases[1]. According to the World Health Organization[2], 80% of the world population use medicinal plants in the remedy of diseases and this rate is much higher amongst African countries[3]. Currently, about 25% of drugs contained in modern pharmacopoeia are derived from plants[4] while 50% of drugs in clinical practice are of natural product origin[5]. Furthermore, medicinal plants play an important role in the development programs of several major pharmaceutical companies and reputable research institutes such as the US National Cancer Institute[6]. Therefore, any contribution to the discovery of templates for the treatment and or management of disease conditions such as HIV/AIDS, malaria, diabetes, microbial infections and most especially cancers or tumors will be of particular interest to the scientific world and of immense benefits to mankind. In the light of this reality, 'benchtop' bioassays such as the brine- shrimp lethality test[7] which makes use of the *Artemia Salina* larvae is routinely employed as a pre-screen for plant extracts in toxicological studies. It provides a quick, inexpensive and desirable alternative to complexities encountered with animal models. In addition, the brine- shrimp bioassay predicts possible anti-cancer activity because a positive correlation exists between this test and the

9KB (human nasopharyngeal carcinoma) cytotoxicity[7,8]. This present study was carried out on some Nigerian medicinal plants with the aim of searching for potential templates for detailed anticancer research in subsequent research.

MATERIALS AND METHODS

Collection of Plant Materials

Different organs of ten medicinal plants native to Nigeria were collected around June, 2012 from the Uyo Local Government Area of Akwa Ibom State, Nigeria. The plants were identified by Mr. E. Okon, Taxonomist, Department of Pharmacognosy and Natural Medicine, University of Uyo, Nigeria while authentication was done by comparison with herbarium samples at the National Institute of Horticulture (NIHORT) and Forestry. Research Institute of Nigeria (FRIN) both in Ibadan, Oyo State, Nigeria. Afterwards, voucher specimens labelled No H92 to No H101 were deposited in the Herbarium Unit. Sea water was collected from Kuramo Beach, Lagos while a plastic case and brine-shrimp eggs (*Artemia Salina* Leach) were obtained from the San Francisco Bay Brand Inc., Newark, CA 94560, USA.

Extraction and Processing of Plant Materials

Fresh seeds were peeled and pulverized a wooden mortal using a pestle to obtain the ground powder. However, other organs such as leaves, stem and roots were individually oven-dried (40^0C) and then separately ground into coarse powders on an electric mill (Gallen kamp, UK). The resultant powders were then extracted with cold 96% ethanol at room temperature (27 ± 2^0C) for 72h. The obtained filtrates were also separately evaporated to dryness *in-vacuo* on a rotary evaporator (Buchi CH -920, Laboratorium Technic, Flawk/ SG, Switzerland). The resultant dried extracts were then stored in appropriately labelled amber bottles in a refrigerator (-4^0C) prior to the tests.

Phytochemical Screening

The dried crude extract of each plant was separately investigated for secondary metabolites (alkaloids, saponins, tannins, anthraquinones, cardiac glycosides, terpenes, flavonoids and cyanogenic glycosides) according to the laid down procedures[9-19].

Brine-shrimp Lethality Assay

Hatching of the Eggs: Some sea water was placed in a small plastic tank with perforated dividing dam which was fabricated from a plastic case. Some shrimp eggs were added to one side of the divided tank. This side was darkened by covering it with a plastic lid while the other compartment was exposed to illumination. The set up was left for 48h for shrimp eggs to hatch and mature as nauplii. Mature nauplii usually swim to the exposed compartment.

Preparation of Vials for Testing: A stock solution of sample was prepared by dissolving 20 mg of extract in 2ml of 50% methanol. To obtain the desire final concentrations such as 1000 µg/ml, 100 µg/ml and 10 µg/ml; 0.5ml, 0.05ml and 0.005ml of the stock solution were transferred into the three vials respectively. The solvent was then evaporated by drying the vials in a vacuum desiccator for 24h. Ten[10] shrimp nauplii were counted into each vial (i.e. 30 nauplii/ dilution). The total volume of solution in each vial was adjusted to 5ml by adding sea water (5ml / vial). The control (50% methanol) was prepared in the same way except that the sample (extract) was omitted. The vials were maintained in the laboratory with normal fluorescent illumunation and the set-up left for 24h. The number of survivors usually swimming was counted with the aid of a magnifying lens for each of the vials at the end of 24h. Thus, the number of the dead was computed; hence the LD_{50} in ppm (parts per million) was determined using the Finney probit analysis software[7,20].

Statistical Analysis: Statistical analysis was done by the analysis of variance (ANOVA); mean difference of the treatment significance was calculated at $p < 0.05$. Results are means of three replicates.

RESULTS AND DISCUSSION

Processing of Plant Materials

The rules governing collection of different plant parts were strictly adhered to in the course of this study. Subsequently, the plants were identified and authenticated by following the prescribed guidelines. Also, the principles of extraction and phytochemical screening of extracts were obeyed, thereby preventing any changes to the chemical composition of the extracts[10,11].

Phytochemical Tests

The phytochemical screening indicated that all the plants tested negative to both anthraquinones and cyanogenic glycosides except *A. Indica* which tested positive for cyanogenic glycosides. This was not surprising because acalyphin, a cyanogenic glycoside had previously been isolated from the roots of the plant[12]. However, each plant showed either the presence or absence of alkaloids, saponins, tannins, cardiac glycosides, terpenes and flavonoids (Table 1).

Secondary metabolites such as saponins, cardiac glycosides, alkaloids, tannins and flavonoids have demonstrated in several previous studies[21-33]. They prove to be responsible for the cure or management of different kinds of disease conditions in traditional medicine.

Table 1. Phytochemical Screening of Plant Extracts

Plant/organ	ALKA	SAPO	TANN	CARD	TERP	ANTR	FLAV	CYAN
Acalypha ciliata (L)	-	-	+	+	+	-	-	-
Acalypha hispida (L)	-	+ +	+ +	+ +	+	-	+	-
Acalypha indica (L)	-	-	+	+	+	-	-	+ + +
Acalypha wilkesiana Red var.(L)	-	+ +	+ +	+ +	+	-	+	-
Acalypha wilkesiana Golden- yellow var.(L)	-	-	+	+ +	+	-	-	-
Acalypha wilkesiana Lace var.(L)	-	-	+ +	+	+	-	-	-
Carica papaya (L)	+	+	+	+	+	-	+	-
C. papaya (S)		+	+	+	+	-	+	-
C. papaya (R)	+	+ +	+	+	+	-	+ +	-
Calotropis procera (L)	+	+ +	+	+ + +	+ + +	-	+	-
C. procera (S)	+ +	+ +	+	+ + +	+ + +	-	+	-
Garcina kola (SE)	+ +	+ + +	+ + +	+ +	+ +	-	+ + +	-
Pycnanthus angolensis (L)	-	+	-	+	+	-	-	-
P. angolensis (S)	-	+	-	+	+	-	-	-
P. angolensis (R)	-	+	-	+	+	-	-	-

Key: = Absent**; +** = Trace ; **++** = Moderately present; **+++** = Abundant; **L** = Leaf extract; **S** = Stem extract; **R** = Root extract; **SE** = Seed extract; **ALKA** =Alkaloids; **SAPO** = Saponins; **TANN** = Tannins; **CARD** = Cardiac glycosides; **TERP** = Terpenes; **ANTR** = Anthraquinones; **FLAV** = Flavonoids; **CYAN** = Cyanogenic glycosides

Table 2: Brine-shrimp Lethality of Plant Extracts (Experiments in Triplicates) Dead Average (DA)

Plant/organ	**1000 µg/ml**				**100 µg/ml**				**10 µg/ml**				**LD_{50} (ppm)**
	1st	**2nd**	**3rd**	**(DA)**	**1st**	**2nd**	**3rd**	**(DA)**	**1st**	**2nd**	**3rd**	**(DA)**	
Acalypha ciliata (L)	10	10	10	(10)	2	2	2	(2)	0	1	0	(0.3)	164.26
Acalypha hispida (L)	7	6	7	(6.7)	1	1	1	(1)	1	1	2	(1.3)	555.92
Acalypha indica (L)	10	10	10	(10)	7	7	7	(7)	6	6	6	(6)	76.56
Acalypha wilkesiana Red var.(L)	10	10	10	(10)	2	2	2	(2)	1	1	1	(1)	NR
Acalypha wilkesiana Golden- yellow var.(L)	10	9	10	(9.7)	3	3	2	(2.7)	0	1	1	(0.7)	NR
Acalypha wilkesiana Lace var.(L)	7	7	8	(7.3)	2	1	1	(1.3)	0	1	1	(0.7)	339. 47
Carica papaya (L)	10	10	10	(10)	10	10	10	(10)	8	8	8	(8)	2.73
C. papaya (S)	6	6	6	(6)	4	4	4	(4)	3	3	3	(3)	384.45
C. papaya (R)	8	8	8	(8)	7	7	7	(7)	6	6	6	(6)	272.75
Calotropis procera (L)	10	10	10	(10)	9	7	9	(8.3)	7	7	5	(6.3)	192.56
C. procera (S)	10	10	10	(10)	8	9	9	(8.7)	7	7	7	(7)	182.45
Garcina kola (SE)	6	6	7	(6.7)	2	1	2	(1.7)	2	1	1	(1.3)	567.76
Pycnanthus angolensis (L)	10	10	10	(10)	9	9	9	(9)	7	7	7	(7)	2.56
P. angolensis (S)	7	7	7	(7)	3	3	3	(3)	2	2	2	(2)	362.67
P. angolensis (R)	9	9	9	(9)	7	7	7	(7)	2	2	2	(2)	310.45
**Persia major* (Bark)													*2.60
**Pogonopus speciosus* (Dry sap)													*50.09
**Myrsine africanus* (R)													*114.07

Values are statistically significant at ($p < 0.05$).

Key:

L = Leaf extract; **S** = Stem extract; **R** = Root extract; **SE** = Seed extract; **DA** = Average number of dead larvae (nauplii); * = Refer to the LD_{50} values of some plants in literature; **NR** = Data did not converge and therefore could not be regressed by the Finney probit analysis software program;**LD_{50}** = Concentration at which 50% dose becomes lethal to the the brine-shrimp larvae (nauplii); p**pm** = Parts per million.

Processing of Plant Materials

The rules governing collection of diferent plant parts were strictly adhered to in the course of this study. Subsequently, the plants were identified and authenticated by following the prescribed guidelines. Also, the principles of extraction and phytochemical screening of extracts were obeyed, thereby preventing any changes to the chemical composition of the extracts[10,11].

Phytochemical Tests

The phytochemical screening indicated that all the plants tested negative to both anthraquinones and cyanogenic glycosides except *A.* I*ndica* which tested positive for cyanogenic glycosides. This was not surprising because acalyphin, a cyanogenic glycoside had previously been isolated from the roots of the plant[12]. However, each plant showed either the presence or absence of alkaloids, saponins, tannins, cardiac glycosides, terpenes and flavonoids (Table 1).

Secondary metabolites such as saponins, cardiac glycosides, alkaloids, tannins and flavonoids have demonstrated in several previous studies[21-33]. They prove to be responsible for the cure or management of different kinds of disease conditions in traditional medicine.

Brine-shrimp Lethality Test

The brine-shrimp assay determines the lethalities of plant extracts toward brine-shrimp larvae (nauplii) and in doing so predicts the ability to kill cancer cell cultures, pests and also exerts a wide range of pharmacological effects[7,8]. The shrimp (nauplii) have been used for a number of bioassay systems in which natural product extracts, fractions or pure isolates are tested at concentrations 1000 µg/ml, 100 µg/ml and 10 µg/ml in vials containing 5ml of brine and ten nauplii in each of the replicates[34]. The LD_{50} values in ppm are estimated with 95% confidence using the appropriate mathematical estimates; the Finney probit analysis program being the model routinely employed. The LD_{50} values of plant extracts obtained in this study compare with those of some plants in literature such as the values of *Myrsine Africanus* (114.07 ppm), *Pogonopus speciosus* (50.09 ppm and *Persia major* (2.60 ppm) which are below 200 ppm but generally considered as 'significant'[32,35-40] as presented in Table 2. The brine-shrimp lethality (i.e. potential anti-cancer activity) is rated 'marginal' or 'significant' depending on the computed LD_{50}. Values below 200 ppm are considered 'significant' (strongly active) while those above it are taken as 'marginal' (weakly active). Consequently, the results presented in Table 2 show that the leaf and stem extracts of *C. Procera* gave 'significant' LD_{50} values of 192.56 and 182.45 ppm respectively. Also, the leaf extracts of *A. Ciliata*, *A. Indica, C. Papaya* and *P. Angolensis* were strongly active at 164.24, 76.56, 2.73 and 2.56 ppm respectively. However, the leaf extracts of *A. Hispida*, *A. Wilkesiana* (Lace variety) and seed extract of *G. kola* gave 'marginal' LD_{50} values of 555.92, 384.45 and 567.76 ppm while the stem and root extracts of *P. Angolensis* and *C. Papaya* were equally weakly active at 362.67, 310.45, 384.75 and 272.75 ppm respectively. These results are not surprising because the extracts of these plants tested positive for cardiac glycosides, terpenes and saponins as displayed in Table 1**.** These classes of compounds have been shown in previous studies to be cytotoxic[31,35,36,37,41,42].

CONCLUSION

The findings of the brine-shrimp lethality assay have revealed potential plant templates such as *A. Ciliata, A. Indica, C. Papaya*, *C. Procera* and *P. Angolensis* which could further be investigated in detailed *in-vitro* and *in-vivo* studies for anti-cancer activity.

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