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<sup>1</sup>Applied Chemistry Research  
Laboratory, Department of Chemistry,  
University of Abuja, FCT, Nigeria

<sup>2</sup>Department of Metrology and Climate  
science, African Aviation and Aerospace  
University, Abuja, FCT, Nigeria

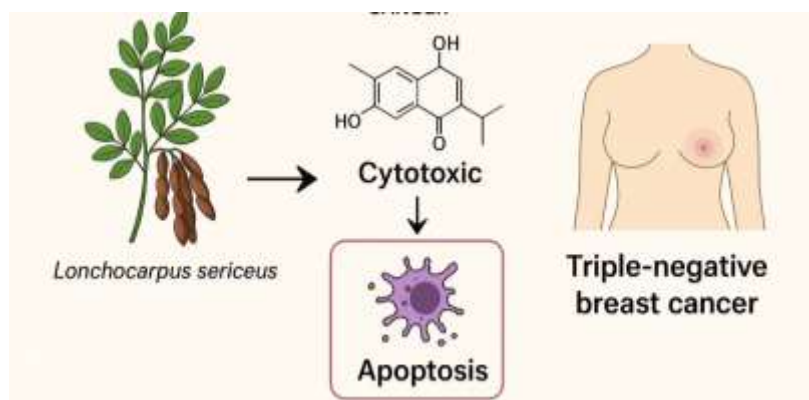
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**CYTOTOXIC AND APOPTOSIS-INDUCING****EFFECTS OF *LONCHOCARPUS SERICEUS* ROOT EXTRACT  
S: TOWARD SUSTAINABLE THERAPEUTICS FOR TRIPLE-  
NEGATIVE BREAST CANCER.**

\*Bilkisu A. Adedoyin<sup>1,2</sup>, Onuche S. Ugbedeajo<sup>1</sup>, Omenka U. Peter<sup>1</sup>, Ahmed Umar<sup>1</sup>

**Abstract**

This study evaluated the cytotoxic and apoptosis-inducing effects of *Lonchocarpus sericeus* root extracts on selected triple-negative breast cancer (TNBC) cell lines. The increasing resistance of TNBC to conventional therapies necessitates the exploration of alternative treatment options, particularly those derived from natural sources. Root extracts obtained using methanol and ethyl acetate were assessed through brine shrimp lethality assay (BSLT), MTT cell viability assay, TUNEL apoptosis assay, and caspase-3 activation analysis. The methanol extract exhibited higher biological activity, with a lower LC<sub>50</sub> value of 57.86 µg/mL compared to the ethyl acetate extract (83.26 µg/mL), indicating stronger cytotoxic potential. In vitro cytotoxic evaluation against breast cancer cell lines including MCF7 (luminal A breast adenocarcinoma), Hs578T (triple-negative breast carcinoma), SkBr3 (HER2-positive breast adenocarcinoma), and MDA-MB-231 (triple-negative breast adenocarcinoma) demonstrated dose-dependent growth inhibition. The methanol extract produced IC<sub>50</sub> values ranging from 69.1–88.4 µg/mL, comparable to etoposide (68.0–78.0 µg/mL). TUNEL assay results revealed significant DNA fragmentation, while caspase-3 activation confirmed apoptosis through caspase-dependent pathways. Although both extracts demonstrated biological activity, the methanol extract showed comparatively stronger cytotoxic effects in the MTT and caspase-3 assays. However, the ethyl acetate extract poses higher apoptotic percentages in selected TUNEL assay results, indicating possible differences in phytochemical composition and mechanisms of action. These findings suggest that *L. sericeus* possesses promising anticancer potential and may serve as a source of bioactive compounds for sustainable plant-based therapeutic development.



**Keywords:** *Lonchocarpus sericeus*, Triple-negative breast cancer, Cytotoxicity, Apoptosis, Sustainability

## 1. Introduction

Cancer remains one of the most significant global health challenges, contributing substantially to morbidity and mortality worldwide. Despite advances in diagnosis and treatment, it continues to limit improvements in life expectancy across many regions. Among the various cancer types, breast cancer is the most commonly diagnosed in women, with triple-negative breast cancer (TNBC) representing one of its most aggressive subtypes. TNBC is characterised by the absence of estrogen, progesterone, and human epidermal growth factor receptor 2 (HER2), which makes it unresponsive to hormonal and targeted therapies. As a result, treatment options are limited, and the disease is often associated with high recurrence rates and poor prognosis, particularly among younger women and populations of African descent. (WHO, 2019 : Sung *et al.*, 2021).

The increasing burden of TNBC, coupled with the limitations of conventional chemotherapy, highlights the urgent need for alternative therapeutic strategies. In recent years, natural products derived from medicinal plants have attracted considerable attention due to their chemical diversity, biological activity, and relatively low toxicity. These plant-derived compounds offer a promising approach for the development of novel anticancer agents, especially in resource-limited settings where access to advanced therapies may be restricted. (Carey *et al.*, 2006; Feng *et al.*, 2018).

*Lonchocarpus sericeus* (family Fabaceae) is a medicinal plant widely distributed across tropical Africa and traditionally used for the management of various ailments, including inflammatory conditions, infections, and parasitic diseases (Uwemedimo *et al.*, 2020). Different parts of the plant have been employed in ethnomedicine, with reports indicating the presence of bioactive constituents such as flavonoids, alkaloids, saponins, and phenolic compounds (Uwemedimo *et al.*, 2020; Sung *et al.*, 2021). These phytochemicals are known to exhibit a range of pharmacological activities, including antioxidant, anti-inflammatory, and anticancer effects. Previous studies suggest that such compounds can interfere with key cellular processes involved in cancer progression, including cell proliferation and apoptosis. (Addotey & Amoo, 2016).

Given its ethnopharmacological relevance and phytochemical composition, *L. sericeus* represents a potential source of bioactive compounds for anticancer drug discovery. In

addition to therapeutic benefits, the use of locally available medicinal plants aligns with the principles of sustainable development by promoting the utilisation of renewable biological resources. This approach supports global efforts toward improving healthcare outcomes while encouraging environmental conservation and innovation in drug development.

Therefore, this study aims to evaluate the cytotoxic and apoptosis-inducing effects of *L. sericeus* root extracts on selected triple-negative breast cancer cell lines. The findings are expected to provide scientific evidence supporting its traditional use and contribute to the search for sustainable and effective anticancer agents.

## 2. Materials and Methods

### 2.1 Plant Collection and Identification

Roots of *L. sericeus* were collected from Saye village, Zaria, Kaduna State, Nigeria. The plant was authenticated at the Department of Biological Sciences, Ahmadu Bello University, Zaria, and a voucher specimen was deposited (Voucher No. ABU01085).

### 2.2 Sample Preparation and Extraction

The collected roots were cleaned, air-dried, and pulverised into fine powder. Approximately 300 g of the powdered material was extracted separately with 1,500 mL of methanol and ethyl acetate using cold maceration for 72 h at room temperature. The extracts were filtered and concentrated under reduced pressure using a rotary evaporator at 35 °C. The resulting crude extracts were air-dried and stored in airtight containers until analysis.

### 2.3 Bioassay Analysis

#### 2.3.1 Brine Shrimp Lethality Test (BSLT)

The cytotoxic potential of the extracts were initially screened using the BSLT. Brine shrimp (*Artemia salina*) eggs were incubated in seawater for 48 h to hatch into larvae. Extracts were prepared at concentrations of 1, 10, 100, and 1,000 µg/mL. Ten larvae were introduced into each test vial in triplicate, and mortality was recorded after 24 h. The median lethal concentration (LC<sub>50</sub>) was determined by Finney's probit analysis.

#### 2.3.2 MTT Cell Viability Assay

Breast cancer cell lines MCF7 (luminal A breast adenocarcinoma), Hs578T (triple-negative breast

carcinoma), SkBr3 (HER2-positive breast adenocarcinoma), and MDA-MB-231 (triple-negative breast adenocarcinoma) were cultured in DMEM with 10% FBS at 37 °C and 5% CO<sub>2</sub>. Cells were seeded in 96-well plates and treated with varying concentrations of each extract for 24h. Cell viability was assessed by the MTT assay at 560 nm. Etoposide served as the reference control. All experiments were performed in triplicate (n = 3), and results were expressed as mean ± standard deviation (SD). IC<sub>50</sub> values were calculated using GraphPad Prism version 9.0.

### 2.3.3 TUNEL Apoptosis Assay

Apoptotic DNA fragmentation was assessed using the APO-BrdU TUNEL assay kit following the manufacturer's instructions. Cells were fixed in 1% paraformaldehyde, treated with ethanol, and analysed by flow cytometry (10,000 events/sample) using FlowJo 10 software.

### 2.3.4 Caspase-3 Activation Assay

Caspase-3 activity was examined by Western blot analysis. Treated cells were lysed in buffer containing Tris-HCl, NaCl, EDTA, Triton X-100, and protease inhibitors. Lysates were separated on 10% SDS-PAGE gels, transferred to nitrocellulose membranes, and probed with antibodies against caspase-3 and β-actin. Protein bands were visualised by enhanced chemiluminescence.

## 2.4 Statistical Analysis

All experiments were conducted in triplicate, and data were expressed as mean ± standard deviation (SD). Statistical analysis was performed using GraphPad Prism version 9.0. Differences between groups were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test. Statistical significance was considered at p < 0.05.

## 3. Results

### 3.1 Brine Shrimp Lethality Test

| Extract Type          | LC <sub>50</sub> (µg/ml) |
|-----------------------|--------------------------|
| Ethyl acetate extract | 83.26                    |
| Methanol extract      | 57.86                    |

The methanol extract demonstrated higher lethality (lower LC<sub>50</sub>), suggesting a greater concentration of bioactive cytotoxic compounds.

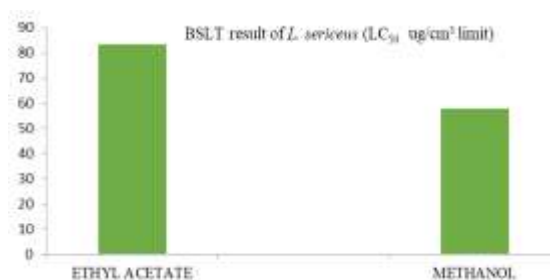


Figure 1: Brine shrimp lethality activity of *L. sericeus* root extracts showing LC<sub>50</sub> values for methanol and ethyl acetate fractions.

### 3.2 MTT Cytotoxicity Assay

| Extract / Standard                 | MCF7       | Hs578 T    | SkBr3      | MDA-MB-231 |
|------------------------------------|------------|------------|------------|------------|
| <i>L. sericeus</i> (ethyl acetate) | 72.4±2.320 | 79.2±2.243 | 84.1±0.055 | 87.5±2.737 |
| <i>L. sericeus</i> (methanol)      | 69.5±2.472 | 88.4±2.167 | 71.1±0.023 | 69.1±2.902 |
| Etoposide (control)                | 68.0±2.471 | 78.9±2.471 | 71.9±2.471 | 68.9±2.471 |

The methanol extract demonstrated comparatively stronger cytotoxic activity against MCF7, SkBr3, and MDA-MB-231 cell lines, with IC<sub>50</sub> values close to that of etoposide. However, the ethyl acetate extract showed slightly stronger activity against Hs578T cells.

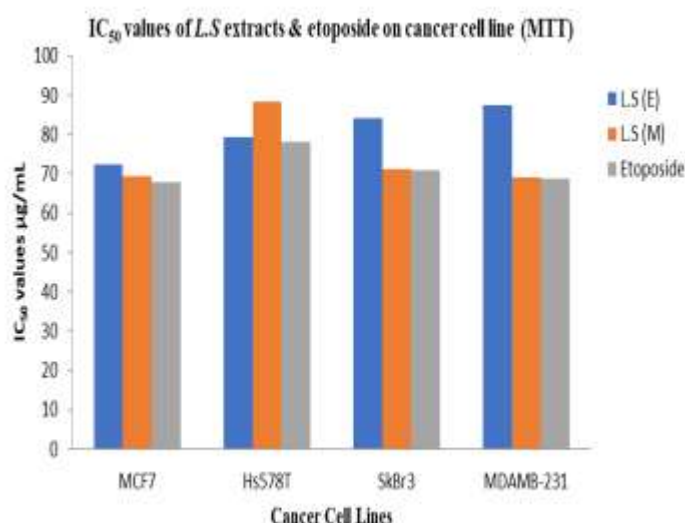


Figure 2: IC<sub>50</sub> values obtained from MTT cytotoxicity assay of *L. sericeus* root extracts against breast cancer cell lines.

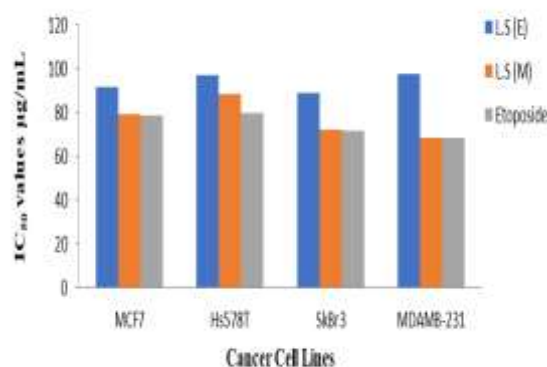
### 3.3 TUNEL Assay (Apoptosis Induction)

| Extract / Standard                 | MCF7            | Hs578T          | SkBr3           | MDA-MB-231      |
|------------------------------------|-----------------|-----------------|-----------------|-----------------|
| <i>L. sericeus</i> (ethyl acetate) | 96.2<br>± 2.220 | 80.5<br>± 1.133 | 74.5<br>± 0.052 | 97.5<br>± 1.631 |
| <i>L. sericeus</i> (methanol)      | 70.5<br>± 2.247 | 77.8<br>± 1.127 | 81.1<br>± 0.032 | 67.1<br>± 1.302 |
| Etoposide (control)                | 65.9<br>± 2.241 | 78.5<br>± 1.471 | 71.9<br>± 0.341 | 68.9<br>± 2.421 |

Both extracts induced apoptosis in the tested cell lines. The methanol extract demonstrated stronger apoptotic activity against MCF7, Hs578T, and MDA-MB-231 cells, whereas the ethyl acetate extract showed slightly stronger activity against SkBr3 cells.

The methanol extract demonstrated stronger caspase-3 activation activity across all tested cell lines compared to the ethyl acetate extract, with values closely comparable to the standard anticancer drug, etoposide.

IC<sub>50</sub> values of *LS* extracts & etoposide on cancer cell line (CASPASE)



IC<sub>50</sub> values of *LS* extracts & etoposide on cancer cell line (TUNNEL)

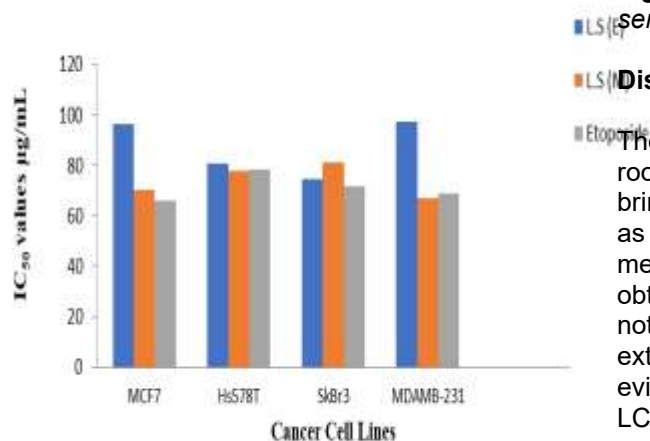


Figure 3: IC<sub>50</sub> values of the crude extracts of *L.sericeus* roots against TNBC

Figure 4: Caspase-3 activation induced by *L. sericeus* root extracts in breast cancer cell lines

### Discussion

The cytotoxic activity of *Lonchocarpus sericeus* root extracts was initially evaluated using the brine shrimp lethality assay **Fig1**, which serves as a rapid and reliable preliminary screening method for bioactive compounds. The results obtained indicate that both extracts possess notable biological activity, with the methanol extract demonstrating greater potency as evidenced by its lower LC<sub>50</sub> value. Extracts with LC<sub>50</sub> values below 100 µg/mL are generally considered biologically active (Meyer *et al.*, 1982), suggesting that the observed effects may be attributed to the presence of potent secondary metabolites. The higher activity observed in the methanol extract may be due to its ability to solubilize polar phytochemicals such as flavonoids, phenolics, and alkaloids, which are widely reported to interfere with essential cellular processes (Krishnaraju *et al.*, 2005).

Further evaluation using the MTT assay revealed that both extracts exhibited inhibitory effects on the proliferation of the tested breast cancer cell lines. The IC<sub>50</sub> values obtained for the methanol extract were slightly lower compared to the ethyl acetate extract, indicating relatively stronger cytotoxic activity (IC<sub>50</sub> values of 72.4- 87.5 µg/mL) **Fig 2**. Notably, the observed effects were comparable to those of the standard anticancer drug, suggesting that the plant extracts possess significant antiproliferative potential. This activity may be associated with the presence of bioactive constituents commonly found in members of the

### 3.4 Caspase-3 Activation

Caspase-3 activation confirmed that apoptosis occurred through caspase-dependent pathways, particularly in cells treated with the methanol extract.

| Extract / Standard                 | MCF7                | Hs578 T             | SkBr3               | MDA-MB-231          |
|------------------------------------|---------------------|---------------------|---------------------|---------------------|
| <i>L. sericeus</i> (ethyl acetate) | 91.7<br>± 1.32<br>0 | 97.1<br>± 1.12<br>9 | 89.0<br>± 1.01<br>3 | 97.7<br>± 1.73<br>7 |
| <i>L. sericeus</i> (methanol)      | 79.5<br>± 1.42<br>2 | 88.7<br>± 1.14<br>4 | 72.1<br>± 1.01<br>2 | 68.4<br>± 1.90<br>2 |
| Etoposide (control)                | 78.8<br>± 1.43<br>1 | 79.9<br>± 1.27<br>9 | 71.9<br>± 1.34<br>1 | 68.7<br>± 1.43<br>1 |

Fabaceae family, including isoflavonoids and related compounds known for their ability to modulate cell growth and survival pathways. (Sanz-Biset & Cañigual, 2011).

The induction of apoptosis is a key mechanism through which anticancer agents exert their effects. In this study, the TUNEL assay **Fig 3** demonstrated that treatment with the extracts resulted in increased DNA fragmentation, particularly in cells exposed to the methanol extract. This finding indicates that the reduction in cell viability observed in the MTT assay may be partly due to the activation of programmed cell death pathways. Apoptosis is considered a desirable mode of cell death in cancer therapy because it allows for the controlled elimination of malignant cells without triggering inflammatory responses (Elmore, 2007; Saleem *et al.*, 2014).

The involvement of apoptotic pathways was further supported by the results of the caspase-3 activation assay **Fig 4**. Increased caspase-3 activity was observed in treated cells, confirming that cell death occurred through a caspase-dependent mechanism. Caspase-3 plays a central role in the execution phase of apoptosis, and its activation is often associated with both intrinsic and extrinsic apoptotic pathways. The stronger effect observed with the methanol extract suggests that it may contain higher concentrations of compounds capable of triggering these signaling pathways. (Bianchini *et al.*, 2016).

Compared with previous studies on medicinal plants used in cancer therapy, the present findings support the growing evidence that plant-derived compounds possess valuable anticancer properties. Similar cytotoxic activities have been reported for flavonoid-containing medicinal plants investigated against breast cancer models (Bianchini *et al.*, 2016; Saleem *et al.*, 2014).

Despite the promising findings, this study has certain limitations. The investigation was restricted to in vitro assays, and the precise molecular mechanisms responsible for the observed cytotoxic effects were not fully explored. In addition, isolation and characterization of the active phytochemical constituents were not performed. Further in vivo studies and mechanistic investigations are therefore necessary to validate the therapeutic potential of *L. sericeus*.

Overall, the findings of this study demonstrate that *L. sericeus* root extracts possess significant

cytotoxic and pro-apoptotic properties against triple-negative breast cancer cell lines. The comparatively higher activity of the methanol extract highlights the importance of solvent polarity in the extraction of bioactive compounds. These results support the potential of this plant as a source of natural compounds for anticancer drug development.

In addition to its biological activity, the use of *L. sericeus* offers advantages from a sustainability perspective. As a plant species that is locally available and not currently endangered, it represents a renewable resource that can contribute to the development of affordable therapeutic agents. The integration of such plant-based approaches into drug discovery aligns with current efforts to promote sustainable healthcare solutions while conserving biodiversity.

## Conclusion

The findings of this study demonstrate that *Lonchocarpus sericeus* root extracts possess notable cytotoxic and apoptosis-inducing activities against breast cancer cell lines. The methanol extract exhibited stronger cytotoxic effects in the MTT assay, whereas the ethyl acetate extract demonstrated higher apoptotic activity in selected TUNEL assay results. These observations suggest that both extracts contain bioactive compounds capable of inducing cancer cell death through apoptosis-related pathways.

The study provides scientific evidence supporting the medicinal potential of *L. sericeus* and highlights its relevance as a possible source of novel anticancer compounds. However, additional studies involving compound isolation, mechanistic evaluation, and in vivo validation are required before therapeutic application can be considered.

## Authors' Contributions

Bilkisu A. A. conceived the study. Onuche S. U. and Omenka U. P. conducted the experiments. Bilkisu A. A., Onuche S. U., and Ahmed U. contributed to data analysis and manuscript preparation. All authors reviewed and approved the final version of the manuscript.

## Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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