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Comparative cytotoxicactivity of selected Nigerian medicinal plant extracts on Ehrlich ascites carcinoma cells

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ABSTRACT

Background: Nigerian medicinal plants have long been reported to manage cancer and its symptoms. Some of the frequently mentioned plants used for the treatment of cancer reported in Nigerian Ethnobotanical surveys include *Securidaca longepedunculata*, *Tetrapleura tetraptera*, *Morinda lucida*, *Spondias mombin* and *Nymphaea lotus*. The cytotoxicities of some of these plants have been reported in literature. However, there is no comparison of the cytotoxicities of these plants on Ehrlich ascites carcinoma (EAC) cells. This research aims to compare the *in vitro* cytotoxic activities of the anti-cancer plants using EAC cells.
Method: Aqueous extracts of *Securidaca longepedunculata*, *Tetrapleura tetraptera*, *Morinda lucida*, *Spondias mombin* and ethanol extracts of *Tetrapleura tetraptera, Spondias mombin*, *Nymphaea lotus* were prepared. The different extracts were used to test for cytotoxicity on Ehrlich ascites carcinoma cells using the Trypan blue dye exclusion principle.
Results: All extracts caused dose-dependent increase in mortality of EAC cells. IC_{50} values of the extract range from 11.48 for Aqueous extract of *Tetrapleura tetraptera* to 2691µg/ml for ethanol extract of *Nymphaea lotus*.
Conclusion: Aqueous extract of Tetrapleura tetraptera was the most cytotoxic with an IC_{50} of 11.48 µg/ml compared to 5-fluorouracil with 2.88 µg/ml.

Key words: *Securidaca longepedunculata*, *Spondias mombin*, *Tetrapleura tetraptera*, *Nymphaea lotus*, *Morinda lucida*, cytotoxic, plants, Nigeria

INTRODUCTION

Medicinal plants have long been reported to be used in the management of non-infectious ailments like Cancer[1]. Nigeria has a large flora of medicinal plants implicated in the treatment and management of cancer[2]. Some of these plants include *Securidaca longepedunculata*[3,4,5,6], *Tetrapleura tetraptera*[7], *Morinda lucida*[8], *Spondias mombin*[9] and *Nymphaea lotus*[8,10]. Plants used in the treatment and management of cancer exert this effect through mechanisms which include telomerase inhibition[11], cytotoxicity[9], pro-apoptosis[12], alkylating[6] and anti-mitotic[13]. The in vivo and in vitrocytotoxic activity of *Securidaca longepedunculata* aqueous root bark extract on Ehrlich ascites carcinoma (EAC) cells has been reported in a previous work by Lawal *et al*.[3] while the cytotoxicity of the extract on EAC cells and components of the root bark extract were also reported[5]. The *in vitro* and *in vivo* cytotoxic activity of ethanol extract of *Tetrapleura tetraptera* on EAC cells were equally reported in a previous work by Ozaslan *et al*.[7]. The cytotoxic effect of *Morinda lucida* and *Nymphaea lotus* on Brine shrimps has also been reported[11]. EAC cells have been severally used as a model for breast cancer (3,7). However, there has been no comparison of the in vitro cytotoxicities of major Nigerian anti-cancer medicinal plants on EAC cells, a model for breast cancer. Hence, this research aims to compare the in vitro cytotoxicities of *Securidaca longepedunculata*, *Tetrapleura tetraptera*, *Morinda lucida*, *Spondias mombin* and *Nymphaea lotus* on EAC cells.

MATERIALS AND METHODS Chemicals

The Trypan blue used for cytotoxicity assay was obtained from Sigma-Aldrich (St. Louis, US), 5-Fluorouracil purchased from Kocak Farma (Turkey) was used as a standard cytotoxic drug. All other chemicals used were of analytical grade available locally.

Ehrlich ascites carcinoma cells

EAC cells used in this research were obtained from the Molecular Biology Division, Department of Biology, University of Gaziantep, Gaziantep, Turkey.

Collection and authentication of plant materials

The root bark of *Securidaca longepedunculata*,

the fruits of *Tetrapleura tetraptera*, root bark of M*orinda lucida*, stem bark of *Spondias mombin* and *Nymphaea lotus* leaves were collected from Osogbo, South-West, Nigeria in 2010. The plants were identified and authenticated by Dr George Nodza. Voucher specimen of each plant was deposited in the University Herbarium, University of Lagos, Lagos, Nigeria with the following voucher number; *Securidaca longepedunculata* (LUH 3593), *Tetrapleura tetraptera* (LUH 4197), *Morinda lucida* (LUH 8392), *Spondias mombin*() and *Nymphaea lotus* (LUH 3493) .

Preparation of plant extracts

The plant materials were shade dried for 3 days and pulverized into powder. Aqueous extracts of the coarsely powdered material of *Securidaca longepedunculata* (SLW), *Morinda lucida* (MLW), *Spondias mombin* (SMW) and *Tetrapleura tetraptera* (TTW) was prepared by macerating 1kg of root bark in 1 L of distilled water for 72 hours. The macerate was filtered and the filtrate was concentrated using the Rotary Evaporator (Rotavapor® R-300, BUCHI, Switzerland) and further concentrated to constant weight in vacuo using a lyophilizer (Lyotrap, LTE, England). Ethanol extract of the coarsely powdered material of Spondias mombin (SME), *Tetrapleura tetraptera* (TTE) and *Nymphaea lotus* (NLE) was prepared by macerating 1kg of root bark in 1 L of ethanol for 72 hours. The macerate was filtered and the filtrate was concentrated using the Rotary Evaporator (Rotavapor® R-300, BUCHI, Switzerland) to constant weight.

Cytotoxicity study

The determination of *in vitro* cytotoxic activity was carried out using the Trypan Blue dye exclusion method. Briefly, aqueous and ethanol extracts in Phosphate buffered saline (1000, 100, 10, 1 and 0.1 µg/ml) were incubated with aliquot of 100 µL of EAC with a concentration of 106 cells/mL at 370C for 25 h. The standard cytotoxic drug, 5-Fluorouracil (5-FU) with concentrations of 1000, 100, 10 and 1 µg/mL was used as the positive control and equally incubated with aliquot of 100 µL of EAC with a concentration of 106 cells/mL at 370C for 25h. The cells were stained with trypan blue dye (Sigma-Aldrich, St. Louis, USA). Ascitic tumour cell counts were done in a Cedex Cell Counting machine (Roche, California) in which viable cells were unstained while damaged and non-viable cells were stained blue. Results were expressed as percentage cell viability. Percentage mortality was calculated as % Mortality = 100 - % cell viability (14).

Statistical Analyses

Graphpad prism 15 was used to draw charts. The IC50 was calculated using the Finney Probit Analysis method (15).

RESULTS

The results of this study indicated that the aqueous extract of *Tetrapleura tetraptera* caused the highest mortality for both the lowest and highest doses with percentage mortalities of 18.4 and 90% respectively. All extracts concentrations showed varying cytotoxicities to Ehrlich ascites carcinoma cells in vitro. The extracts exhibited a dose-dependent increase in cytotoxicity to Ehrlich ascites carcinoma cells *in vitro* i.e the higher the dose of the extracts, the higher the percentage mortality of Ehrlich ascites cells (Table 1).

The aqueous extract of T*etrapleura tetraptera* had the least IC_{50} of 11.48 µg/ml. Fluorouracil had an IC_{50} of 2.88 µg/ml.Aqueous extracts of *Securidaca longepedunculata* (SLW), *Morinda lucida* (MLW) and *Spondias mombin* (SMW) had IC_{50} of 67, 75.85 and 33.11µg/ml. Ethanol extract of *Spondias mombin* (SME), *Tetrapleura tetraptera* (TTE) and Nymphaea lotus (NLE) had IC_{50} values of 74.13, 13.18 and 2691 µg/ml (Table 2).

Table 1: *In vitro* cytotoxic activity of Nigerian medicinal plants extracts on Ehrlich Ascites Carcinoma cells.

Concentration (µg/ml)	Mortality (%)							
	SLW	MLW	SMW	SME	TTW	TTE	NLE	5-FU
1000	82.5	75.0	73.2	82.6	90.0	74.8	40.8	51.9
100	45.1	48.2	70.5	50.0	83.2	62.2	39.6	39.8
10	27.9	29.0	39.5	16.9	29.8	40.5	7.8	29.5
1	10.6	16.4	29.6	10.1	22.2	32.5	5.8	9.6
0.1	7.3	14.6	7.3	9.0	18.4	21.4	4.0	5.0

*Results are expressed as Mean of 3 determinations

Keys: **SLW** – Aqueous extract of *Securidaca longepedunculata*, **MLW** – Aqueous extract of *Morinda lucida*, **SMW** – Aqueous extract of *Spondias mombin*, **SME** – Ethanol extract of *Spondias mombin*, **TTW** -Aqueous extract of *Tetrapleura tetraptera*, **TTE** - Ethanol extract of *Tetrapleura tetraptera*, **NLE** – Ethanol extract of *Nymphaea lotus*, **5-FU** – 5-fluorouracil.

Table 2: Comparative cytotoxicities of Nigerian medicinal plant extracts

Extract	SLW	MLW	SMW	SME	TTW	TTE	NLE	5-FU
IC_{50} (µg/ml)	67.00	75.85	33.11	74.13	11.48	13.18	2691.00	2.88

Keys: IC_{50} – Inhibitory concentration that will kill 50% of the cells, **SLW** – Aqueous extract of *Securidaca longepedunculata*, **MLW** – Aqueous extract of *Morinda lucida*, **SMW** – Aqueous extract of *Spondias mombin*, **SME** – Ethanol extract of *Spondias mombin*, **TTW** -Aqueous extract of *Tetrapleura tetraptera*, **TTE** - Ethanol extract of *Tetrapleura tetraptera*, **NLE** – Ethanol extract of *Nymphaea lotus*, **5-FU** – 5-fluorouracil.

DISCUSSION

The trypan blue exclusion assay has been used to assess cytotoxicity of plant extracts[3, 4, 6,16]. The results of the assay indicate percentage of viable cells. However, the number of non-viable or dead cells can be obtained if the number of viable cells and total number of cells is known. The percentage mortality was converted to probit values and used to plot a graph against the log of extract concentrations. The equation of the line of the graph can be used to determine the IC50, which is the inhibitory concentration that will kill fifty percent of EAC cells. The results of the cytotoxic assay of Nigerian plant extracts on EAC cells indicate a dose-dependent increase in mortality for all extracts tested. This indicates that EAC cells exposed to higher doses of the extract had a larger percentage of their cells stained blue[17].The aqueous extract of *Tetrapleura tetraptera* was observed to have the least IC_{50}, hence the most cytotoxic, amongst the extracts tested. The ethanol extract of *Tetrapleura tetraptera* had slightly lower cytotoxicity to the aqueous extract. The little difference in cytotoxicities observed between the two extracts may be due to the differences in polarity of the solvents (water and ethanol) used in extraction. The IC_{50} of the ethanol extract of *Tetrapleura tetraptera* on EAC reported here is quite different from and much lower than the values obtained in the work of Ozaslan *et al*.[7]. The disparity in values might have been due to experimental differences and mathematical methods used in the studies. *Tetrapleura tetraptera* has been reported to containpolar bioactive agent that could be implicated in cytotoxic activity[18]. Aqueous extract of Spondias mombin was observed to be the third most cytotoxic extract. The ethanol extract of *Spondias mombin* had a much higher IC_{50} compared to the aqueous extract. This indicates that water is a much better solvent in extracting the cytotoxic principles, which are likely polar compounds. Spondias mombin has a lot of potential active principles reported in literature[19] which could be responsible for the observed cytotoxic activity. The fourth most cytotoxic extract is the aqueous extract of *Securidaca longepedunculata* with an IC_{50} value much higher than the aqueous extract of Spondias mombin but lower than the IC_{50} of the ethanol extract of *Spondias mombin*. Polar compounds extracted from *Securidaca longepedunculata* have been implicated in its cytotoxic activity (5). This further corroborates previous study (3) that reported the ability of *Securidaca longepedunculata* to cause an increase in life span of tumour-bearing mice model of breast cancer. However, *Nymphaea lotus* had the lowest IC_{50} and the least cytotoxic of the extracts tested on EAC cells. This contrasts with the suggestion of Sowemimo *et al*.[11] that ethanol extract of Nymphaea lotus had a low LD_{50}. In that study, *Nymphaea lotus* displayed strong anti-cancer activity through its telomerase inhibiting activity. This further supports the assertion that medicinal plants display anti - cancer activity through different mechanisms[20]. In another study[12], Nymphaea lotus was shown to have amino acids which are thought to be responsible for the telomerase inhibiting ability of the extract. Our present study indicates that the anti-cancer mechanism of Nymphaea lotus is not cytotoxic as depicted by the very high IC_{50} value obtained with EAC cells. The IC_{50} of 5-fluoruracil, a known anti-cancer drug, calculated in this study is however lower compared to the most cytotoxic of the extracts used in this study. *Tetrapleura tetraptera* had the IC_{50} value closest to the standard anti-cancer drug. Aqueous extract of *Tetrapleura tetraptera* exhibited comparable cytotoxicity to the standard anti-cancer drug.

CONCLUSION

Aqueous extract of *Tetrapleura tetraptera* showed the least IC_{50} of 11.48 µg/ml compared to 5-fluorouracil with 2.88 µg/ml.

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