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Research Article

# Apoptotic and Antibacterial Activities of Terminalia catappa Ethanolic Extract

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**Abstract:** Prostate cancer remains a significant contributor to morbidity and mortality rates in men due to resistance to conventional treatments. Natural products such as *Terminalia catappa* have emerged as promising candidates with their antiproliferative and proapoptotic activities. Ethanolic extract of *T. catappa* leaves were tested against PC-3 and LNCaP prostate cancer cells. Antiproliferative activity was investigated using the WST-1 assay. Caspase-3, -8, -9, Bcl-2, p53, and survivin expression were investigated by ELISA and Western blot methods. The antibacterial activity of the extract was tested by the well diffusion method. *T. catappa* extract inhibited prostate cancer cell proliferation in a concentration and duration dependent profile, with $IC_{50}$ values of 49.54 µg/mL (24 h) and 30.41 µg/mL (48 h) in PC-3 cells and 61.53 µg/mL (24 h) and 43.64 µg/mL (48 h) in LNCaP cells. Microscopic analysis revealed apoptotic effects, while HUVECs maintained their viability and morphology. Caspase-3, -8, and -9 activities were significantly increased, indicating activation of apoptotic pathways. Western blotting revealed a strong increase in p53 and a decrease in Bcl-2 and survivin. Additionally, the extract was found to exhibit stronger antibacterial activity against Gram-positive strains. These findings highlight the dual anticancer and antibacterial potential of the *T. catappa* ethanol extract.

***Keywords:*** *Terminalia catappa, Prostate cancer, Apoptosis*

## I. INTRODUCTION

Prostate cancer ranks one of the most frequently diagnosed cancers in men globally and is a primary contributor to cancer-related mortality. Its development and progression are highly dependent on genetic, environmental, and lifestyle factors, and androgen receptor signaling plays a pivotal role in disease initiation and progression, particularly in androgen-sensitive prostate cancers such as LNCaP cells (Wilson & Zishiri, 2024; Tan et al., 2015). In contrast, androgen-independent PC-3 cells represent a more aggressive and treatment-resistant form of the disease (Chavali et al., 2025). For that reason, there is an immediate requirement for novel therapeutic approaches that can target all subtypes of prostate cancer.

Conventional treatments such as androgen deprivation therapy, chemotherapy, and radiotherapy are often restricted by the therapeutic resistance, systemic toxicity, and low selectivity against malignant cells (Zafar et al., 2025). Therefore, there is increasing interest in naturally derived compounds with high anticancer potential that may offer safer and more effective complementary treatment alternatives (Newman & Cragg, 2020). Therefore, medicinal plants rich in polyphenols and flavonoids are in high demand due to their diverse biological activities, including antiproliferative, proapoptotic, antioxidant, and anti-inflammatory effects (Jomova et al., 2025).

*Terminalia catappa* L. (Combretaceae), commonly known as Indian almond or tropical almond, is widely distributed in Asia, Africa, and tropical regions. Traditionally, its leaves, bark, and fruit have been utilized in ethnomedicine for the treatment of diverse diseases such as hepatitis, dysentery, dermatitis, and cancer (Habibullah et al., 2023). Phytochemical analyses of *T. catappa* leaves have revealed the presence of bioactive compounds including flavonoids (e.g., kaempferol, quercetin), tannins, triterpenoids, and phenolic acids, which are associated with strong antioxidant and anticancer properties (Das et al., 2020; Warnasih et al., 2024). Studies have demonstrated that extracts of *T. catappa* induce apoptosis, inhibit cell proliferation, and suppress metastasis in several cancer cell models, including hepatocellular carcinoma (Yeh et al., 2012), breast cancer (Zarredar et al., 2021), and colon cancer (Shanehbandi et al., 2021).

Apoptosis is regulated by intrinsic and extrinsic pathways involving key mediators such as caspases, Bcl-2 family proteins, p53, and survivin. Caspase-3, -8, and -9 are the central executors of apoptosis, while anti-apoptotic proteins promote tumor cell survival. Conversely, p53, a well-characterized tumor suppressor, promotes apoptosis by inducing pro-apoptotic signaling cascades and cell cycle arrest (Elmore, 2007). Loss of regulation of the apoptotic pathway is a key feature of prostate cancer and this mechanism contributes to tumor growth and treatment resistance (Campbell & Leung, 2021).

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Based on the traditional medicinal use of *T. catappa* and its promising anticancer potential, we analyzed the antiproliferative and proapoptotic effects of *T. catappa* ethanolic leaf extract on PC-3 and LNCaP cell lines. The expression of crucial proteins (caspase-3, -8 and -9, Bcl-2, p53 and survivin) involved in apoptosis induction and inhibition were also investigated. These results offer new informations about the molecular effects of *T. catappa* in prostate cancer and support its potential as a candidate for complementary therapeutic development.

## II. MATERIAL AND METHOD

### A. *Preparation of T. Catappa Ethanol Extract*

The dried leaves of *T. catappa* were purchased from a petshop in Istanbul, Türkiye. Dried and powdered plant material (50 g) was extracted with ethanol (150 mL, 95%, Merck, Darmstadt, Germany) for 24 h using a Soxhlet apparatus. Filter paper No. 1 (Whatman) was used to drain the extract. The rotary evaporator running in vacuum at 55 °C was employed to vaporize the drained solvent (yield 18.4% for ethanol). The obtained extract was preserved at −20 °C in tagged, sterile jars. To achieve a concentration of 200 mg/mL, the extract was dissolved in dimethyl sulfoxide (DMSO).

### B. *Microorganisms and Antibacterial Assay*

*Escherichia coli* ATCC 10536, *Enterobacter cloacae* ATCC 23355, *Proteus vulgaris* ATCC 8427, *Pseudomonas aeruginosa* ATCC 27853, *Klebsiella pneumoniae* UC57, *Salmonella typhimurium* ATCC 14028 as Gram negative bacteria and *Staphylococcus aureus* ATCC 6538P, *Staphylococcus epidermidis* ATCC 12228, *Streptococcus pyogenes* ATCC 19615, *Bacillus cereus* ATCC 7064, *Listeria monocytogenes* ATCC 15313, *Micrococcus luteus* CCM 169, *Mycobacterium smegmatis* CCM 2064, as Gram positive bacteria were used as test bacterial strains. The test bacteria strains were maintained in Mueller Hinton Broth (Oxoid Ltd. UK) to prepare the inoculum and cultured at 37 °C for 24 h. The cultures were subsequently diluted to the 0.5 McFarland turbidity standard. The colonies were diluted in normal saline and subsequently seeded to Mueller Hinton Agar (Oxoid Ltd. UK). The agar well method was employed to evaluate the ability of each extract to inhibit the growth of the test bacteria. Inoculated Mueller Hinton Agar plates were allowed to dry. Subsequently, a 4 mm cork borer was employed to create wells in the surface of the inoculated agar plates. 0.2 mL of each extract at varying concentrations was added to the wells with a Pasteur pipette. The resulting zones of inhibition did not overlap due to sufficient separation between the wells. The zones of inhibition observed from the triplicate replication of the experiment was reported. Penicillin, Ampicillin, Ceftazidime, Vancomycin, Ofloxacin, and Tetracycline were utilized as positive controls and comparative agents in the study of bacterial antibiotics.

### C. *Cell Culture and Cell Viability Assay*

In this study, human prostate cancer cells (PC-3; androgen-independent and LNCaP; androgen-dependent), and normal healthy control HUVEC (human umbilical cord endothelial cells) cells were used. PC-3 and LNCaP cell lines were cultured in RPMI-1640 medium (Capricorn Scientific, Germany), while HUVECs were maintained in DMEM (Capricorn Scientific, Germany). 10% fetal bovine serum (FBS, Capricorn Scientific, Germany) and 1% penicillin-streptomycin were added to all media. Cells were maintained in a humidified environment containing 5% $CO_2$ at 37 °C and subcultured using 0.25% trypsin-EDTA when they reached 70–80% confluence. The antiproliferative effect of *T. catappa* extract was evaluated using the WST-1 assay (Abcam, Cambridge, UK). PC-3 and LNCaP cells were plated in 96-well plates at a density of $5 \times 10^3$ cells per well and incubated for 24 h. Cells were subsequently exposed to different concentrations of the extract (0–200 µg/mL) for durations of 24 and 48 h. Following treatment, 10 µL of WST-1 reagent was introduced to the wells and incubated for 2 h at 37 °C. The absorbance was quantified at 450 nm utilizing a microplate reader (Allsheng Instruments, Hangzhou, China). Cell viability was quantified as a percentage compared to untreated controls, and IC50 values were determined using GraphPad Prism 9.0 (GraphPad Software, San Diego, CA, USA).

### D. *Morphological Observations*

Morphological changes associated with *T. catappa* extract treatment were examined in PC-3, LNCaP, and HUVEC cells. After 24 h of treatment, morphological alterations were visualised with an inverted microscope (Euromex, Arnhem, Netherlands) equipped with a CMEX-5 Pro camera at 20x magnification. Cells were seeded at a density of 3 × 105 cells per well in 6-well plates and cultured until 90% confluence. Then, cells were subsequently treated with 50 µg/mL and 100 µg/mL of the extract for durations of 24 h and 48 h. Untreated cells served as controls. Apoptotic morphology in PC-3 and LNCaP cells was identified by features such as cell shrinkage, rounding, detachment, and membrane blebbing. HUVEC morphology was evaluated for potential cytotoxicity of the extract to normal healthy cells.

### E. *Protein Isolation and ELISA Assay*

Apoptosis induction was further quantified by measuring caspase-3, -8, and -9 activity using commercial ELISA kits (BT LAB, Shanghai, China), adhering to the manufacturer's guidelines. PC-3 cells were seeded at a density of $3 \times 10^5$ cells per well in 6-well plates and exposed to 50 µg/mL and 100 µg/mL of the extract for a duration of 24 h. Following treatment, cells were lysed, and supernatants were collected by centrifugation. Caspase activity was determined by

antibody-based colorimetric detection, and absorbance was quantified at 450 nm with the microplate reader. (Allsheng Instruments, Hangzhou, China). Results were showed as fold changes compared to untreated controls.

*F. Western Blot Analysis*

Bcl-2, p53, and Survivin expression levels were assessed by Western blotting. After the PC-3 cells treated with the extract (50 µg/mL and 100 µg/mL) for 24 h, cells were harvested, and lysed in RIPA buffer (A.B.T, Ankara, Türkiye). Protein concentration was determined by the Bradford assay. Equivalent quantities of protein (30–40 µg) were resolved by SDS-PAGE (10–12%) and transferred to PVDF membranes (Macherey-Nagel GmbH, Düren, Germany). Membranes were blocked with 5% non-fat dry milk in TBST and incubated with the primary antibodies (overnight at 4°C; dilution rate: 1:1000) targeting Bcl-2, p53, survivin, and GAPDH (as loading control) (Affinity Biosciences, Cincinnati, USA). Following washing, the membranes were incubated for 1h at room temperature with secondary antibodies (HRP-conjugated). The blotting bands were imaged by enhanced chemiluminescence (ECL, ABP Biosciences, Beltsville, USA) and imaged using a chemiluminescence detection system (Pop-Bio Imaging, Cambridge, UK). Densitometric analysis was performed using ImageJ software.

*G. Statistical Analysis*

All tests were independently repeated at least three times. Data were presented as mean ± SD (standard deviation). Statistical analyses were conducted with GraphPad Prism 9.0 software. Statistical significance was assessed through one-way ANOVA and two-way ANOVA, followed by Dunnett's post-hoc test. Significance levels were presented as *p <0.05, **p <0.01, ***p <0.001, and ****p <0.0001.

## III. RESULTS AND DISCUSSIONS

Table 1 is showed that the inhibition zones formed by the plant extracts against tested bacterial strains and the standard antibacterial comparison antibiotics. As clearly seen in Table 1, the plant extracts have a strong antibacterial effect against both Gram positive and Gram negative bacterial cultures. The extracts demonstrated inhibitory effects on all tested bacteria, resulting in zones of inhibition ranging from 13.2 to 18.8 mm. The best antibacterial effects were obtained with EtOH extract against *Staphylococcus aureus* (18.8 mm) and *S. epidermidis* (17.4 mm), which were stronger than those of the comparison antibiotics P10, SAM20, CTX30 and V30. The next most effective result against Gram negative bacterium *Escherichia coli* and Gram positive bacterium *Streptococcus pyogenes* in the same inhibition zone at 16.8 mm. It is seen that this value is well above the antagonistic effect of some comparison antibiotics used in the study. The third highest value determined is that the effect activity of the extract is against Gram negative bacterium *Proteus vulgaris* (16.6 mm), which was greater than those of P10 and SAM20.

Table 1. Summary of antibacterial activity of the plant extract and the standard comparison antibiotics

| | **Mean diameter of inhibitory zones (mm)** | | | | | | |
|---|---|---|---|---|---|---|---|
| **Microorganisms** | **EtOH extract** | **P10** | **SAM20** | **CTX30** | **V30** | **OFX5** | **TE30** |
| **Gram (-) Bacteria** | | | | | | | |
| *Escherichia coli* | 16.8±1.3$^{e}$ | 15.2±0.6$^{f}$ | 11.8±0.9$^{h}$ | 18.6±0.8$^{c}$ | 19.2±1.1$^{b}$ | 20.8±1.4$^{a}$ | 20.8±0.8$^{a}$ |
| *Enterobacter aerogenes* | 13.4±0.2$^{f}$ | 15.8±0.9$^{d}$ | 16.2±0.5$^{c}$ | 14.2±1.3$^{e}$ | 16.6±0.9$^{c}$ | 18.6±0.2$^{b}$ | 22.2±0.7$^{a}$ |
| *Proteus vulgaris* | 16.6±0.9$^{d}$ | 10.8±1.2$^{h}$ | 15.6±0.3$^{e}$ | 18.4±0.6$^{c}$ | 20.2±1.2$^{b}$ | 21.4±0.9$^{a}$ | 18.6±1.3$^{c}$ |
| *Salmonella typhimurium* | 12.4±0.5$^{e}$ | 16.0±0.7$^{c}$ | 14.6±1.2$^{d}$ | 13.8±1.3$^{d}$ | 19.8±0.4$^{a}$ | 10.6±0.6$^{g}$ | 20.0±0.4$^{a}$ |
| *Pseudomonas aeruginosa* | 13.2±1.2$^{g}$ | 11.2±0.9$^{h}$ | 15.2±0.3$^{f}$ | 12.6±0.9$^{h}$ | 18.6±1.4$^{e}$ | 20.4±0.7$^{d}$ | 30.2±1.2$^{a}$ |
| *Klebsiella pneumoniae* | 14.8±0.6$^{e}$ | 17.8±1.0$^{c}$ | 16.2±0.5$^{d}$ | 19.8±0.1$^{b}$ | 16.0±0.5$^{d}$ | 22.4±0.6$^{a}$ | 22.6±0.9$^{a}$ |
| **Gram (+) Bacteria** | | | | | | | |
| *Staphylococcus aureus* | 18.8±1.5$^{b}$ | 14.8±0.8$^{e}$ | 16.4±1.1$^{d}$ | 11.8±0.4$^{f}$ | 12.8±0.5$^{f}$ | 18.6±0.2$^{b}$ | 22.2±1.0$^{a}$ |
| *Staphylococcus epidermidis* | 17.4±0.8$^{b}$ | 11.6±0.1$^{e}$ | 13.8±0.6$^{d}$ | 16.4±0.7$^{c}$ | 14.6±0.7$^{d}$ | 18.3±0.9$^{b}$ | 20.2±0.3$^{a}$ |
| *Bacillus cereus* | 12.4±0.3$^{e}$ | 15.8±0.8$^{c}$ | 14.0±0.5$^{c}$ | 13.2±0.2$^{d}$ | 13.8±1.2$^{d}$ | 18.8±0.7$^{a}$ | 18.6±0.9$^{a}$ |
| *Micrococcus luteus* | 14.4±0.7$^{f}$ | 20.8±0.5$^{b}$ | 22.4±1.1$^{a}$ | 20.2±0.6$^{b}$ | 20.2±1.1$^{b}$ | 21.4±0.4$^{b}$ | 19.2±0.6$^{c}$ |
| *Listeria monocytogenes* | 14.6±0.5$^{g}$ | 19.6±0.4$^{c}$ | 14.8±0.2$^{g}$ | 15.2±0.5$^{f}$ | 19.8±0.9$^{c}$ | 24.1±0.3$^{a}$ | 18.0±0.2$^{d}$ |
| *Streptococcus pyogenes* | 16.8±1.4$^{a}$ | 17.4±1.0$^{a}$ | 15.2±0.4$^{b}$ | 15.6±0.3$^{b}$ | 15.2±1.3$^{b}$ | 16.8±0.7$^{a}$ | 17.6±0.5$^{a}$ |

1: P10 (Penicillin G) (10 units); 2: SAM20 (Ampicillin 10 mg); 3: CTX30 (Cefotaxime 30 mg); 4: V30 (Vancomycin 30 mg); 5: Ofloxacin 5 mg; 6: TE30 (Tetracycline 30 mg).
In each case triplicate tests were performed and the average was taken as the final reading.
*Different letters within a row indicate statistically significant differences among compounds (one-way ANOVA, Tukey's post-hoc test, p<0.05). Compounds with the same letter are not significantly different.

The extract is resistant to *Klebsiella pneumoniae* (14.8 mm) and *Listeria monocytogenes* (14.6 mm). Notably, *Pseudomonas aeruginosa* (13.2 mm).is susceptible to the extract as compared to P10, SAM20 and V30. The other tested

bacterial strains were significantly less effective than the comparative antibiotics compared to the effect of the extract. Similar results were shown by Babayi et al. (2004), Kaneria et al. (2009) and Chanda et al. (2011). Gram-positive bacteria exhibited greater susceptibility according to Gram-negative bacteria. This aligns with prior studies indicating that plant extracts showed considerable activity against Gram-positive bacteria relative to Gram-negative bacteria (Kelmanson et al., 2000; Parekh & Chanda, 2006; Kumar et al., 2006). The observed differences can be ascribed to the structural characteristics of bacterial cell walls: Gram-positive bacteria possess a single, thick layer, while Gram-negative bacteria exhibit a multilayered and more complex structure. *T. catappa* has been found to include a diverse range of bioactive compounds. According to Mwangi et al. (2024), the plant contains alkaloids, tannins, steroids, cardiac glycosides, flavonoids, phenols, saponins, and coumarins. These compounds have been shown to have anti-inflammatory, antimicrobial and antioxidant features. Further analysis by Anand et al. (2015) revealed that the presence of phenols, flavonoids and carotenoids in *T. catappa*.

As demonstrated in Figure 1A, the extract significantly reduced the viability of PC-3 cells in a manner dependent on concentration and time. The calculated $IC_{50}$ values were 49.54 µg/mL at 24 h and decreased to 30.41 µg/mL at 48 h, indicating enhanced potency with longer exposure. Similarly, in Figure 2A, the $IC_{50}$ values for LNCaP cells were 61.53 µg/mL at 24 h and 43.64 µg/mL at 48 h, confirming that the extract is effective against both androgen-independent and androgen-dependent prostate cancer phenotypes. Cell viability measurements (Figures 1B and 2B) further confirmed this result. At 24 h, treatment with 50 µg/mL reduced PC-3 viability to 55% and LNCaP viability to 65% compared to control. At higher concentrations (100–400 µg/mL), cell viability dropped dramatically below 40% in both cell lines. After 48 h, the inhibitory effect was significantly stronger, with proliferation suppressed to less than 20% at 200–400 µg/mL. Statistical analyses revealed highly significant differences compared to untreated controls, with $p<0.05$ to $p<0.0001$ depending on the concentration. Together, these findings demonstrate that *T. catappa* ethanol extract exerts time- and dose-dependent antiproliferative effects on prostate cancer cells, with PC-3 cells being slightly more sensitive than LNCaP cells.

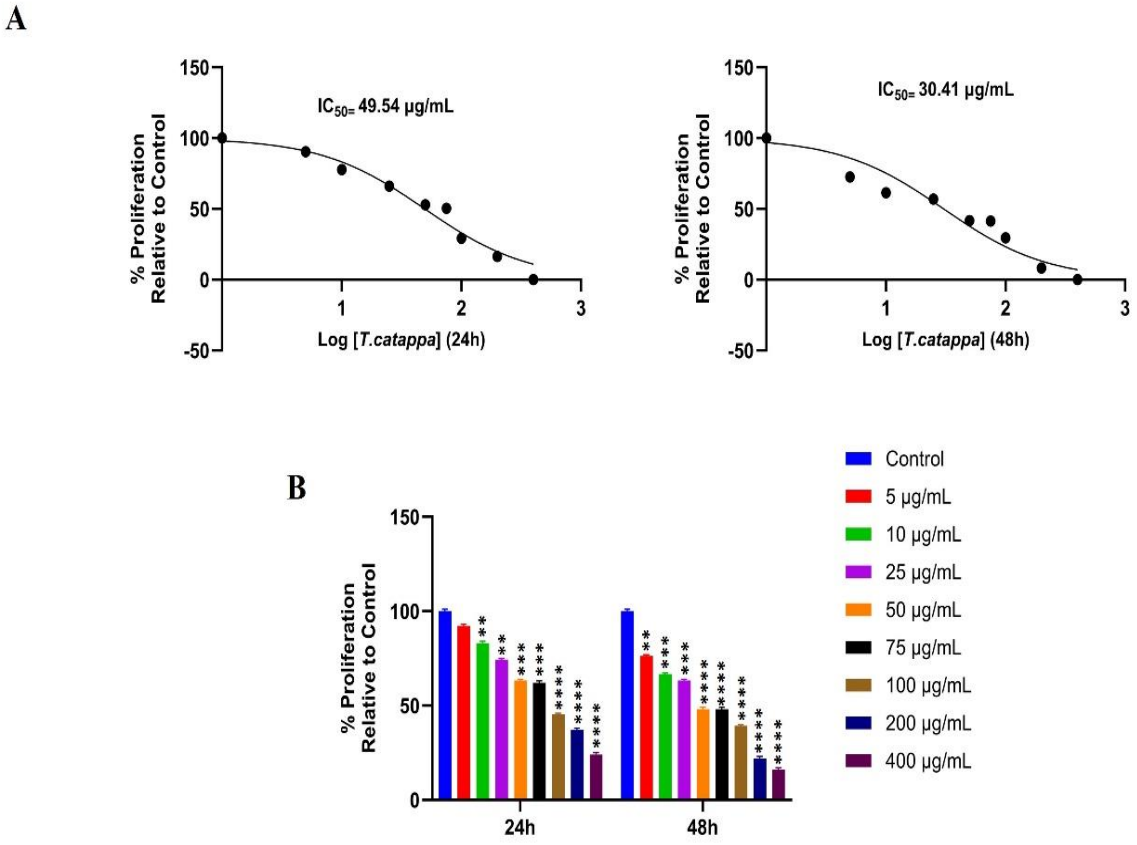



Figure 1. Antiproliferative effect of *T. catappa* ethanol extract on PC-3(androgen-independent) prostate cancer cell line. (A) Analysis of dose–response curves and determination of $IC_{50}$ values following 24 h and 48 h treatments. (B) Percentage of viable cells following treatment with increasing concentrations of the extract. (**p<0.01, ***p<0.001, ****p<0.0001).

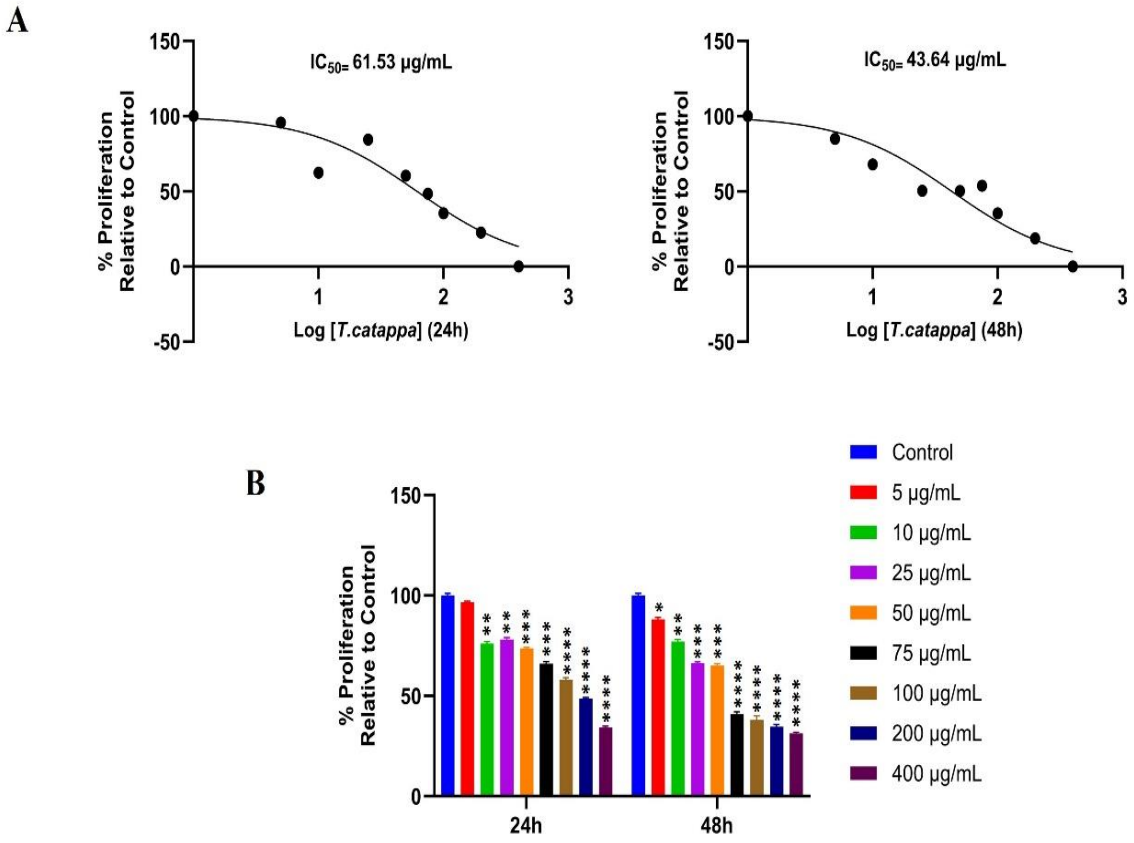



Figure 2. Antiproliferative effect of *T. catappa* ethanol extract on LNCaP (androgen-dependent) prostate cancer cell line. (A) Analysis of dose–response curves and determination of $IC_{50}$ values following 24 h and 48 h treatments. (B) Percentage of viable cells following treatment with increasing concentrations of the extract. (*p<0.05, **p<0.01, ***p<0.001, ****p<0.0001).

Our findings showed that ethanol extract of *T. catappa* decreased cell viability in a profile that was dependent on concentration and duration of exposure, with PC-3 cells exhibiting slightly greater sensitivity than LNCaP cells. This differential response aligns with the biological heterogeneity of prostate cancer, where androgen-independent models such as PC-3 often exhibit more aggressive characteristics but may also be sensitive to polyphenolic compounds targeting nonhormonal signaling pathways (Fernandes et al., 2023). Inhibitory effects of *T. catappa* have been observed in hepatocellular carcinoma (Yeh et al., 2012), breast (Zarredar et al., 2021), lung (Zarredar et al., 2021) and colorectal cancer cells (Shanehbandi et al., 2021), reinforcing the plant's broad anticancer potential.

To further investigate the cytotoxic effect of the extract, morphological alterations were investigated with the inverted microscope. As shown in Figure 3, untreated PC-3 and LNCaP cells exhibited normal adherent morphology with well-defined cell–cell contacts and elongated structures. Following 50 µg/mL and 100 µg/mL of the *T. catappa* ethanol extract for 24 h, both prostate cancer cell lines displayed classical apoptotic features, including shrinkage in cells, blebbing of the cellular membrane, and detachment from the culture surface. These effects were more pronounced at the higher concentration, with evident cell fragmentation and apoptotic body formation. In contrast, HUVEC cells (as healthy control cells) retained their typical elongated and spindle-like morphology under identical treatment conditions. Although minimal changes were noted at 100 µg/mL, the majority of HUVEC cells remained viable and morphologically intact, suggesting that *T. catappa* ethanol extract exhibits preferential cytotoxicity toward malignant cells while sparing normal healthy cells. This selective effect highlights the therapeutic potential of the extract with reduced off-target toxicity.

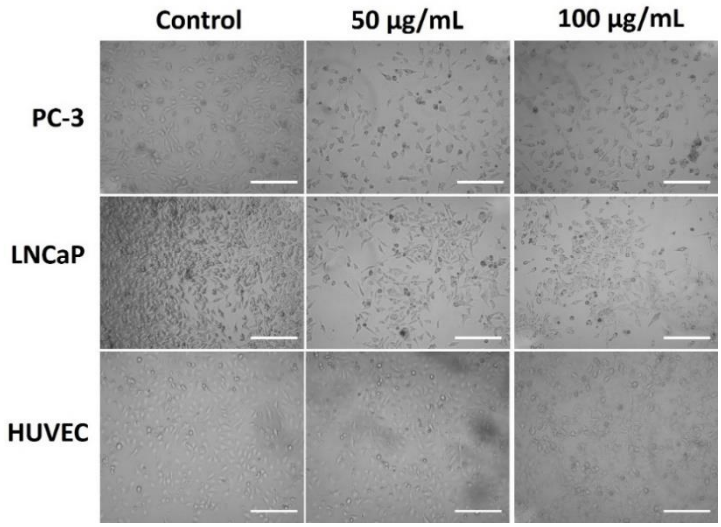



Figure 3. Morphological changes induced by *T. catappa* ethanol extract in PC-3, LNCaP and HUVEC cells (Scale bar = 100 µm).

Microscopic observations revealed classic apoptotic morphology characterized by cell shrinkage, rounding, detachment, and membrane blebbing in PC-3 and LNCaP cells, while HUVEC cells mostly maintained their normal morphology. This selective cytotoxicity is an important finding that highlights the therapeutic index of plant-derived extracts, unlike traditional chemotherapeutic agents that generally do not segregate between malign and normal cells (Dehelean et al., 2021).

To explore whether apoptosis was the underlying mechanism of growth inhibition, the expression levels of caspase proteins were investigated in PC-3 cells. As showed in Figure 4, 50 µg/mL extract treatment significantly increased caspase-3 expression (2-fold compared to control, $p<0.001$), while 100 µg/mL further elevated its levels to more than 3-fold ($p<0.0001$). Similarly, caspase-8 and caspase-9 expression levels were markedly elevated in a concentration-dependent fashion, indicating concurrent activation of extrinsic and intrinsic pathways of apoptosis. Importantly, the merge on caspase-3, the executioner caspase, suggests efficient induction of programmed cell death. These results demonstrated that *T. catappa* ethanol extract triggers apoptosis via dual signaling cascades.

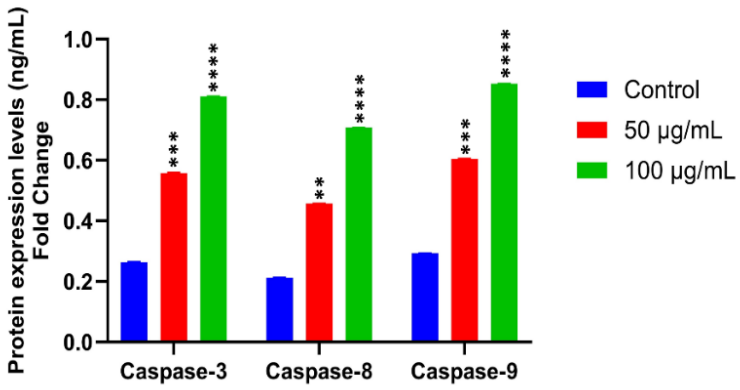



Figure 4. Effect of *T. catappa* ethanol extract on caspase protein expression in PC-3 cells. (**$p<0.01$, ***$p<0.001$, ****$p<0.0001$).

In PC-3 cells, both intrinsic (caspase-9) and extrinsic (caspase-8) pathways were activated, resulting in the activation of caspase-3, which acts as a trigger for apoptosis. The dual activation of apoptotic signaling suggests that T. catappa extract enhances its pro-apoptotic effects in cancer cells by activating various regulatory pathways. Consistent with previous research, phytochemicals in *T. catappa*, including flavonoids and tannins, may directly interact with mitochondria-dependent signaling and regulate death receptor-mediated pathways [24]. Western blot analysis provided further mechanistic insight into the pro-apoptotic activity of the extract in PC-3 cells.

As showed in Figure 5, *T. catappa* extract reduced the expression levels of the Bcl-2 and survivin anti-apoptotic proteins. At 100 µg/mL, Bcl-2 levels were reduced to less than 40% of the control ($p<0.001$), and survivin expression

was similarly downregulated, weakening the cancer cells' survival mechanisms. Conversely, the tumor suppressor protein p53 was strongly upregulated, with nearly a 2.5-fold increase compared with control ($p<0.0001$). This coordinated modulation which is downregulation of pro-survival factors and upregulation of a key tumor suppressor supports the conclusion that apoptosis is induced by direct regulation of critical signaling pathways.

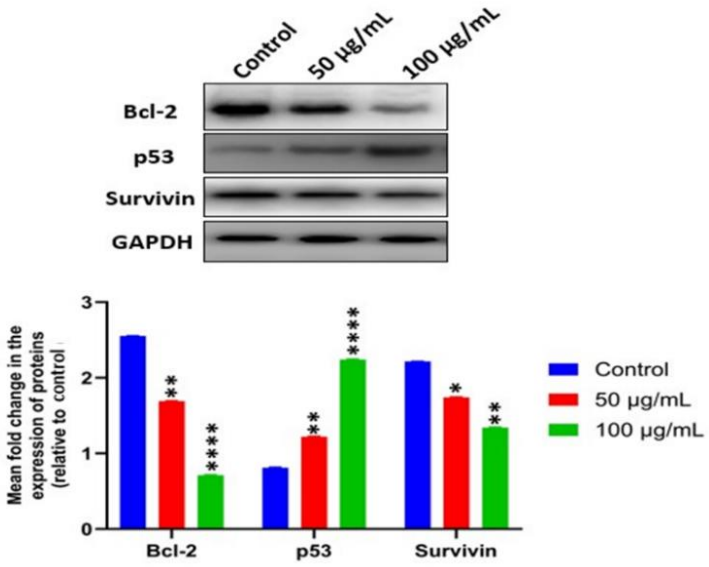



Figure 5. Modulation of apoptosis-related proteins by *T. catappa* ethanol extract in PC-3 cells. (*$p<0.05$, **$p<0.01$, ****$p<0.0001$).

Western blot results showed that the ethanol extract of *T. catappa* decreased the expression levels of the Bcl-2 and survivin in PC-3 cells, while significantly increasing the levels of the tumor suppressor p53. Downregulation of Bcl-2 weakened mitochondrial membrane integrity, promoting cytochrome c release and caspase activation, while suppression of survivin inhibited caspase-dependent inhibitors of apoptosis. Upregulation of p53 suggests cell cycle arrest and apoptosis activation, indicating p53-dependent modulation of transcriptional activity in prostate cancer cell of *T. catappa*. Our data collectively suggest that *T. catappa* regulates apoptosis through coordinated modulation of survival and death signaling pathways. Our findings also suggest that the *T. catappa* ethanol extract is a promising candidate for complementary anticancer strategies by effectively targeting both androgen-sensitive and androgen-resistant prostate cancer cells. However, this study was handled *in vitro*; therefore, *in vivo* pharmacokinetics, bioavailability, and systemic toxicity were not examined. The crude extract contains a complex mixture of phytochemicals; future studies should focus on identifying and isolating the specific active components responsible for the observed effects. Furthermore, due to increasing evidence that natural products may improve the action of standard treatments while reducing side effects, potential synergistic interactions with conventional chemotherapeutics warrant further investigation.

## IV. CONCLUSION

This study elucidates the mechanisms underlying the anticancer activity of *T. catappa*, highlighting its capacity to induce apoptosis through caspase activation, downregulation of survival proteins, and upregulation of p53. *T. catappa* extract inhibited prostate cancer cell proliferation in a concentration and time dependent profile. We also demonstrated that the extract showed limited cytotoxicity toward normal HUVEC cells. With its selective cytotoxicity and antibacterial properties, *T. catappa* may constitute a crucial fount of biologically active compounds with potential applications in the management of prostate cancer. Furthermore, further studies are needed to isolate and characterize the specific phytoconstituents responsible for these important biological effects, to evaluate in vivo bioavailability and toxicity, and to investigate possible synergistic interactions with standard chemotherapeutics.

## DECLARATIONS

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**Author Contributions:** Conceptualization, I.K.; Methodology, I.K., G.D. and B.D.; Validation, I.K and G.D.; Investigation, I.K and G.D.; Resources, I.K., G.D. and B.D.; Data Curation, I.K and G.D.; Writing—Original Draft, I.K and G.D.; Writing—Review & Editing, I.K., G.D. and B.D.; Supervision, B.D. All authors have read and approved the final version of the manuscript.

**Conflict of Interest:** The authors declare no conflict of interest.

**Supporting Institutions:** This research received no external funding.

**Ethical Approval:** This study does not involve human or animal participants. All procedures followed scientific and ethical principles, and all referenced studies are appropriately cited.

**Plagiarism Statement:** This article has been evaluated for plagiarism and no instances of plagiarism were detected.

**Use of AI Tools:** The authors declare that no Artificial Intelligence (AI) tools were used in the creation of this article.

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