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PRELIMINARY CYTOTOXICITY STUDIES ON SOME NIGERIAN MEDICINAL PLANTS USED AS TRADITIONAL ANTICANCER REMEDIES

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ABSTRACT
Crinum jagus, *Icacina trichantha* and *Solanum erianthum* are used in ethno-medicine for the treatment of cancer. This study screened different parts of these plants (leaves and bulb of *C. jagus*; leaves and tuber of *I. trichantha*; and leaves and stem of *S. erianthum*) for cytotoxicity. Dried samples of these plant parts were extracted successively with hexane, ethylacetate and methanol using the maceration method and concentrated to dryness using a rotary evaporator. Cytotoxicity studies on the extracts were carried out using the brine shrimp assay at concentrations of 10 µg/mL, 100 µg/mL and 1000 µg/mL (in triplicates). The response of the nauplii to different concentrations of the extracts was observed after 24 h. The LC_{50} value was used as a measure of the toxicity of the extracts. The LCc_{50} values for the different extracts of the leaves and bulb of *Crinum jagus* ranged between 0.251 µg/ml and 10.280 µg/ml. Values ranging between 9.992 µg/mL and 192.602 µg/mL were obtained for the leaves and tuber of *Icacina trichantha*. The leaves and stem of *Solanum erianthum* gave LC_{50} values between 2.395 µg/mL and 84.924 µg/mL. The plant parts under study had very low LC_{50} values and therefore show promise as potential sources of novel anticancer agents.

Keywords: Cytotoxicity, LC_{50}, Nauplii, Extracts, Anticancer.

INTRODUCTION

Cancer remains one of the major causes of human mortality all over the world (GDB, 2015). Cancer is a complex disease and treatment often involves the use of radiation, surgery and chemotherapy or a combination of these procedures (Abbas & Rehman, 2018). Chemotherapy often involves the use of cytotoxic drugs which act by killing cancerous cells (Bagnyukova *et al*., 2010). Plants have been known to be used widely for the treatment of cancer by traditional medical practitioners and plant-derived compounds have played significant role in the development of chemotherapeutic drugs (Kuruppu *et al*., 2019). Extracts of plants used in drug discovery efforts are normally subjected to a battery of assays in order to identify fractions possessing the required medicinal property. One of such assays is the Brine Shrimp Lethality Assay (BSLA) which gives preliminary information on the cytotoxity of extracts under investigation and therefore their anticancer potentials (Hamidi *et al*., 2014). This assay can be carried out rapidly (within 24 h) and inexpensive. It is simple, cheap, easily mastered and can be carried out with small amount of material (Meyer *et al.,* 1982). In addition, BSLA and other *in vivo* lethality test have been successfully employed for bioassay-guided fractionation of active cytotoxic and antitumor agents (Krishnaraju & Tsay, 2006). The technique has been used to identify over 300 novel antitumor and pesticidal natural products (Krishnaraju & Tsay, 2006).

Crinum species have a great medicinal reputation in traditional medicines of Africa, tropical Asia and South America. They are used as expectorants, laxatives, emetics, anti-malaria, anti-aging, anti-tumour and anti-asthmatics (Levin, 2001; Kapu *et al*., 2001). They are also used in the treatment of inflammatory disorders such as rheumatism, earache, wounds, backache, headache, edema and haemorrhoids (Ahmad, 1996). They also possess antimicrobial properties as they have been used in the treatment of various parasitic skin diseases, abscesses, leprosy, anthrax, dysentery, tonsillitis, laryngitis and sexually transmitted diseases. The plant, *Crinum jagus* belongs to the family, *Amaryllidaceae*. The plant is used in southwestern Nigeria for the treatment of tuberculosis, chronic cough, rheumatism and cancer. Previous studies on *C. jagus* extracts indicate it possesses antihaemorrhagic (Ode *et al*., 2010), anti-venom (Ode & Asuzu, 2006), and liver-protective properties (Nwaehujor *et al*., 2012)

Solanum species have been known to possess antiviral, antimalarial, antimycotic, tetratogenic, molluscidal and cytotoxic properties (Huang *et al.,* 2009). *Solanum erianthum* also known as 'potato tree' belongs to the family *Solanaceae*. The decoction of the leaves is used by traditional medical practitioners in southwestern Nigeria for the treatment of malaria, leprosy and cancer.

Icacina trichantha belonging to the family, *Icacinacea* is a shrub up to 2 m in height. It produces very large tuber of forest and jungle vegetation in southern part of Nigeria (Burkill, 1994). The tuber is used by herbalists for the treatment of poisoning, constipation, induce emesis and to cure malaria. Some traditional medical practitioners claim the leaves are effective in treating cancer.

This study evaluated extracts of different parts of *C. jagus*, *S. erianthum* and *I. trichantha* for cytotoxic properties using the Brine Shrimp Lethality Assay. The aim was to provide preliminary scientific information on whether or not these plants actually possess anticancer activities as claimed by herbalists.

MATERIALS AND METHODS

Sample Collection and Extract Preparation

Samples of the different plant parts under investigation (leaves and bulb of *C. jagus*, leaves and tuber of *I. trichantha*, and leaves and

stem of *S. erianthum*) were collected from the Botanical Gardens of the University of Ibadan. The samples were identified by Mr Kayode Owolabi, a taxonomist working with the Garden.
The samples were dried under mild sunlight and pulverized. Thereafter, 1000g of each of the samples was extracted successively with hexane, ethylacetate and methanol using the maceration technique. Each extraction step lasted for 3 days. The extracts obtained were separated from the residue using Whatman's No 1 filter paper. The extracts were concentrated to dryness using rotary evaporator.

Brine Shrimp Lethality Assay
The experiment was carried out as described by Osho & Lajide (2012) with slight modifications. Brine shrimp eggs were hatched in artificial sea water (5 g of salt in 200 mL of water). Ten living nauplii were counted using a Pasteur pipette and added to vials containing different concentrations (1000 µg/mL, 100 µg/mL and 10 µg/mL) of the extracts in Dimethylsulfoxide (DMSO). The experiments were carried out in triplicates. Control experiment containing a mixture of DMSO and artificial sea water only was also set up. After 24 h, the vials were examined against a lighted background. The average number of nauplii that survived in each vial was thereafter determined. Nauplii were considered dead if they do not exhibit any movement on observation.

Statistical/Data Analysis
The median lethal concentration (LC_{50}), which is the concentration of sample required to kill 50 % of brine shrimp, was obtained by a plot of the percentage of the shrimps killed against the logarithm of the sample concentration (Meyer *et al*., 1982). The LC_{50} values were determined using a probit regression analysis which employs the Finney's statistical method (Finney, 1971). This statistical procedure has been incorporated into the IBM SPSS used for the determination of LC_{50} values in the current study.

RESULTS AND DISCUSSION
In the current study, extracts of the leaves and bulb of *C. jagus* (reconstituted in DMSO) have been screened for cytotoxicity at concentrations of 10, 100 and 1000 µg/mL. Most studies involving assessment of plant extracts for cytotoxicity are carried out at these concentrations (Kamanja *et al*., 2018; Omeke *et al*., 2018). Dimethylsulfoxide is widely employed for the reconstitution of plant extracts because brine shrimp nauplii have been known to show no significant sensitivity to the solvent up to 11 % concentration (Kamba & Hassan, 2010; Musa, 2012; Ahmed *et al*., 2013). Preparation of the stock solution for each of the extracts followed by serial dilution to obtain the different test concentrations made it possible to determine the linear increase in toxicity with increasing extract concentration. The number of survivors in each vial, together with the percentage mortalities obtained at each concentration, is recorded in Tables 1-3. These data were then used to determine the LC_{50} for each extract. The values obtained could serve as a basis for comparison of the potencies of the extracts. Solvents of differing polarities were employed in the extraction of the dried plant samples prior to carrying out the assay. Differences in polarity among various solvents are known to be responsible for the differences in solubility of active plant active properties, hence variations in the degree of activity (Ngo *et al*., 2017). This likely accounted for the differences in the LC_{50} values obtained for different solvent fractions of the same plant parts in the current study. Similar results have been severally reported in literature (Kamanja *et al*., 2018; Omeke *et al*., 2018).

From the results, it could be observed that the degrees of lethality increased with exposure to higher concentrations of the extracts. This is expected as the quantity of the toxic compounds in the extracts would likely increase with the concentration of the extracts. The LC_{50} values obtained from extracts of the leaves and bulb of *C. jagus* ranged between 0.251 (for methanol extract of the leaves, CJLME) and 10.540 (for hexane extract of the bulb, CJTHE). The LC_{50} values for *I. trichantha* extracts ranged between 9.992 (for methanol extract of the tuber, ICTME) and 192.602 (for hexane extract of the leaves, ICLHE). For the stem and leaves extracts of *S. erianthum*, the LC_{50} values obtained ranged between 2.395 (for the hexane extract of the stem, SESHE) and 91.352 (for hexane extract of the leaves, SELHE). From the Tables 1-3, the most toxic among the extracts screened for cytotoxicity are CJLEE, CJLME, CJTEE, CJTME, ICTME and SESHE (all these extracts had LC_{50} values less than 10).

Table 1: Response of Nauplii to Different Concentration of *C. jagus* Extracts

Extracts	Conc(µg/ml)	Living Nauplii			Percentage Mortality	LC_{50} (µg/ml)
		1	2	3		
CJTHE	10	7	5	4	47	10.540
	100	3	2	2	77	
	1000	0	0	0	100	
CJTEE	10	1	5	7	57	9.032
	100	0	0	0	100	
	1000	0	0	0	100	
CJTME	10	4	3	1	73	3.215
	100	1	0	0	97	
	1000	0	0	0	100	
CJLHE	10	6	5	4	50	10.280
	100	3	0	0	90	
	1000	0	0	0	100	
CJLEE	10	2	2	2	80	5.015
	100	0	0	0	100	
	1000	0	0	0	100	
CJLME	10	3	0	2	83	0.251
	100	1	4	1	80	
	1000	0	0	0	100	

Key: CJTHE-Hexane Extract of *C. jagus* bulbs; CJTEE-Ethylacetate Extract of *C. jagus* bulbs; CJTME-Methanol Extract *C .jagus* bulbs; CJLHE-Hexane Extract of *C. jagus* leaves; CJLEE-Ethylacetate Extract of *C.jagus* leaves; CJLME-Methanol Extract *C. jagus* leaves

Table 2: Response of Nauplii to Different Concentrations of *I. trichantha* Extracts

Extracts	Conc(µg/ml)	Living Nauplii			Percentage Mortality	LC_{50} (µg/ml)
		1	2	3		
ICTHE	10	9	5	7	30	48.378
	100	4	6	6	47	
	1000	0	0	0	100	
ICTEE	10	6	7	7	33	32.526
	100	2	4	6	60	
	1000	0	0	0	100	
ICTME	10	4	3	5	53	9.992
	100	5	5	4	60	
	1000	0	0	1	97	
ICLHE	10	8	9	9	13	192.602
	100	10	10	5	17	
	1000	0	3	0	90	
ICLEE	10	7	6	9	27	92.168
	100	7	7	8	27	
	1000	1	0	0	97	
ICLME	10	3	7	6	47	17.131
	100	3	4	3	67	
	1000	0	0	0	100	

Key:ICTHE-Hexane Extract of *I. trichantha* tuber; ICTEE-Ethylacetate Extract of *I. trichantha* tuber; ICTME-Methanol Extract *I. trichantha* tuber; ICLHE-Hexane Extract of *I. trichantha* leaves; ICLEE-Ethylacetate Extract of *I. trichantha* leaves; ICLME-Methanol Extract *I. trichantha* leaves

Table 3: Response of Nauplii to Different Concentration of *S. erianthum* Extracts

Extracts	Conc(µg/ml)	Living Nauplii			Percentage Mortality	LC_{50} (µg/ml)
		1	2	3		
SESHE	10	3	1	3	77	2.395
	100	1	0	0	97	
	1000	0	0	0	100	
SESEE	10	10	7	8	17	84.924
	100	6	6	7	37	
	1000	0	0	0	100	
SESME	10	6	10	0	47	13.371
	100	0	0	6	80	
	1000	0	0	0	100	
SELHE	10	9	7	9	17	91.352
	100	7	5	5	43	
	1000	4	3	2	70	
SELEE	10	4	5	4	50	14.967
	100	4	6	5	57	
	1000	0	0	0	100	
SELME	10	6	7	2	50	12.159
	100	4	2	1	77	
	1000	0	0	0	100	

Key: SESHE-Hexane Extract of *S. erianthum* stem; SESEE-Ethylacetate Extract of *S. erianthum* stem; SESME-Methanol Extract *S. erianthum* stem; SELHE-Hexane Extract of *S.erianthum* leaves; SELEE-Ethylacetate Extract of *S. erianthum* leaves; SELME-Methanol Extract *S. erianthum* leaves

Two most commonly used criteria for measuring the toxicity of herbal extracts are the Meyer's and Clarkson's toxicity indices. Meyer's toxicity index indicates that extracts with LC_{50} < 1000 µg/ml are considered toxic while those with LC_{50} < 1000 µg/ml are non-toxic (Meyer *et al*., 1982). Clarkson graded extract toxicity in the following order: extracts with LC_{50} > 1000 µg/ml are non-toxic, LC_{50} between 500 - 1000 µg/ml are low toxic, extracts with

LC_{50} of 100 - 500 µg/ml are medium toxic, while extracts with LC_{50} of 0 - 100 µg/ml are highly toxic (Clarkson *et al*., 2004). Using the Meyer index, all the extracts under investigation are toxic. However, with Clarkson's classification, all the extracts from the different plant parts under investigation are toxic (LC_{50} < 100 µg/ml) except the hexane extract of the leaves of *I. trichantha* (LC_{50} = 192.602) which has medium toxicity.

Literature search provides several pieces of evidences that suggest that plant extracts with very low LC_{50} values have a likelihood of yielding anticancer compounds. For example, the plant, *Phyllanthus engleri, in* a cytotoxicity study using brine shrimps, gave an LC_{50} of 0.47 µg/ml (Moshi *et al.*, 2004). Recently englerin A, a selective anti-cancer compound against kidney cancer cells was isolated from the plant (Ratnayake *et al.*, 2009). Similarly, the root extract of *Ozoroa insignis* with LC_{50} of 2.21 µg/ml in brine shrimp assay showed moderate activity in KB, A 549 and MDA-MB cell lines, with IC_{50} values of 30.5, 22.0 and 15.5 µg/ml, respectively (Moshi *et al*., 2004; Abreu *et al*., 1999). Also six dihydrofuranoxanthone epoxides isolated from *Psorospermum febrifugum* were found to exhibit significant *in vitro* cytotoxic activity against 9PS cells in culture. *Psorospermum febrifugum* had LC_{50} value of 12.7 µg/ml in brine shrimp assay (Abou-Shoer *et al*., 1988; Moshi *et al*., 2006). These previous studies provide some evidences that the extracts of plants used in the current study may actually be potential sources anticancer compounds.

Conclusion

The results of this preliminary study appear to support the utilization of the plants by herbalists in the treatment of cancer. However, this must be confirmed by screening the extracts for activity against actual cancer cell lines.

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