IN-VITRO CYTOTOXIC POTENTIAL OF CARICA PAPAYA LEAVES ON BJ-HUMAN FIBROBLAST, CERVICAL-HELA AND PROSTATE CELL LINES

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How to cite this article

Asadullah, Saeed, Asif, Unar MA. In-Vitro Cytotoxic Potential of Carica Papaya Leaves On Bj-Human Fibroblast, Cervical-Hela and Prostate Cell Lines. J Gandhara Med Dent Sci.2025;12(4):18.-22. http://doi.org/10.37762/jgmds.12-4.736

Date of Su bmission: 29-05-2025
Date Revised: 30-08-2025
Date Acceptance: 30-08-2025

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ABSTRACT

OBJECTIVES

To investigate the in vitro cytotoxic potential of Papaya Leaves on 3 distinct cell lines: BJ, cervical, and prostate.

METHODOLOGY

Papaya (Red Lady) samples were obtained. Acetone and n-hexane extracts were used in a Soxhlet extractor at the University of Karachi. The cytotoxic activity was assessed using the MTT (3- (4,5-dimethylthiazol-2-yl)- 2,5-diphenyltetrazolium bromide) assay. Cells were cultured in 75cm² flasks with 5% CO2 at 37°C, 5% fetal-bovine serum, 100IU/ml penicillin, and 100µg/ml streptomycin. Cells were counted using a hemocytometer, diluted, and then added to 96-well plates at a concentration of 6 × 10^4 cells/mL. After 48 hours, MTT reduction to formazan was measured using a microplate reader.

RESULTS

The acetone extract exhibited potent cytotoxic activity against the BJ cell, with a % inhibition (mean ± SD) of 60.4 ± 0.85 at a concentration of 30 µM, and an IC50 of 18.19 ± 0.19 µM ($p < 0.001$). In contrast, the n-hexane extract revealed minimal activity. In cervical, both extracts fell below the 50% inhibition threshold, unlike the effective Doxorubicin. The acetone extract showed moderate activity for prostate, while the n-hexane extract was inactive.

CONCLUSION

The acetone extract displays significant activity against BJ cells, while the n-hexane extract is inactive. Both extracts have minimal effects on HeLa and none on PC3 cells.

KEYWORDS: *Fibroblasts, Cervical Cell Lines, Prostate Cell Lines*

INTRODUCTION

Cancer is a leading global cause of mortality, marked by uncontrolled cell proliferation. Lung cancer is prevalent in males, while breast cancer affects females.[1] Treatment options vary by cancer type, stage, and location, including surgery, chemotherapy, radiotherapy, immunotherapy, and combination therapies.[2] Chemotherapy, using drugs like Irinotecan and Doxorubicin, is particularly effective for highly metastatic cancers.[3] Despite their effectiveness, chemotherapeutic drugs have drawbacks like limited bioavailability, toxicity, lack of specificity, rapid clearance, and adverse effects such as cytotoxicity, neuropathy, cardiovascular issues, and gastrointestinal disturbances.[4] Consequently, to address these challenges, researchers are exploring alternative treatments with minimal or no side effects.[5] Extensive studies suggest phytochemicals are a promising anti-cancer option due to their potential efficacy and fewer side effects compared to traditional medicine.[6] The tropical fruit papaya is a family of caricaceae and several varieties have been utilized as ethnomedicine against a different of disorders.[7] Papaya is cultivated for its roots, leaves, seeds, and fruits, which are extensively used in conventional medicine to treat a wide range of disorders.[8] Papaya is revered as a nutritionally plentiful source of several vitamins, such as A, B, and C, providing a reasonable quantity of Ca and Fe.[9] It has papain, an enzyme that helps with the digestion and is utilized to treat ulcers and some microbial illnesses.[10] Benzyl isothiocyanate, found in papaya fruit seed extract, has bacteriostatic, fungicides, and bactericide effects at a safe dose of 4-5g of seeds.[11] Papaya has strong antioxidant properties that help scavenge free radicals and stop the onset of disease.[12] Papain, glycyl-endopeptidase, chymopapain, and caricain are among the many proteinases found in latex, which is one of the most significant components of papaya.[13] New attention has been drawn to the rich phytochemicals found in tropical plants like papaya, prompting global research efforts towards developing phytochemical-based treatments with minimal side effects for combating cancer.[14] Papaya leaves have been used to treat several illnesses, such as asthma, beriberi, colic, fever, and jaundice. In addition, Papaya leaves have been conventionally utilized for anti-cancerous activities in the native Gold Coast of Australia, based

on a few sketchy pieces of information of significant cases acknowledged in various publications.[15] Different varieties of papaya are cultivated worldwide, varying in color and size. The Red Lady variety is commonly grown globally due to its excellent yield.[16] Consequently, this study aimed to explore the potential of Red Lady papaya leaves as a therapeutic option for cancer by evaluating the cytotoxic effects of acetone and n-hexane extracts against various cell lines.

METHODOLOGY

An in vitro experimental study was conducted at the Department of Pharmacology and Therapeutics, Baqai Medical College and University of Karachi, spanning a duration of 6 months. Selected plant was collected from University of Karachi, and authenticated by an ethno-botanist (G.#H97627). Extraction was used at the International Centre for Chemical and Biological Sciences (ICCBS) University of Karachi, Industrial Analytical Centre. The technique involved inserting the ground sample into the Soxhlet apparatus thimble chamber. Heating the acetone and n-hex extraction solution in the bottom flask caused it to evaporate into the sample thimble, condense in the condenser, and drip back. The procedure was repeated as the fluid filled the bottom flask and cleared into it as it approached the siphon arm. The extraction process takes place in a Soxhlet apparatus for 72 hours, after weighing between 10g and 82g of the semi-solid sample. By evaporating the extract at 60°C, the extract was concentrated.[17] Acetone and n-hexane were used for the extraction of distinctly different classes of phytochemicals. Acetone, with a moderate polarity, extracts flavonoids, phenolics, and other moderately polar compounds that potentially contribute to bioactivity. N-hexane, being a nonpolar solvent, facilitates the separation of lipophilic constituents, such as fatty acids, terpenoids, and chlorophyll derivatives. Together, this might offer a complementary option for polar-non-polar portfolios. This choice of solvents is selected because most ethanol, methanol, and water, as well as other solvents used in previous studies on papaya leaves, have already been utilized, which prompted consideration of less-explored fractions for novel activities. The cell lines were obtained from the Bio-bank facility of ICCBS, which were purchased from the American Type Culture Collection: i.e., HeLa (cervix), PC3 (prostate), and BJ (normal human skin fibroblast). HeLa and BJ cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM), supplemented with 10% heat-inactivated fetal bovine serum (FBS), 2 mM L-glutamine, penicillin (100 U/mL), and streptomycin (100 µg/mL). The PC3 cell line was maintained in RPMI-1640, supplemented with 10% (v/v) FBS, penicillin (100U/mL), streptomycin (100µg/mL), and 1% of non-essential amino acids. All cell lines were incubated in a T-75 cell culture-treated flask with 10mL of complete medium at 37°C in a humidified incubator with 5% CO2 until the medium was replaced. A cell culture was prepared and added to 96-well plates (100 µL/well) at a concentration of 6×10^4 cells/mL. Following an overnight incubation period, the medium was removed and 200µL of new medium containing a variable dosage of compounds (1-30µM) was added. Each well received 200 µL of MTT (0.5mg/mL), and after 2 days and 4 more hours of incubation, the cells were required. Then, 100 µL of DMSO was added to every well, and the absorbance at 570nm was measured using a microplate reader (Spectra-Max plus, Molecular Devices, CA, USA) to determine the degree of MTT reduction to formazan within the cells. The concentration that caused 50% growth inhibition (IC_{50}) for BJ, HeLa, and PC-3 was used to measure the cytotoxicity. The subsequent equation was used for calculating the % Inhibition: %Inhibition=100-(mean of test compounds O.D.-mean of negative-control O.D.)/ (mean of positive-control O.D.-mean of negative-control O.D.)*100).[18,19] For Statistical Analysis, all cytotoxicity values were compared using one-way ANOVA to measure differences between treatment groups. In instances where a difference was detected ($p < 0.05$), pairwise significance was evaluated by Tukey's HSD post hoc test. The cytotoxic activity was defined with reference to the 50% inhibition cut-off. This threshold is widely applied in cytotoxicity and natural product screening studies for separating active extracts from inactive ones. Although the choice of 50% inhibition seems somewhat arbitrary, it is consistently applied in other works, allowing some comparisons of the results obtained. In this work, such a comparison was made possible by adopting the same threshold as used in prior literature.

RESULTS

The results for BJ Human fibroblast cell lines in Table 1 suggest that the acetone extract of Papaya leaves (RL) demonstrates remarkable cytotoxic effects, with a %Inhibition (mean±SD) of 60.4 ± 0.85, at a concentration of 30µM. The IC_{50}±SD value for this extract was calculated to be 18.19±0.19 µM, with p <, highlighting its efficacy in impeding cell growth. In contrast, the n-hexane extract from the same Papaya variety showed insignificant activity, with a 40.5 ± 0.70 inhibition (mean ± SD) at a 30 µM concentration, falling below the 50% threshold and thus deemed inactive. Instead, the positive control, Doxorubicin, showed vigorous activity, with a 93.7 ± 0.45% Inhibition (mean ± SD) at a 30 µM concentration and

an exceptionally low IC50 ± SD value, emphasizing its potent ability to suppress cell growth. For the cervical-HeLa cell line, the results shown in Table 2 exhibited that the acetone extract displayed a 26.9 ± 0.72% Inhibition (mean ± SD). In comparison, the n-hexane extract illustrated a 38.9 ± 0.64% Inhibition (mean ± SD). Despite these findings, both extracts fell short of the predefined cut-off value of 50%, rendering them inactive at the tested concentrations. In contrast, the standard anti-cancer drug Doxorubicin showed notable efficacy against cervical cancer cells, with a remarkable % inhibition (mean ± SD) of 98.7 ± 0.47 at a concentration of 30 µM. The low IC_{50} value of 1.13±0.16µM for Doxorubicin emphasizes its potent ability to suppress cancer cell growth. For the prostate PC3 cell line, the results, as demonstrated in Table 3, illustrated that the acetone extract, when administered at a concentration of 30 µM, showed a moderate % inhibition (mean ± SD) of 30.5 ± 0.69. Although it showed significant activity, it did not meet the required threshold of 50% inhibition for classification as active. Similarly, the n-hexane extract, administered at a concentration of 30 µM, showed a relatively low percentage of inhibition (mean ± SD), 8.0 ± 0.44, classifying it as inactive. In contrast, Doxorubicin, at a concentration of 30 µM, revealed a significant % inhibition (mean ± SD) of 80.8 ± 0.62, indicating a positive effect against prostate cancer cells.

Table 1: Cytotoxic Effect of Papaya Leaves Variety of Red Lady Extract on BJ-Human Fibroblast Cell Lines (Conc.30 µM)

Sample	%Inhibition (mean±SD)	IC50± SD (µM)	One-way ANOVA	Tukey's HSD Post Hoc Test (Pairwise comparisons)
Acetone ext. of CPL. Var.RL	60.4 ± 0.85	18.19± 0.19	P<0.001	Acet vs n-Hex ~20(P<0.001) Acet vs Std~33.0 (P<0.01)
N-hexane ext. of CPL. Var.RL	40.5 ± 0.70	Inactive		n-Hex vs Std ~53.0(P<0.001)
Doxorubicin (Std)	93.7 ± 0.45	0.16±0. 19		

Table 2: Cytotoxic Effect of Papaya Leaves Variety of Red Lady Extract on Cervical-Hela Cell Line (Conc. 30 µM)

Sample	%Inhibition (Mean± SD)	IC50± SD (µM)	One-way ANOVA	Tukey's HSD Post Hoc Test (Pairwise Comparisons)
Acetone ext. of CPL. Var.RL	26.9 ± 0.72	Inactive	P<0.001	**Acet vs n-Hex**~12(P<0.001) **Acet vs Std**~71.7(P<0.01)
N-hexane ext. of CPL. Var.RL	38.9 ± 0.64	Inactive		**n-Hex vs Std**~59.8(P<0.001)
Doxorubicin (Std)	98.7 ±0.47	1.13±0. 16		

Table 3: Cytotoxic Effect of Papaya Leaves Variety of Red Lady Extract on Prostate-Pc3 Cell Line (Conc. 30µM)

Sample	%Inhibition (mean± SD)	IC50± SD (µM)	One - way ANOVA	Tukey's HSD Post Hoc Test (Pairwise Comparisons)
Acetone ext. of CPL. Var. RL	30.5 ± 0.69	Inactive	P<0.0 01	Acet vs nHex~22.8(P<0.0 01)Acet vs Std~50.0(P<0.01)
N-hexane ext. of CPL. Var. RL	8.0 ± 0.44	Inactive		n-Hex vs Std~72.8(P<0.001)
Doxorubicin (Std)	80.8 ± 0.62	1.18±0. 21		

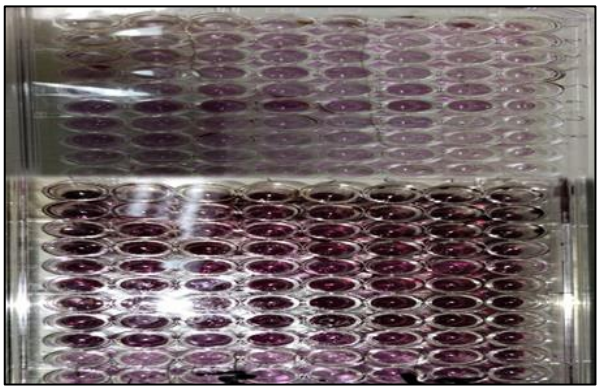

Figure 1: MTT method in PC3, HeLa, and BJ cells exposed to different concentrations of Carica Papaya Leaves variety of Red Lady extracts, and assays were performed in triplicate.

DISCUSSION

Many anti-cancer drugs derived from botanical sources are currently recognized as successful treatments in medical settings. Vinca alkaloids, Taxanes, Podophyllotoxin and its analogues, and Camptothecin and the derivatives are the four main categories into which these agents fall.[20] In the leaves of papaya, numerous compounds have been reported to possess potential anti-tumor activity, including α-tocopherol, lycopene, flavonoids, and benzyl-isothiocyanate.[21,22] The results for BJ-Human fibroblast cell lines suggest that the acetone extract of these leaves showed important activity, as indicated by a substantial % Inhibition (mean±SD) of 60.4 ± 0.85, at a concentration of 30 30µM, suggesting that Papaya leaves could be a valuable source of agents. The relatively low IC_{50} value indicates a strong inhibitory effect on cell growth, underscoring the therapeutic potential of the acetone extract from Papaya leaves. Whereas the n-hexane extract from the Papaya (RL) Leaf demonstrated moderate activities, with a 40.5 ± 0.70 %Inhibition (mean±SD) at (30µM conc.), falling below the 50% threshold and thus deemed inactive. In

the research examination of Pawlicka, MA et al. Reported in 2022 that the experienced isoflavonoid is intimately connected to its strength. Increasing the concentrations of Genistein (50µM and 100µM) reduces the number of fibroblast cells with longer contact times (48 hours). However, the decrease in strength of Genistein (10 & 20µM) exhibited a higher number of dermal fibroblasts. 23 Our research study results further support the thought that the cytotoxic activities of Papaya leaves (RL) might vary depending on the extraction solvent utilized. Overall, our findings highlight the potential of Papaya leaves, particularly the acetone extract from the RL variety, as a source of novel anti-proliferative agents. The research findings reveal the cytotoxic potential of Papaya Leaves against the cervical HeLa cell line. Both acetone and n-hexane extracts exhibited cancer cell growth inhibition; their potency was insignificant at the tested concentrations. The result for the prostate-PC3 cell line showed that the acetone extract of Papaya RL Leaf yielded a negligible effect when dispensed at a concentration of 30µM, illustrating a reasonable % inhibition (mean ± SD) of 30.5 ± 0.69. Even though it demonstrated considerable activity. The cytotoxicity of the acetone extract is recorded against BJ fibroblasts, but not in HeLa or PC3 cells, possibly due to the presence of mid-polarity phytoconstituents that exert nonspecific toxic properties on normal cells. In contrast, cancer cells evade death by modulating apoptosis or efflux mechanisms. Further phytochemical studies are necessary to understand the selectivity better. Likewise, the n-hexane extract of Papaya Leaf, at a concentration of 30 µM, showed a comparatively low inhibition rate of 8.01%, indicating it to be inactive. In 2020, Abankwa JK et al. investigated the antioxidant and anti-cancer activities of Moringa oleifera, Phyllanthus amarus, and Carica papaya. Aqueous extract of Carica papaya in particular exhibited significant anti-prostate cancer activity, selective enough toward PC3 prostate cancer cells to record an IC_{50} value of 45.68 ± 1.16 µg/mL and a very high selectivity index (SI = 18). This provides evidence for the therapeutic promise of C. papaya as a rich source of bioactive compounds capable of selectively targeting cancer cells with minimal effects on normal cells, thereby supporting its traditional application in complementary medicine. There remains a further need to deepen mechanistic and in vivo studies to validate its application in the management of prostate cancer.[25] Further optimization of Papaya leaf extracts is recommended to enhance their cytotoxic efficacy across different cell lines. Future studies should focus on identifying and isolating specific bioactive components responsible for the observed moderate activity against the BJ-cell line, with the goal of developing targeted therapies.

LIMITATIONS

This study is limited by its reliance on in-vitro assays, which may not fully replicate the complex interactions in living organisms. Only two extraction solvents were tested, which may not capture the complete phytochemical potential of papaya leaves. Furthermore, the study focused on a limited number of cell lines, and the sample size for assays was relatively small, restricting the generalizability of the findings. Additional in-vivo studies, broader solvent systems, and mechanistic analyses are required to validate and expand upon these results.

CONCLUSIONS

The acetone extract displays significant activity against BJ-cells (59.5, 60.48, and 61.2%), while the n-hexane extract is inactive, based on a cut-off value of 50%. Both extracts have minimal effects on HeLa cells and none on PC3 Cells, falling short of the predefined cut-off value of 50%.

CONFLICT OF INTEREST: None

FUNDING SOURCES: None

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AUTHORS CONTRIBUTION

Asadullah - *Concept & Design; Data Acquisition; Drafting Manuscript; Final Approval*
Saeed - *Concept & Design; Data Acquisition; Data Analysis/Interpretation; Drafting Manuscript; Final Approval*
Asif - *Concept & Design; Data Acquisition; Drafting Manuscript; Critical Revision; Final Approval*
Masood Ahmed Unar- *Concept & Design; Data Acquisition; Drafting Manuscript; Final Approval*

The authors accept responsibility for all aspects of the work and will ensure that any concerns regarding the accuracy or integrity of any part are properly investigated and resolved.