Archives of Pharmaceutical Sciences and Biotechnology Journal

Volume 6 Issue 1 June, 2026 ISSN: 2971_611X

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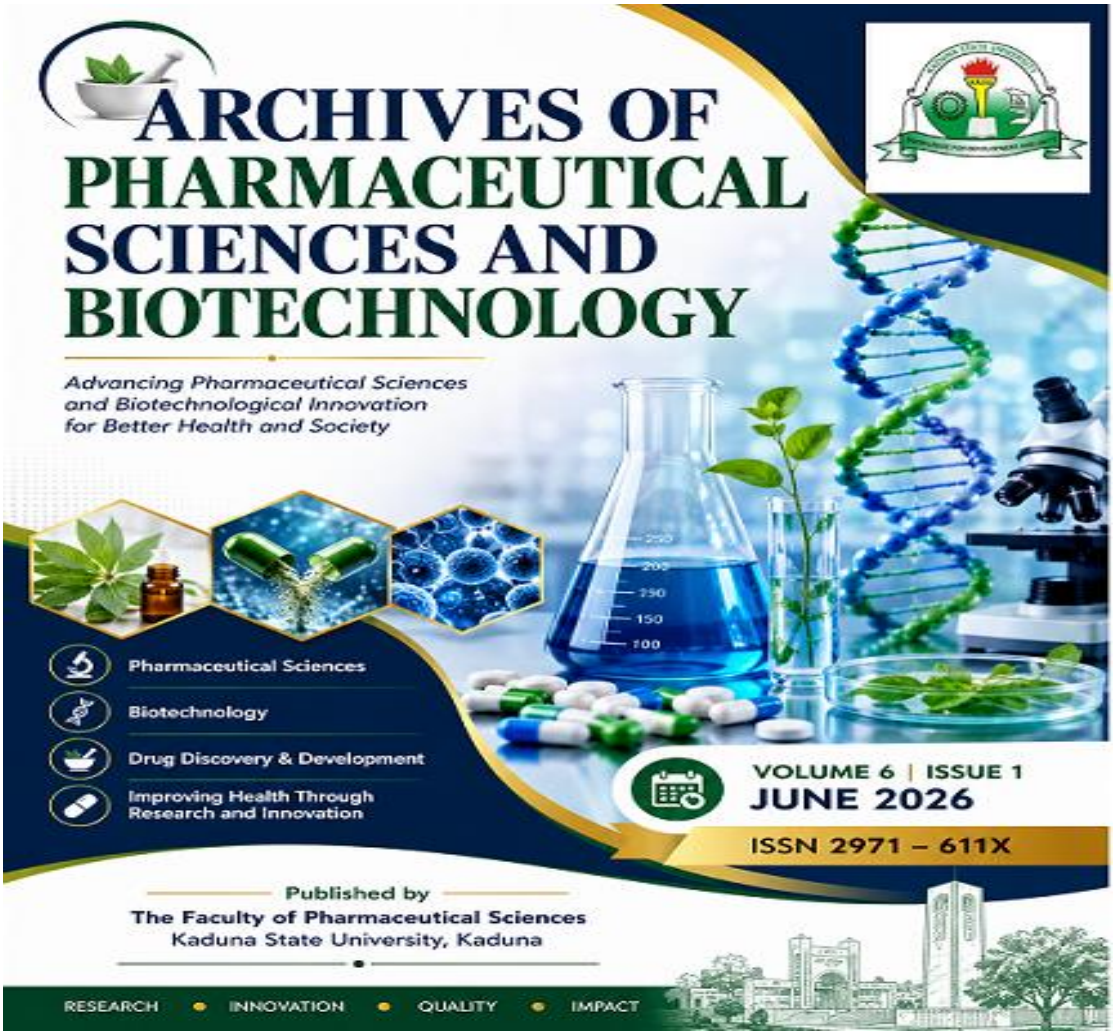

ARCHIVES OF PHARMACEUTICAL SCIENCES AND BIOTECHNOLOGY JOURNAL

VOLUME 6 ISSUE 1, JUNE 2026

ISSN 2971 – 611X

Published by the Faculty of Pharmaceutical Sciences, Kaduna State University, Kaduna

Archives of Pharmaceutical Sciences and Biotechnology Journal
Volume 6 Issue 1 June, 2026 ISSN: 2971_611X
Page 1 - 10 Submitted 8/4/2026 Accepted 30/5/2026
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JOBELYN® INDUCES APOPTOSIS OF MCF-7 BREAST CANCER CELLS THROUGH MODULATION OF BAX AND BCL-2 GENES

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ABSTRACT

Jobelyn® is a standardized herbal supplement derived from the pigmented leaf sheaths of *Sorghum bicolor*. Although previous studies have reported its antioxidant and immunomodulatory activities, information on its direct anticancer effects remains limited. This study evaluated the cytotoxic and apoptosis-inducing effects of Jobelyn® on the human breast adenocarcinoma cell line (MCF-7). Cytotoxicity of the Jobelyn was determined using the MTT assay after 48 h exposure of cells to Jobelyn® at concentrations of 31.25-500 µg/mL. Morphological changes were examined by phase-contrast microscopy, and the expression of apoptosis-related genes (*Bax* and *Bcl-2*) was assessed by quantitative real-time PCR. Jobelyn® caused a concentration-dependent reduction in MCF-7 cell viability with an IC_{50} value of 26.90 µg/mL. Microscopic examination showed reduced cell density, altered cell morphology, and detachment of cells from the culture surface after treatment. Gene expression analysis showed increased expression of the pro-apoptotic gene *Bax* and decreased expression of the anti-apoptotic gene *Bcl-2*, resulting in a pronounced increase in the Bax/Bcl-2 ratio. These findings indicate that Jobelyn® exerts cytotoxic effects on breast cancer cells and induces apoptosis through modulation of Bax and Bcl-2 gene expression.

Keywords: Jobelyn, apoptosis, bax, bcl-2, IC_{50}, qPCR, MCF-7

INTRODUCTION

Jobelyn® is a standardized herbal dietary supplement derived from the intensely pigmented leaf sheaths of a unique variety of *Sorghum bicolor* (L.) Moench, a cereal crop native to the sub-Saharan regions of West Africa (Oghenekevwe *et al.*, 2025). In traditional African medicine, *S. bicolor* is locally known as dawa (Hausa language), *okababa* (Yoruba language) and *soro* (Igbo language). The concoctions of this plant have been used for centuries to manage diverse ailments ranging from severe anemia and chronic pain to inflammatory conditions (Adedeji, 2020). Jobelyn®, which is commercially formulated as a syrup for children and capsules for adults, is produced, branded, and distributed by Health Forever Products Ltd., Lagos, Nigeria (Ayuba *et al.*, 2014; Aluko, 2015).

The use of Jobelyn® has increased significantly due to its status as one of the most potent natural antioxidants currently known. Brunswick Laboratories have

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categorized Jobelyn as a food product with an exceptionally high Oxygen Radical Absorbance Capacity (ORAC) value, surpassing well-known "superfoods" such as the acai berry. This biological potency is largely attributed to its unique phytochemical profile, characterized by high concentrations of rare 3-deoxyanthocyanidins, specifically apigeninidin and luteolinidin, alongside other polyphenols like quercetin, luteolin, and naringenin (Makanjuola *et al.*, 2017).

While literature has extensively documented the pharmacological efficacy of Jobelyn® in neuroprotection (Umukoro *et al.*, 2019), immunomodulation (Ayuba *et al.*, 2014; Makanjuola *et al.*, 2016), and hematological recovery (Ani and Imaga, 2011; Diaku-Akinwumi *et al.*, 2024), there is a conspicuous lack of empirical data regarding its direct anti-neoplastic potential. Despite its inclusion in the National Cancer Institute (NCI) drug dictionary for its chemopreventive properties (NCI, n.d.), no study to date has established its direct cytotoxic threshold or its ability to trigger programmed cell death in human malignancy. The molecular mechanisms that enable Jobelyn® to protect healthy neurons, such as the modulation of p53 and NF-κB signaling, suggest a dual capacity to also influence apoptotic pathways in cancer cells (Charles *et al.*, 2016; Umukoro *et al.*, 2019). Consequently, there is a need to evaluate the anti-breast cancer effects of this standardized formulation. Therefore, this study investigated the cytotoxicity of Jobelyn® capsules against the MCF-7 human breast adenocarcinoma cell line and elucidated the underlying mechanism of its potential anti-cancer action, via expression profiles of the pro-apoptotic Bax and anti-apoptotic Bcl-2 genes, providing the first direct evidence of Jobelyn®-induced apoptosis in a human cancer model.

MATERIALS AND METHODS

Drug and Reagents

Jobelyn® capsules (Health Forever Products Ltd, Lagos, Nigeria), cyclophosphamide, trypsin-EDTA (Millipore Sigma), Dulbecco's Modified Eagle Medium ((DMEM; Pricella, Elabscience, Houston, TX, USA)) medium, phosphate-buffered saline (PBS), 3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide (MTT) (Sigma-Aldrich, Germany), trypan blue, dimethyl sulphoxide (DMSO), molecular grade water, cDNA synthesis kit (Toyobo, Japan), Luna® Universal qPCR Master Mix.

Cell Culture

Human breast cancer cell line (MCF-7) was obtained from the American Type Culture Collection (ATCC, Va, USA) and cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin in T25/75 culture flasks. The cells were maintained in a CO_2 incubator (CHP-16) at 5% CO_2 and 95% relative humidity as described by (Waziri *et al.*, 2024).

Cell Cytotoxicity Assay

Cytotoxicity effect of Jobelyn® on MCF-7 cancer cells was assessed as described by Waziri *et al.* (2016) using 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT). Monolayer cells were detached with trypsin-EDTA to make a single cell suspensions and viable cells were counted after staining with Trypan blue to

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exclude dead cells using a haemocytometer. Cells were then grown overnight in a 96-well plate at a density of 3×10^3 cells/well at 37°C and 5% CO_2. The cultured cells were then treated with 100 µL of the drug at different concentrations (31.25, 62.5, 125, 250 and 500 µg/mL). Cyclophosphamide was used a standard while medium alone served as negative control. After 48 hours of treatment, 20 µL of MTT solution (5mg/mL concentration) was added to each well (giving final concentration of 0.5 mg/mL) and the plate, wrapped with foil paper was incubated for 4 hours. Afterwards, the medium was replaced with 150 µL/well of dimethyl sulfoxide (DMSO) to solubilize the formazan crystals. The optical density was measured at 490 nm using an ELISA plate reader. The experiment was performed in triplicates and percentage cytotoxicity calculated using the formula:

$$\text{Cytotoxicity } (\%) = \frac{ODt}{ODc} x\ 100$$

Where ODt = optical density of treatment well and ODc = optical density of negative control well.

Phase Contrast Imaging

Phase contrast micrographs were acquired prior to the addition of MTT reagent to assess morphological changes. Images were captured at 100× total magnification using a 10×/0.25 NA phase-contrast objective (AmScope) coupled with a MU303 digital CMOS camera under halogen illumination.

Gene Expression Studies

Primer design

Primer sequences (Table 1) for housekeeping gene (β-actin) and target genes (Bax and Bcl-2) as designed by (Promise *et al.*, 2025) were synthesized by Integrated DNA Technologies (IDT, Singapore).

Table 1: Sequence of Primers Used

Gene	Primer sequence
β-actin	F: 5'-ACCTAACTTGCGCAGAAAACAAGA-3' R: 5'-ACTGCTGTCACCTTCACCGT-3'
Bax	F: 5'-GAGTGTCTCAAGCGCATCGG-3' R: 5'-AGTAGAAAAGGGCGACAACCC-3'
Bcl-2	F: 5'-ATCGCCCTGTGGATGACTGAG-3' R: 5'-AGGGCCAAACTGAGCAGAGTC-3'

RNA extraction

MCF-7 cells were seeded in 6-well plates at a density of 5×10^4 cells/well and incubated overnight. The cells were then treated with 50, 25 and 12.5 µg/mL of Jobelyn® for 48 hours, with untreated cells serving as the control. RNA was extracted from the cells using TRIpure Total RNA extraction kit (ELK Biotechnology, USA) according to Manufacturer's instruction. The purity and concentration of RNA was measured using a NanoDrop® One Microvolume UV-Vis Spectrophotometer (Thermo Fisher Scientific, USA).

cDNA synthesis

Reverse transcription of the RNA was performed using RT Master mix with gDNA remover (Toyobo, Japan) under the following conditions: genomic DNA removal at 37°C for 5 min, cDNA synthesis at 37°C for 15 min, and the reaction was terminated by incubating at 98°C to inactivate the reverse transcription enzyme.

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qPCR analysis

Quantitative real-time PCR was carried out using Luna® Universal qPCR Master Mix (New England Biolabs, M3003). Each 20 µL reaction contained 10 µL of Luna mix, 0.5 µL each of forward and reverse primers (10 µM), template cDNA, and nuclease-free water to volume. The cycling conditions included an initial denaturation at 95°C for 60 seconds, followed by 40 cycles of denaturation at 95°C for 15 seconds and annealing/extension at 60°C for 30 seconds with plate reading. Relative gene expression was calculated using the $2^{\wedge-\Delta\Delta Ct}$ method (Waziri *et al.*, 2026). To assess the apoptotic threshold, the Bax/Bcl-2 ratio was determined by dividing the relative fold change of the pro-apoptotic Bax gene by that of the anti-apoptotic Bcl-2 gene for each treated group (Putri, 2025).

Statistical Analysis

All experiments were carried out in triplicates and half maximum inhibitory concentration (IC_{50}) was determine through nonlinear regression dose response curve using GraphPad Prism software (Ver.10.4.2, USA). One-way analysis of variance (ANOVA) was applied to compare variation in fold change between groups with p-value set at ≤0.05 and 95% confidence interval.

RESULTS

Cytotoxicity (MTT) Assay

The cytotoxicity effect of Jobelyn® capsules on breast cancer cells (MCF-7) is presented in Figure 1. Jobelyn induced a dose dependent cytotoxic effect on the MCF 7 cells. A nonlinear regression analysis of the percentage activity gave half-maximum inhibitory concentration (IC_{50}) values of 26.90 µg/mL for Jobelyn® and 27.69 µg/mL for standard drug.

Phase Contrast Microscopy

The microscopic examination of the effect of Jobelyn® capsules on the breast cancer cells revealed a severe alteration in cell morphology, density, and viability characterized by a concentration-dependent reduction in the number of adherent cells (Figure 2). In addition, jobelyn treatment led loss of normal cell morphology, and widespread cell detachment or lysis unlike in the untreated control cells (Figure 2).

Gene expression study

Jobelyn® treatment increased the expression of the pro-apoptotic gene, Bax and a consequent decrease on anti-apoptotic gene, Bcl-2 (Figure 3). Specifically, Bax gene levels increased over 15-fold than the untreated control, while Bcl-2 levels showed a sharp and significant decrease ($p<0.0001$) across all treated concentrations lower than the control. Analysis of the Bax/Bcl-2 ratio revealed a sharp, concentration-dependent increase compared to the untreated control (Figure 4). The ratio rose significantly from 1.0 in the control to 58.8, 417.0, and 4773.3 following treatment with 12.5, 25, and 50 µg/mL of Jobelyn®, respectively.

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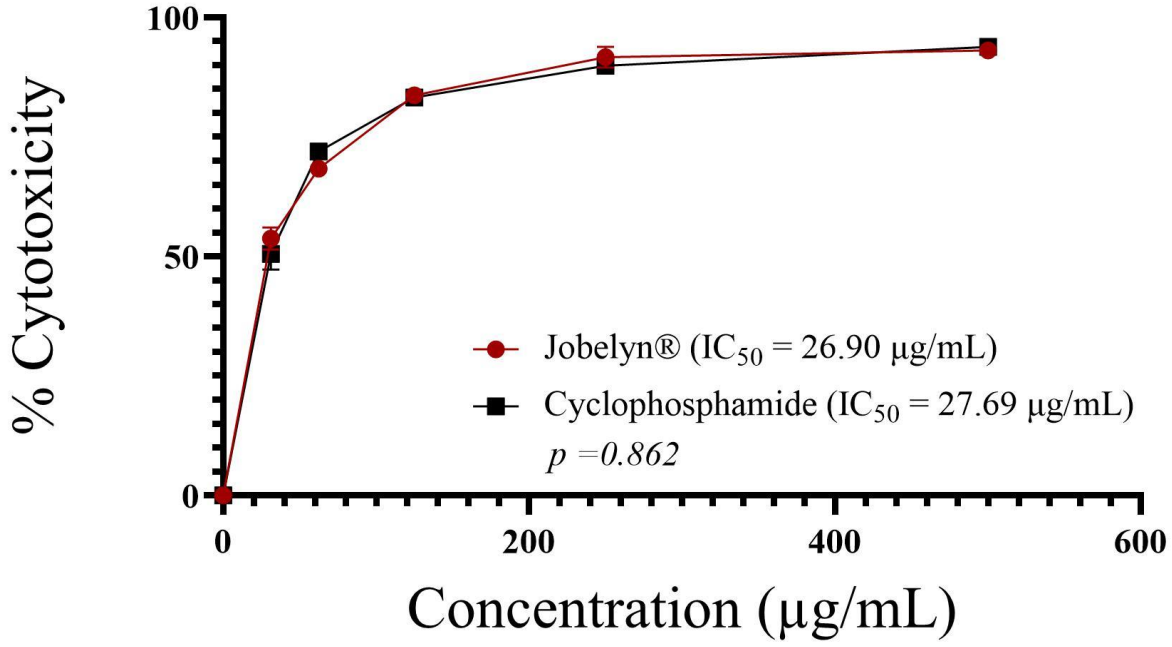

Figure 1: Cytotoxicity effect of Jobelyn® capsules on MCF-7 cells.

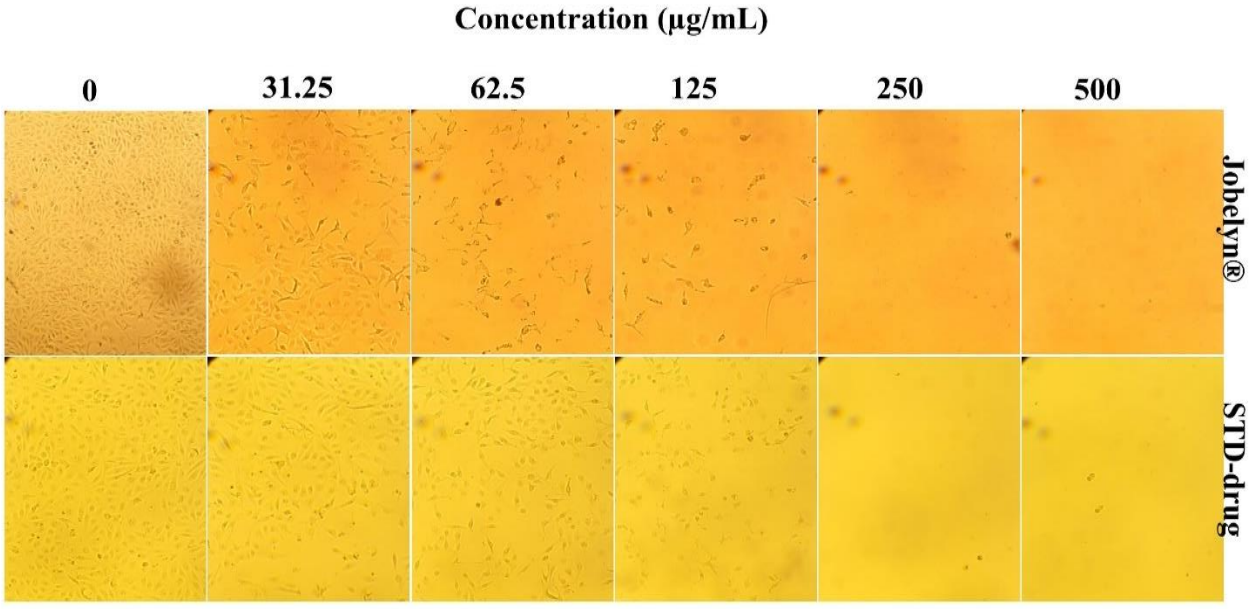

Figure 2: Effect of Jobelyn on MCF-7 cell morphology.

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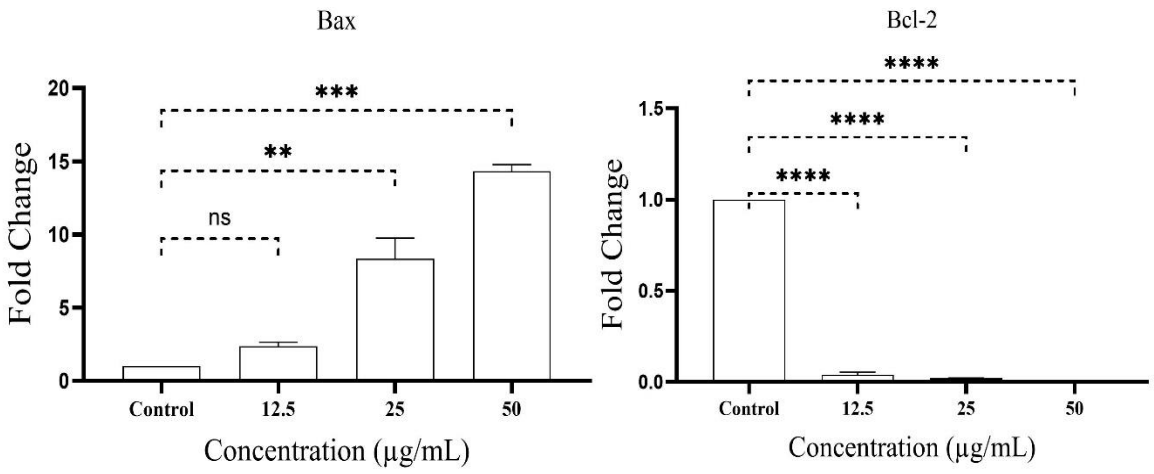

Figure 3: Effect of Jobelyn on pro-apoptotic Bax and anti-apoptotic Bcl-2 genes in MCF-7 cells

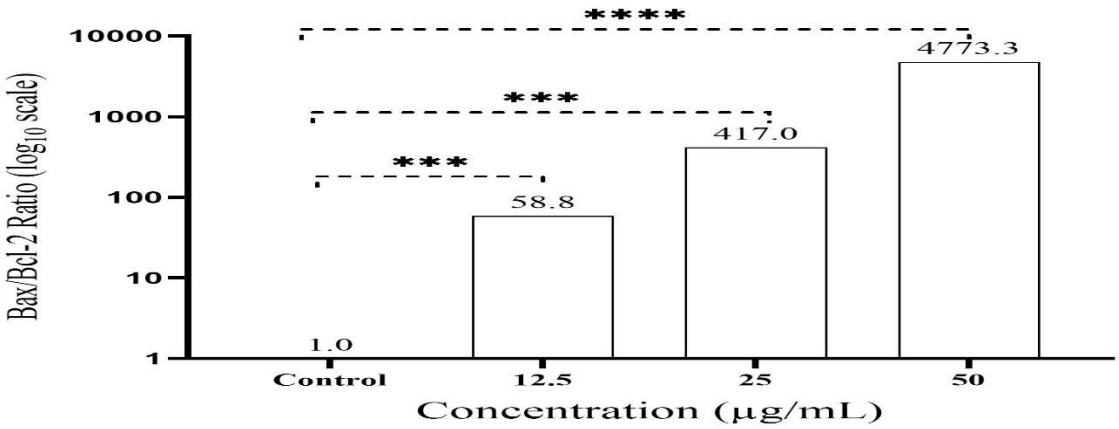

Figure 4: Impact of Jobelyn® on the pro-apoptotic index (Bax/Bcl-2 ratio) in MCF-7 cells.

DISCUSSION

The present study demonstrates that Jobelyn® capsules exhibits significant cytotoxic activity on MCF-7 human breast cancer cells, evident in the concentration-dependent reduction in cell viability, with an IC_{50} value of 26.90 µg/mL. The inhibitory effect observed relative to cyclophosphamide suggests that the phytochemical constituents of Jobelyn® possess notable anti-proliferative potential. Although direct studies on the anticancer activity of Jobelyn® are scarce, previous investigations have shown that phenolic compounds derived from *Sorghum bicolor* can significantly inhibit the growth of human cancer cell lines, including hepatocarcinoma (HepG2) and colorectal adenocarcinoma (Caco-2) cells, through mechanisms involving cell-cycle arrest and apoptosis (Chen *et al.*, 2021).

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These findings support the present observation that sorghum-derived phytochemicals may exert potent anticancer effects, likely mediated by their high polyphenolic content as Jobelyn® is particularly rich in rare 3-deoxyanthocyanidins such as apigeninidin and luteolinidin, compounds known to possess strong antioxidant and chemopreventive properties that can interfere with tumor cell proliferation (Makanjuola *et al.*, 2017).

The pronounced structural alterations including loss of cellular integrity, reduced cell density, and detachment from the culture surface following treatment with Jobelyn® are all characteristic morphological hallmarks of apoptosis or severe cytotoxic stress in adherent cancer cell lines. Similar morphological features have been reported in studies evaluating plant-derived anticancer compounds, where treated cancer cells exhibit cell shrinkage, membrane blebbing, and eventual detachment from the substrate during apoptotic progression (Kwan *et al.*, 2016; Promise *et al.*, 2025). The dose-dependent morphological changes observed in this study therefore corroborates the cytotoxicity result obtained from the MTT assay and suggests that the inhibitory effect of Jobelyn® is associated with induction of programmed cell death rather than simple growth suppression.

At the molecular level, the present study shows that Jobelyn® significantly modulates apoptotic regulatory genes, with a concentration-dependent up-regulation of the pro-apoptotic gene, *Bax* and concomitant down-regulation of the anti-apoptotic gene, *Bcl-2*. This shift resulted in a significant increase in the Bax/Bcl-2 ratio, which is considered a critical determinant of mitochondrial membrane permeabilization and apoptosis initiation (Putri, 2025). When Bax expression predominates over Bcl-2, mitochondrial outer membrane permeability increases, leading to cytochrome-c release and activation of downstream caspase cascades that ultimately drive apoptotic cell death (Misao *et al.*, 1996). A previous study showed that treated MCF-7 cells with *Dioscorea* extract similarly reported increase in Bax expression and decreased Bcl-2 expression, ultimately promoting apoptotic cell death in breast cancer cells (Doulaby *et al.*, 2023). Similarly, studies by Kazemi and Shahrestani (2018) reported saffron extract (a potent source of phytochemicals), induces a dose-dependent elevations in the Bax/Bcl-2 ratio, thus promoting apoptosis in the cancer cells. The gene expression pattern observed in the present study therefore strongly suggests that Jobelyn® induces apoptosis through activation of the intrinsic mitochondrial apoptotic pathway.

CONCLUSION

Jobelyn® showed potent cytotoxic activity against MCF-7 human breast cancer cells and induced apoptosis through concentration-dependent modulation of selected mitochondrial apoptotic regulators. Treatment resulted in significant up-regulation of the pro-apoptotic gene Bax and down-regulation of the anti-apoptotic gene Bcl-2, leading to a substantial increase in the Bax/Bcl-2 ratio, a critical determinant of apoptosis. These findings provide the first experimental evidence that the standardized *Sorghum bicolor* derived supplement Jobelyn® can directly trigger apoptotic signaling in human breast cancer cells, suggesting potential relevance for further investigation as a natural anticancer candidate.

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Funding

The research is funded by TETFUND-IBR Research grant (TETF/DR&D/CE/UNI/KADUNA/IBR/2022/VOL 1, BATCH 5)

Acknowledgement

The authors are grateful to Precision Biomedical research Laboratory for the support offered in the course of this research.

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