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In Vitro Cytotoxicity of *Lonchocarpus laxiflorus* for Breast Cancer Therapy

Fagge Khadija Ali*, Bilkisu Adedoyin, Ahmed Umar, Jamimah Simon Wakawa, and Olutola Adeola Olayemi

Applied Chemistry Research Laboratory, University of Abuja, FCT, Abuja, Nigeria.
Corresponding Author's email: Khadeejafagge4646@gmail.com Tel: 07061959125

ABSTRACT

Lonchocarpus laxiflorus, a medicinal plant native to Nigeria and West Africa, contains bioactive compounds with reported therapeutic potential. This study evaluates the in vitro cytotoxic effects of crude extracts (n-hexane, ethyl acetate, and methanol) from *L. laxiflorus* stem bark against TNBC cell lines. The Brine Shrimp Lethality Test (BSLT) was conducted to assess general toxicity, and the MTT assay was conducted on four TNBC cell lines (*MCF7, Hs578T, SkBr3, and MDAMB-231*) with etoposide as the standard drug. The BSLT results showed high cytotoxicity across all extracts, with LC_{50} values of 67.42 µg/mL (n-hexane), 61.59 µg/mL (ethyl acetate), and 51.5 µg/mL (methanol), indicating the highest toxicity in the methanol extract. Similarly, the MTT assay revealed that the methanol extract exhibited the strongest cytotoxicity against MCF7 cells (IC_{50} = 67 µM), comparable to etoposide (IC_{50} = 68 µM), suggesting potent anti-cancer activity. In contrast, ethyl acetate and n-hexane indicate toxic value, but not as high as the methanol extract, indicating lower cytotoxic effects than methanol. These results reinforce the scientific basis for the plant's traditional medicinal use and its potential application in modern breast cancer therapy and drug development.

KEYWORDS: Cytotoxicity, BSLT, MTT, TNBC, Anti-cancer agents.

1. INTRODUCTION

Cancer is a large group of interconnected diseases that arises as a result of the body's inability to control cell division, manifested by body cells dividing uncontrollably and spreading to surrounding tissues[1]. Several studies have stated that, globally, cancer ranks as the most prevalent cause of mortality. The World Health Organization defined breast cancer as a disease in which abnormal breast cells grow out of control and form tumours, which can spread throughout the body and become fatal if left unchecked and untreated and a diagnosis of triple negative breast cancer means that the three most common types of receptors known to fuel most breast cancer growth oestrogen, progesterone, and the HER-2/neu gene– are not present in the cancer tumour[2]. This has necessitated the need for the search for ways of curbing the dreadful menace of cancer. Chemotherapy, the treatment of cancer, is not without side effects, which also contribute to the severe complications associated with cancer treatment[3]. Consequently, the demand for plant-based therapeutics has grown, especially due to their affordability and accessibility in many developing countries.

Lonchocarpus laxiflorus, a Fabaceae member, is widely used in African traditional medicine. It has been employed in managing mental disorders, skin infections, jaundice, intestinal worms, and reproductive disorders across countries such as Nigeria, Benin, Kenya, and Senegal[4, 5]. Despite its widespread ethnomedicinal use, limited scientific literature exists on its cytotoxic properties. This study aims to bridge that gap by evaluating the *in vitro* cytotoxicity of its stem bark extracts against TNBC cells using the Brine Shrimp Lethality Test and MTT assay.

2. MATERIALS AND METHODS

2.1 Plant collection and identification

The plant sample stem-bark was carefully collected in the morning and evening immediately after sunset for a period of 5days from area B-Z Samaru in ABU campus, Kaduna State, and presented for verification and authentication with voucher number ABU01083 obtained at the herbarium Botany section of the Department of Biological Sciences, Ahmadu Bello University Zaria, Kaduna, Nigeria.

2.2 Sample Preparation

The freshly collected stem bark of the plant was carefully separated, air-dried at room temperature, and pulverized in the laboratory using a wood milling machine. Thereafter, the powdered sample was placed in a clean polythene bag and stored at room temperature for the experiment.

2.3 Collection of TNBC cell lines for bioassay study. (Triple Negative breast cancer)

Four cell lines were used in the analysis, which include **invasive ductal carcinoma (MCF7)**, **Adenocarcinoma (MDA-MB-231)**, **invasive ductal carcinoma (Hs578T)**, and **Invasive ductal carcinoma (SKBr3),** respectively representing different types of breast cancer cells collected from the cell-culture laboratory, Center for Natural Product Discovery. School of Biochemical Science, Liverpool. John Moores University (LJMU), Liverpool. UK.

2.4 Extraction of Crude Extracts

A weighted 500g portion of the pulverized plant samples was separately macerated using Hexane, Ethyl acetate, and methanol (100%) as solvents for seven days using 1.5L of each solvent, respectively, then decanted and filtered. Evaporated to dryness and weighed.

2.5 Cytotoxicity Assay

The Brine shrimp cytotoxicity test: The toxicity level test of the crude extracts was carried out according to[6, 7]. To ascertain their toxicity against the brine shrimps.

2.6 MTT assay protocol [3-(4.5-Dimethylthiazol-2-yl)-2-5-Diphenyltetrazolium Bromide]

The MTT assay protocol is to measure the toxicity of viable cells, and the MTT assay was carried out according to [8], with little modification from [9]. The results were evaluated against the underlisted TNBC cell lines: - MCF7 invasive ductal carcinoma, -MDAMB-231 Adenocarcinoma, - Hs578T Invasive ductal carcinoma, - SkBr3 Invasive ductal carcinoma, and Cells were treated with graded concentrations of extracts, followed by MTT incubation, solubilization of formazan crystals, and absorbance measurement at 560 nm.

2.7 Anticancer Activity

The anticancer activity of the crude extracts was carried out on triple-negative breast cancer (TNBC) cell lines to determine the anticancer activity of each of the crude extracts. This was conducted using MTT, bioassays on the TNBC cell line.

3. RESULTS AND DISCUSSION

3.1 Results

3.1.1 Brine Shrimp Lethality Test (BSLT) Results for *Lonchocarpus laxiflorus* Crude Extracts

LC_{50} of the crude extracts of *Lonchocarpus laxiflorus* stem bark of ethyl acetate, methanol, and n-hexane

CRUDE EXTRACTS	SOLVENT OF EXTRACTION	LC_{50}(µ/L)
K100	n-Hexane	67.42(43.8-515.49)

K200	Ethyl acetate	61.59(47.06-377.31)
K300	Methanol	51.5(45.49-377.99)

LC_{50}> 1000µg/cm³ = Toxic, LC_{50}>1000µg/cm³ =Not Toxic *High/Low 95% Confidence interval

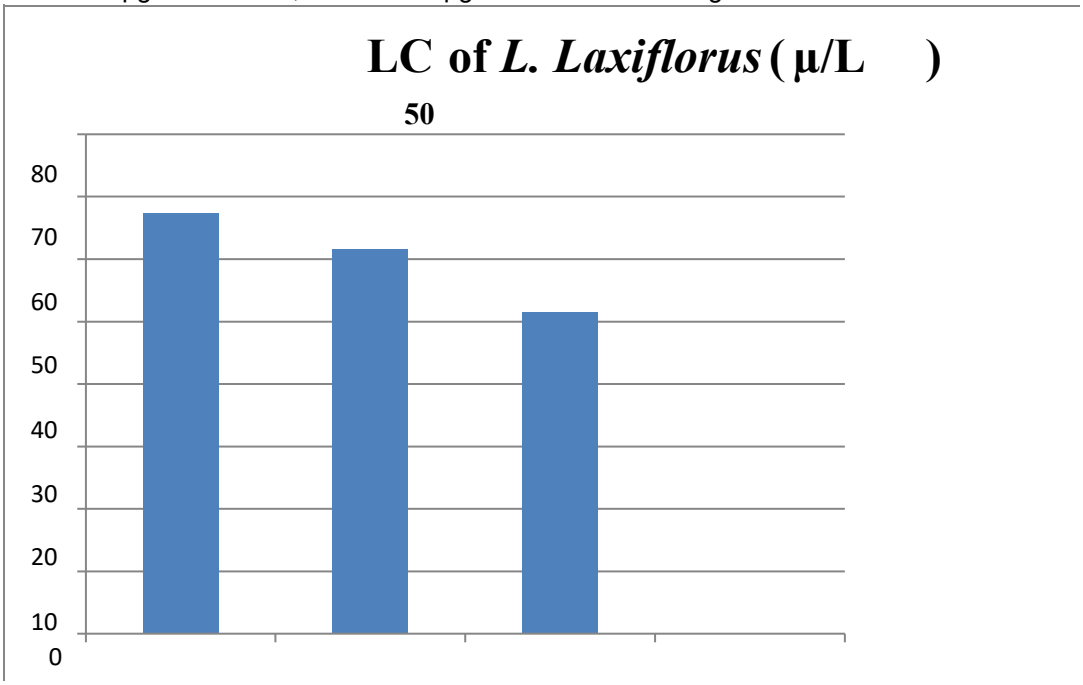

Figure 1: LC_{50} of crude extract *of L. laxiflorus* stem-bark

Table 2: IC_{50} values of the crude extract of the stem bark of *Lonchocarpus laxiflorus*

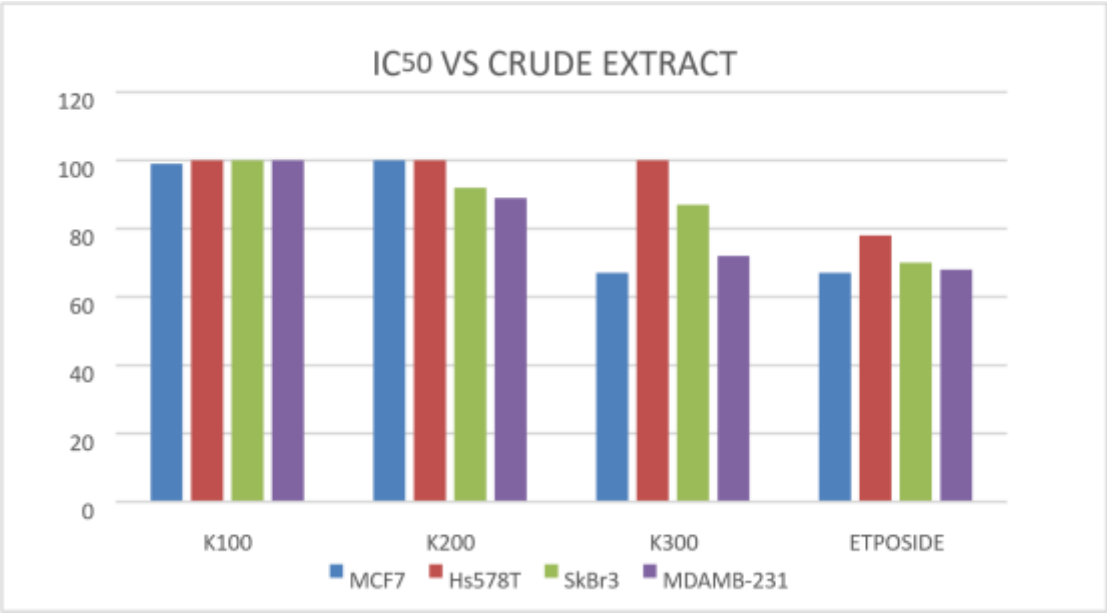

Figure 2: Graph of crude extract versus IC_{50}

3.2 Discussion

This study investigated the cytotoxicity of *Lonchocarpus laxiflorus* stem-bark extracts using both the Brine Shrimp Lethality Test (BSLT) and MTT ([3-(4.5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide] assay. All extracts showed significant toxicity in the BSLT, with survival rates decreasing across concentrations of 1000, 100, and 10 g/cm³. Ethyl acetate and methanol extracts caused the highest mortality (30 larvae), indicating greater toxicity compared to n-hexane. Correspondingly, the LC_{50} values were lowest for methanol (51.51 µg/mL) and ethyl acetate (61.59 µg/mL), suggesting strong cytotoxic potential. To the best of our knowledge, no prior study has reported the cytotoxicity of *Lonchocarpus*

laxiflorus using the Brine Shrimp Lethality Test (BSLT). However, some studies have been conducted on the species *Lonchocarpus* and the family to which the plant belongs, and a few in vivo cytotoxicity studies have been conducted on *L. laxiflorus* and the species.

Using the MTT assay, extracts (K100, K200, K300) were tested on four breast cancer cell lines (MCF7, Hs578T, SKBr3, and MDAMB-231). K300 displayed the most potent activity, especially against MCF7 (67 µg/mL) and MDAMB-231 (72 µg/mL), closely matching the standard drug Etoposide (68 µg/mL). K100 and K200 were less effective, particularly against Hs578T, with IC_{50} values exceeding 100 µg/mL. No prior study has reported the MTT cytotoxicity of *L. laxiflorus* stem bark. This represents the first report of *L. laxiflorus* stem bark cytotoxicity using BSLT and MTT assays. The results align with previous findings from other *Lonchocarpus* species and Fabaceae members [10]. Thus, this study confirms that methanol and ethyl acetate extracts show promising anti-cancer properties. Further anticancer investigations can be done.

4. CONCLUSION

This study demonstrates that *Lonchocarpus laxiflorus* stem bark extracts possess significant *in vitro* cytotoxic activity against triple-negative breast cancer (TNBC) cell lines. Among the three solvents used, the methanol extract exhibited the highest cytotoxicity in both the Brine Shrimp Lethality Test and the MTT assay, with IC_{50} values comparable to those of the standard chemotherapeutic drug, etoposide. These findings suggest the presence of potent bioactive compounds in the methanol extract capable of inhibiting breast cancer cell proliferation. The study provides the first scientific evidence supporting the anticancer potential of *L. laxiflorus* and justifies its traditional use in herbal medicine.

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CONFLICT OF INTERESTS

The authors declare no conflict of interest.

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