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The Cytotoxic Effects of Extracts of Different Solvents of *Lonchocarpus cyanescens'* Stem Against Triple-Negative Breast Cancer (TNBC) Cell Lines

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ABSTRACT

Lonchocarpus cyanescens, a plant widely used in traditional African medicine, has shown promise for cancer therapy. This study evaluates the cytotoxic effects of extracts from different solvents (n-hexane, ethyl acetate and methanol) from *L. cyanescens* against four triple-negative breast cancer cell lines (MCF7, MDA-MB-231, Hs578T, and SkBr3). Brine Shrimp Lethality Test (BSLT) was carried out as preliminary toxicity test and MTT assay for invitro cytotoxicity against the four TNBC cell lines respectively. In the BSLT, methanol showed the highest toxicity (LC_{50} = 51.19 µL), followed by A20E (LC_{50} = 72.26 µL) and A10N (LC_{50} = 81.27 µL), indicating that methanol highest concentration of cytotoxic bioactive compounds compared to the hexane and ethyl acetate extracts. The MTT assay further supported these findings, revealing that methanol extract had the lowest IC_{50} values: 77 µM for MCF7, 69 µM for MDA-MB-231, and 71 µM for SkBr3, making it the most potent extract. In contrast, n-hexane extract, had IC_{50} values >100 µg/mL across all cell lines, except for MDA-MB-231 where it has 97 µM while ethyl acetate exhibited moderate activity with an IC_{50} of 87 µM against MDA-MB-231 and 71 µM against Hs578T respectively. These findings confirm the cytotoxic potential of *L.C* and highlight its prospective role in TNBC therapy. This study establishes a strong foundation for additional investigations into isolation and characterizing of active compounds from the crude extracts as well as mechanistic studies to determine its mode of action against breast cancer cells.

KEYWORDS: Lonchocarpus cyanescens (L.C), Triple Negative Breast Cancer (TNBC), Cytotoxicity, MTT, BSLT.

1. INTRODUCTION

Globally, the prevalence of cancer and its associated mortality rate is still rising, particularly in undeveloped and developing nations. In Nigeria, it is constantly increasing and this may be attributed to lack of affordable healthcare for early diagnosis and treatment. Breast and prostate cancer have the highest incidence in women and men, respectively [1]. Different plant extracts contain phytochemicals that can be utilized as chemotherapeutics. These phytochemicals have demonstrated a variety of antitumor, anti-inflammatory, anti-oxidant, anti-cancer, and antibacterial properties [2]. Researchers have attributed the anticancer activity of medicinal plants to the presence of antioxidants [3]. TNBC is the most aggressive kind of breast cancer, accounting for about 15–25% of all occurrences. Because TNBC lacks ER, PR, and HER2 receptor expression, it is resistant to hormones and HER2-therapies, making treatment difficult [4]. *Lonchocarpus cyanescens* is part of the *Lonchocarpus* species belonging to the Fabaceae family. The plant has historically been utilized for medical purposes. In Senegal, the leaves are served as a side dish with couscous. It is believed in Ghana that L.C roots are more effective than leaves for treating skin disorders and ulcers. In Sierra Leone and Guinea-Bissau, leprosy has also been thought to be treatable using the leaves and roots of this plant. Laxatives can be made from leaves. Leaf sap is consumed in Benin to treat diarrhea and gastrointestinal problems. Women are given a decoction of leafy twigs and roots during or after childbirth, and it is also used as an aphrodisiac. Yaws are treated with ground root, and sores are treated by washing in water containing powdered root [5]. The antioxidant property of its leaf extract using bio-assay was investigated [6]. Acute ulcerous pain in rats with aqueous root extract of *L. cyanescens*, which exhibited both antiulcer and analgesic effects, justifying the folkloric claim for the health benefits of this plant was studied [7]. The anti-cancer property of the plant has not been fully investigated. Hence, the present study is aimed to investigate the cytotoxicity of the crude extracts from Nigeria *Lonchocarpus cyanescens* for breast cancer therapy.

2. MATERIALS AND METHODS

2.1 Plant Collection and Identification

The stem bark of the plant was collected at random during the morning and evening over a period of five 5days from Dumbi Zaria, Kaduna State. It was subsequently presented for verification and authentication at the Botany section of the Department of Biological Sciences, Ahmadu Bello University

Zaria, Kaduna, Nigeria. A sample with identification number ABU090058 has been deposited at the herbarium section of the Department.

2.2 Sample Treatment

Freshly collected stem bark was washed to remove dirt, rinsed and air dried at a room temperature. This was pulverized using a wood milling machine, sieved, weighed and stored in clean containers at ambient temperature and stored for further utilization.

2.3 Extraction of Crude Extracts

The crude extracts of Lonchocarpus cyanescens' stem was extracted by cold maceration continuosly with n-hexane, ethyl acetate and methanol (1500ml each). A portion (500g) of the pulverized plant stems part was soaked using 1500 mL of n-hexane and allowed to stand for seven (7) days at room temperature, filtered with filter paper to get the crude extracts of Lonchocarpus cyanescens and concentrated in vaccuo. Then the residue was dried, soaked with ethyl acetate and allowed to stand for another 7 days. The same procedure was carried out for the ethyl acetate and Methanol.

2.4 Collection of Tnbc Cell Lines and Artemia Salina Shrimp Eggs.

Artemia salina shrimp eggs were collected for cytotoxicity test from standard laboratory abroad for BSLT. Four TNBC cell lines were used in the course of this study which includes: invasive ductal carcinoma (MCF7), Adenocarcinoma (MDA-MB-231), invasive ductal carcinoma (Hs578T), and Invasive ductal carcinoma (SKBr3) respectively and were collected from cell-culture laboratory, centre for Natural Product Discovery. School of Biochemical Science, Liverpool John Moones University (LJMU) Liverpool, UK.

2.5 Cytotoxicity Assay of the Crude Extracts.

2.5.1 The BSLT assay protocol

The cytotoxicity of n-hexane (A10N), Ethyl acetate (A20E) and Methanol (A30M) crude extracts, were evaluated using the Brine Shrimp Lethality assay (BSLT) [8].

2.5.2 MTT Assay of the Crude Extracts

The cell lines were trypsinized, cleaned with phosphate buffer saline (PBS), plated on 96-well plates, and incubated for 24 hours at 37°C with 5% CO_2. Various quantities of L.C. crude extracts were applied to the cells. In order to get final extract concentrations, stock solutions were diluted in culture media, with a final DMSO content of 0.1%. The identical batch of cells was subjected to each concentration in triplicate. The ability of living cells to convert the yellow dye 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl2H-tetrazolium bromide (MTT) into a blue formazan product was used to measure the growth of tumor cells. Following a 24-hour incubation period, MTT solution was added to each well's medium, and the mixture was then incubated for an additional two hours. The formazan crystals formed by live cells were dissolved in iso-propanol and gently agitated after the MTT reagent was withdrawn. A plate reader was then used to measure the absorbance at 560 nm.

3. RESULTS AND DISCUSSION

3.1 Results

3.1.1 Brine–shrimp lethality test result of n-Hexane (A10N), Ethyl acetate (A20E) and Methanol (A30M) Crude extracts of *L. cyanescens*.

The LC_{50} value of n-Hexane, ethyl acetate and methanol are shown as 81.27, 72.26 and 51.19 respectively in table 1.

Table 1: LC_{50} values of n-Hexane (A10N), Ethyl acetate (A20E) and Methanol (A30M) Crude extracts of *L. cyanescens*

Conc.	**1000 µg/cm³**		**100 µg/cm³**		**10 µg/cm³**		**Control**		**LC_{50}U/L Limit**
Sample Extract	Survivor	Dead	Survivor	Dead	Survivor	Dead	Survivor	Dead	
A10N	2	28	5	25	16	14	10	0	81.27
A20E	5	25	6	24	14	16	10	0	83.79
A30M	0	30	5	25	12	18	10	0	51.19

Key: LC_{50}< 1000µg/cm³ = Toxic, LC $_{50}$ >1000µg/cm3 =Not Toxic *High/Low 95% Confidence interval.

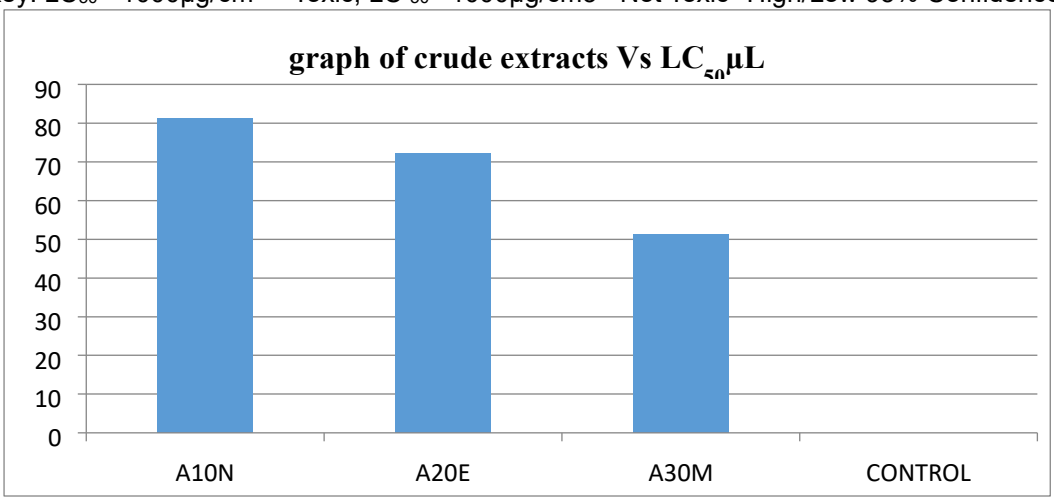

Figure 1: A Graph showing the LC_{50} values for the crude extracts of *L. cyanescens*

3.1.2 The MTT assay protocol of n-Hexane, Ethyl acetate and Methanol Crude extracts

Table 2: IC_{50} values of n-Hexane, Ethyl acetate and Methanol Crude extracts compared with the IC_{50} values of Etoposide, a standard reference.

Cell type	**A10N**	**A20E**	**A30M**	**Etoposide**
		IC_{50} values (µg/mL)		
MCF7	>100	>100	77	68
Hs578T	>100	71	98	78
SKBr3	>100	>100	>71	70
MDA-MB-231	97	87	69	68

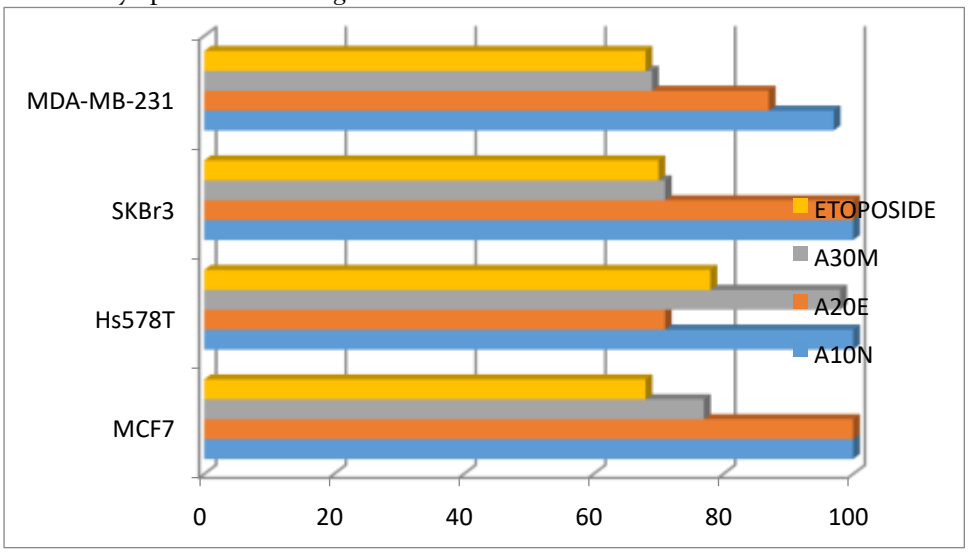

Figure 2: A bar graph showing the IC_{50} values of A10N, A20E and A30M on TNBC cell lines against the standard drug, ETOPOSIDE

3.2 Discussion

From table 1, Methanol extract has the highest (potent) activity, followed by ethyl-acetate and then nhexane extracts, indicating that methanol had the highest concentration of cytotoxic bioactive compounds compared to the hexane and ethyl acetate extracts. Lower IC_{50} value indicates more potent inhibition. The MTT assay supported these findings, revealing that methanol extract had the lowest IC_{50} values: 77 µM for MCF7, 69 µM for MDA-MB-231, and 71µM for SkBr3, making it the most potent and effective extract in table 2. Thus, it can be said that the methanol crude extracts pose good activity (Highest activity) followed by Ethyl acetate and then the n-Hexane, however, lower than that of standard drugs Etoposide. The high cytotoxicity recorded may be partly attributed to the presence of secondary metabolite groups of tannin, saponin, terpenoid, steroid, cardiac glycoside, flavonoids and phlobatannins [9]. A compound is categorized as very toxic if it has an LC_{50} value of less than 30 ppm, is categorized as toxic if it has an LC_{50} value of 30-1000 ppm, and is categorized as non-toxic if it has an LC_{50} value above 1000 ppm [10]. No prior study has reported the cytotoxicity of *Lonchocarpus cyanescens'* stem using the Brine Shrimp Lethality Test (BSLT) and MTT assay. However, some studies have been conducted on species *lonchocarpus* and family of the plant and a few in vivo cytotoxicity studies have been conducted on *L. cyanescens* and species.

4. CONCLUSION

This study evaluated the cytotoxic effects of extracts of different solvents from *L. cyanescens'* stem against four TNBC cell lines comparing their efficacy to the standard drug, Etoposide. These findings highlight methanol extract's superior activity compared to the hexane and ethyl acetate extracts. The findings from this study provide the first BSLT- MTT based toxicity evaluation of the stem of *L. cyanscens*, demonstrating significant cytotoxic activity across all solvent extracts.

These findings confirm the cytotoxic potential of *L. cyanescens* and highlight its prospective role in TNBC therapy. This study establishes a strong foundation for further investigations into the isolation and characterization of active compounds from the crude extracts as well as mechanistic studies to determine its mode of action against breast cancer cells.

CONFLICT OF INTERESTS

The authors declare no conflict of interests.

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