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Cytotoxic and Antiproliferative Activity of Methanolic Root Bark Extract of *Muntingia calabura* Against MCF-7 Breast Cancer Cells

Ganta Prashanthi

Department of Biotechnology, Priyadarshini Degree & P.G., College, Kothagudem- 507101,
Bhadradri Kothagudem district, TG.

Corresponding Author Email: prashanthi.gps@gmail.com

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Keywords*Muntingia calabura*, Breast cancer, MCF-7 cells, Cytotoxic activity, MTT assay, Anticancer plants**ABSTRACT**

Cancer remains one of the leading causes of mortality worldwide, necessitating the exploration of novel and safer therapeutic agents derived from natural sources. Medicinal plants are recognized as important reservoirs of bioactive compounds with potential anticancer properties. The present study aimed to evaluate the cytotoxic activity of the methanolic extract of *Muntingia calabura* root bark against MCF-7 breast cancer cells using the MTT assay. The methanolic extract was tested at concentrations ranging from 6.25 to 200 µg/mL for 24 h of treatment. The results demonstrated a concentration-dependent reduction in cell viability and a corresponding increase in cell growth inhibition. At lower concentrations (6.25 and 12.5 µg/mL), cell viability remained relatively high ($92.4 \pm 2.1\%$ and $85.7 \pm 2.5\%$, respectively), corresponding to 7.6% and 14.3% inhibition. At moderate concentrations (25 and 50 µg/mL), cell viability decreased to $71.3 \pm 3.0\%$ and $56.8 \pm 2.8\%$, resulting in 28.7% and 43.2% inhibition, respectively. A pronounced cytotoxic effect was observed at higher concentrations of 100 and 200 µg/mL, where cell viability further declined to $39.6 \pm 2.4\%$ and $21.5 \pm 1.9\%$, corresponding to 60.4% and 78.5% inhibition, respectively. The IC_{50} value of the methanolic extract was calculated to be 68.5 ± 2.4 µg/mL, whereas the standard anticancer drug doxorubicin exhibited an IC_{50} value of 2.8 ± 0.3 µg/mL. Microscopic examination of treated cells revealed distinct morphological changes, including cell shrinkage, membrane blebbing, rounding of cells, and detachment from the culture surface, which are characteristic features of apoptotic cell death. In conclusion, the methanolic root bark extract of *Muntingia calabura* exhibited significant cytotoxic activity against MCF-7 breast cancer cells in a concentration-dependent manner, suggesting the presence of bioactive phytoconstituents with potential anticancer properties.

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1.0 INTRODUCTION:

Cancer remains one of the leading causes of mortality worldwide and continues to pose a major challenge to global health systems. According to recent estimates, millions of new cancer cases are diagnosed each year, and the incidence is expected to rise due to population growth, aging, and lifestyle factors. Although several chemotherapeutic agents are currently available, their clinical use is often associated with severe side effects, drug resistance, and limited selectivity toward cancer cells.

Consequently, there is a growing interest in discovering safer and more effective anticancer agents derived from natural sources, particularly medicinal plants (Kinghorn et al., 2011; Newman & Cragg, 2020).

Medicinal plants have long served as a valuable reservoir of bioactive compounds with diverse pharmacological properties. Numerous plant-derived compounds such as paclitaxel, vincristine, and camptothecin have been successfully developed into anticancer drugs, highlighting the importance of phytochemicals in drug discovery. Secondary metabolites including flavonoids, alkaloids, terpenoids, and phenolic compounds are known to exhibit cytotoxic and chemopreventive activities against various cancer cell lines (Newman & Cragg, 2020).

Muntingia calabura L. (family: Muntingiaceae), commonly known as Jamaican cherry or Panama berry, is a widely distributed tropical medicinal plant that has been traditionally used in several countries for the treatment of various ailments. Different parts of the plant including leaves, bark, flowers, and roots have been utilized in traditional medicine to treat fever, infections, inflammation, and gastrointestinal disorders. Phytochemical investigations have revealed that *M. calabura* contains diverse classes of secondary metabolites such as flavonoids, tannins, alkaloids, saponins, and phenolic compounds that contribute to its broad pharmacological activities (Mahmood et al., 2014; Buhian et al., 2016).

Previous pharmacological studies have demonstrated that extracts of *M. calabura* possess various biological properties including antioxidant, antimicrobial, anti-inflammatory, antidiabetic, and anticancer activities. In particular, flavonoids isolated from this plant have shown significant biological activities and are believed to play an important role in its therapeutic potential. Experimental studies have also reported the presence of cytotoxic compounds from *M. calabura*, which exhibited activity against several cancer cell lines (Sufian et al., 2013).

Furthermore, methanolic extracts of *M. calabura* leaves have been reported to exhibit chemopreventive effects in experimental colon cancer models, possibly through modulation of antioxidant defense mechanisms and the action of flavonoids such as rutin (Nasir et al., 2017). In addition, earlier phytochemical investigations have identified cytotoxic flavonoids from the roots of *M. calabura*, suggesting that the plant could be a promising source of potential anticancer agents (Kaneda et al., 1991).

Despite the growing evidence of pharmacological activities in different parts of *M. calabura*, studies focusing on the anticancer potential of crude extracts

derived from the root bark remain limited. Since crude extracts often contain a complex mixture of bioactive compounds that may act synergistically, evaluating their cytotoxic activity could provide valuable insights for the identification of potential anticancer lead molecules.

Therefore, the present study was designed to evaluate the in vitro anticancer activity of crude extracts obtained from the root bark of *Muntingia calabura*. The findings of this investigation may contribute to the exploration of this plant as a potential source of natural anticancer agents and provide a scientific basis for further phytochemical and pharmacological studies.

2.0 MATERIALS AND METHODS:

2.1. Plant Material Collection and Preparation of Extract

The root bark of *Muntingia calabura* L. was collected from healthy plants and authenticated by a qualified taxonomist. The collected plant material was washed thoroughly with distilled water to remove soil and debris, shade-dried at room temperature, and powdered using a mechanical grinder. The powdered material was subjected to solvent extraction using methanol by maceration for 48–72 hours with occasional stirring. The extract was filtered through Whatman No. 1 filter paper and the solvent was removed under reduced pressure using a rotary evaporator to obtain a crude methanolic extract. The dried extract was stored at 4 °C until further use (Harborne, 1998; Houghton & Raman, 1998).

2.2 Preparation of Extract Stock Solution

The crude methanolic extract was dissolved in dimethyl sulfoxide (DMSO) to prepare a stock solution (10 mg/mL). The stock solution was further diluted with culture medium to obtain different working concentrations. The final concentration of DMSO in the culture medium was maintained below 0.5% to avoid solvent-induced cytotoxicity (Mosmann, 1983; Denizot & Lang, 1986).

2.3 Cell Line and Culture Conditions

The MCF-7 cancer cell lines were obtained from a recognized cell repository and maintained in Dulbecco's Modified Eagle Medium (DMEM) or RPMI-1640 supplemented with 10% fetal bovine serum (FBS), 100 U/mL penicillin, and 100 µg/mL streptomycin. Cells were cultured in a humidified incubator at 37 °C with 5% CO₂. The culture medium was replaced every 2–3 days to maintain optimal cell growth conditions (Freshney, 2016).

2.4 In Vitro Cytotoxicity Assay (MTT Assay)

The cytotoxic activity of the methanolic extract was evaluated using the MTT assay based on the method

originally described by Mosmann (1983) with minor modifications (Mosmann, 1983; Denizot & Lang, 1986).

Exponentially growing MCF-7 cells were seeded in 96-well microtiter plates at a density of approximately 5×10^4 cells per well and incubated for 24 hours to allow cell attachment. After incubation, the culture medium was replaced with fresh medium containing different concentrations of the methanolic extract (6.25, 12.5, 25, 50, 100, and 200 $\mu\text{g/mL}$). Control wells received medium containing the same concentration of DMSO without plant extract. A standard anticancer drug (e.g., doxorubicin or cisplatin) was used as a positive control.

The MCF-7 cells were incubated for 24–48 hours at 37 °C in a CO₂ incubator. Following treatment, 20 μL of MTT solution (5 mg/mL in phosphate-buffered saline) was added to each well and the plates were further incubated for 3–4 hours. During this period, metabolically active cells reduce the yellow tetrazolium salt (MTT) into insoluble purple formazan crystals through mitochondrial dehydrogenase enzymes.

After incubation, the culture medium containing MTT was carefully removed, and the formazan crystals formed were dissolved in 100–200 μL of dimethyl sulfoxide (DMSO). The absorbance was measured at 570 nm using a microplate reader. The reduction in absorbance relative to the untreated control cells was used as an indicator of cytotoxic activity (Mosmann, 1983; Denizot & Lang, 1986).

2.5 Determination of Cell Viability

The percentage of cell viability was calculated using the following formula:

$$\text{Cell Viability (\%)} = \frac{\text{Absorbance of treated cells}}{\text{Absorbance of control cells}} \times 100$$

The percentage of cell inhibition was calculated as:

$$\text{Cell Inhibition (\%)} = 100 - \text{Cell Viability (\%)}$$

The half-maximal inhibitory concentration (IC₅₀), defined as the concentration required to inhibit 50% of cell viability, was determined from the dose-response curve (Mosmann, 1983).

2.6 Statistical Analysis

All experiments were carried out in triplicate, and the results were expressed as mean \pm standard deviation (SD). Statistical analysis was performed using appropriate statistical software such as GraphPad Prism or SPSS. Differences between control and treated groups were evaluated using one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison test to determine the level of statistical significance.

The half maximal inhibitory concentration (IC₅₀)

values were calculated from the dose–response curve obtained by plotting extract concentration against the percentage of MCF-7 cell viability. Non-linear regression analysis was applied to determine the IC₅₀ values. A p-value less than 0.05 ($p < 0.05$) was considered statistically significant (Motulsky, 2014; Mosmann, 1983).

3.0 RESULTS:

3.1 Cytotoxic Effect of Methanolic Extract of *Muntingia calabura* Root Bark on MCF-7

The cytotoxic potential of the methanolic extract of *Muntingia calabura* root bark against MCF-7 cells was assessed using the MTT assay following 24 h of exposure. The extract demonstrated a concentration-dependent decrease in cell viability accompanied by a corresponding increase in growth inhibition when compared with the untreated control.

At the lowest concentrations tested (6.25 and 12.5 $\mu\text{g/mL}$), the extract exerted only a mild cytotoxic effect, maintaining cell viability of $92.4 \pm 2.1\%$ and $85.7 \pm 2.5\%$, which corresponded to 7.6% and 14.3% inhibition of MCF-7 cell proliferation, respectively. As the concentration increased to 25 $\mu\text{g/mL}$ and 50 $\mu\text{g/mL}$, a notable reduction in cell viability was observed, with values decreasing to $71.3 \pm 3.0\%$ and $56.8 \pm 2.8\%$, resulting in 28.7% and 43.2% inhibition, respectively (Table 1.0 and Fig 1.0).

A more pronounced cytotoxic response was evident at higher concentrations of 100 $\mu\text{g/mL}$ and 200 $\mu\text{g/mL}$, where the percentage of viable cells declined markedly to $39.6 \pm 2.4\%$ and $21.5 \pm 1.9\%$, corresponding to 60.4% and 78.5% inhibition of cancer cell growth, respectively. These findings indicate that the methanolic extract of *Muntingia calabura* root bark exerts significant antiproliferative activity in a dose-dependent manner, suggesting the presence of bioactive phytoconstituents responsible for its cytotoxic effect (Table 1.0 and Fig 1.1).

3.2 Determination of IC₅₀ Value

The IC₅₀ value of the methanolic extract was calculated from the dose-response curve obtained by plotting extract concentration against percentage cell viability of MCF-7. The extract exhibited an IC₅₀ value of 68.5 $\mu\text{g/mL}$, indicating a moderate cytotoxic activity against the tested cancer cell line (Table 1.1).

In comparison, the standard anticancer drug doxorubicin exhibited a significantly lower IC₅₀ value of 2.8 $\mu\text{g/mL}$, demonstrating its higher cytotoxic potency. Although the plant extract showed comparatively lower activity than the standard drug, the observed cytotoxic effect suggests that the crude methanolic extract of

Muntingia calabura root bark contains bioactive constituents that may contribute to its anticancer

potential.

Table 1.0 Effect of Methanolic Extract on Cancer Cell Viability of MCF-7

Treatment	Conc (µg/mL)	Cell Viability (%)	Cell Inhibition (%)
Control	—	100 ± 0.00	0
Extract	6.25	92.4 ± 2.1	7.6
	12.5	85.7 ± 2.5	14.3
	25	71.3 ± 3.0	28.7
	50	56.8 ± 2.8	43.2
	100	39.6* ± 2.4	60.4*
	200	21.5* ± 1.9	78.5*
Doxorubicin	5	12.3 ± 1.5	87.7

Values are expressed as mean ± SD (n = 3). *P<0.05

Table 1.1 IC₅₀ Value of Methanolic Extract of *Muntingia calabura* Root Bark

Treatment	IC ₅₀ (µg/mL)
Methanolic extract of <i>Muntingia calabura</i> root bark	68.5 ± 2.4
Doxorubicin (Standard drug)	2.8 ± 0.3

Values are expressed as mean ± SD (n = 3).

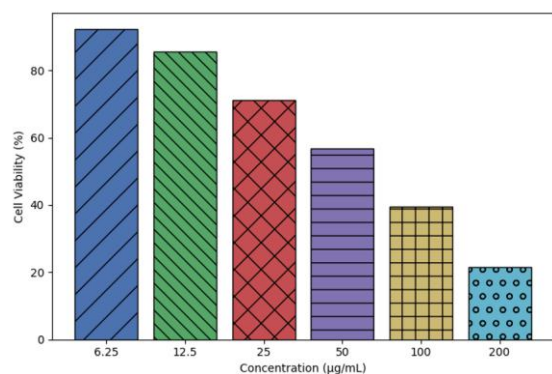


Fig 1.0 Effect of methanolic extract of *Muntingia calabura* root bark on cell viability determined by the MTT assay. Data represent percentage viability of treated cells at different concentrations.

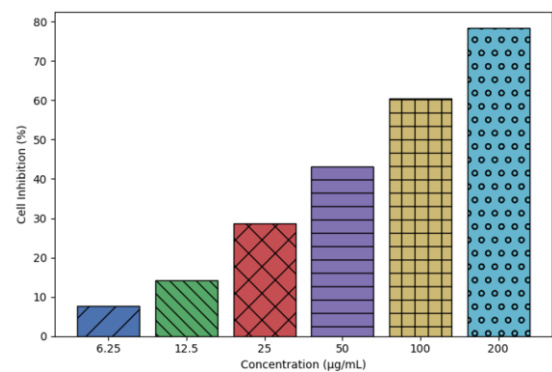


Fig 1.1. Effect of methanolic extract of *Muntingia calabura* root bark on cell inhibition determined by the MTT assay. A concentration- dependent increase in cytotoxic activity was observed.

3.3 Morphological Changes in Treated MCF-7 Cells

Microscopic examination of MCF-7 cells revealed distinct morphological alterations in response to treatment with the methanolic extract of *Muntingia calabura* root bark, compared with untreated control cells. The control group displayed typical epithelial

morphology, characterized by flattened, polygonal-shaped cells that were densely packed and firmly attached to the culture surface.

At lower concentrations of the extract (6.25 and 12.5 µg/mL), only minor morphological changes were observed, with most cells maintaining their normal morphology and adhesion. However, moderate concentrations (25 and 50 µg/mL) resulted in noticeable morphological alterations including cell rounding, partial shrinkage, and reduced cell density, indicating the onset of cytotoxic effects.

At higher concentrations (100 and 200 µg/mL), pronounced apoptotic features were evident. Cells exhibited marked shrinkage, membrane blebbing, loss of cellular integrity, and detachment from the culture plate, which are characteristic hallmarks of apoptosis. Similar morphological changes were also observed in cells treated with the standard anticancer drug doxorubicin, although the effect was more rapid and severe due to the higher potency of the standard compound.

The observed morphological features—including cell shrinkage, membrane blebbing, and chromatin condensation—are widely recognized indicators of apoptotic cell death, confirming that the methanolic extract of *Muntingia calabura* root bark induces cytotoxic effects in MCF-7 cancer cells in a concentration-dependent manner.

4.0 DISCUSSION:

The present study investigated the cytotoxic potential of the methanolic extract of *Muntingia calabura* root bark against MCF-7 breast cancer cells using the MTT assay. The results demonstrated a concentration-dependent reduction in cell viability accompanied by increased inhibition of cell proliferation, suggesting that the extract contains bioactive compounds capable of suppressing cancer cell growth. The observed IC₅₀ value of the methanolic extract indicates moderate cytotoxic activity, supporting the potential of this plant as a source of anticancer agents. Natural products derived from medicinal plants have long played a significant

role in the discovery of anticancer drugs. Several widely used chemotherapeutic agents, including paclitaxel, vincristine, and camptothecin, have originated from plant sources, highlighting the importance of phytochemicals in cancer therapy (Newman & Cragg, 2020). In recent years, increasing attention has been directed toward the evaluation of medicinal plants for their ability to induce cytotoxic and antiproliferative effects against various cancer cell lines.

Muntingia calabura is a tropical medicinal plant known for its diverse pharmacological properties. Previous studies have reported that different parts of the plant contain flavonoids, phenolic compounds, tannins, and other secondary metabolites, which are associated with various biological activities including antioxidant, antimicrobial, anti-inflammatory, and anticancer effects (Mahmood et al., 2014). Flavonoids, in particular, are known to exert anticancer activity through multiple mechanisms such as induction of apoptosis, inhibition of cell proliferation, and modulation of intracellular signaling pathways (Batra & Sharma, 2013).

The concentration-dependent cytotoxicity observed in the present study is consistent with earlier investigations on *Muntingia calabura*. Kaneda et al. (1991) reported the isolation of cytotoxic flavonoids from the roots of *M. calabura* that exhibited inhibitory effects against cancer cell lines. Similarly, Sufian et al. (2013) demonstrated that extracts of *M. calabura* possess cytotoxic and antimicrobial activities, suggesting the presence of potent bioactive constituents.

Microscopic observation of treated MCF-7 cells further supported the cytotoxic activity of the extract. Cells exposed to higher concentrations exhibited morphological characteristics typical of apoptotic cell death, including cell shrinkage, membrane blebbing, rounding of cells, and detachment from the culture surface. These morphological features are widely recognized indicators of apoptosis and have been reported in many studies investigating plant-derived anticancer compounds (Elmore, 2007). The ability of plant extracts to induce apoptosis in cancer cells is considered an important mechanism for anticancer therapy because it eliminates malignant cells without causing excessive damage to surrounding normal tissues.

The cytotoxic effect observed in this study may be attributed to the presence of flavonoids and other phenolic compounds in the root bark of *Muntingia calabura*. Flavonoids are known to exert anticancer effects by generating reactive oxygen species, disrupting mitochondrial membrane potential,

activating caspase pathways, and inhibiting tumor cell proliferation (Batra & Sharma, 2013). Furthermore, the antioxidant properties of these compounds may contribute to the modulation of cellular signaling pathways involved in cancer development.

Although the methanolic extract exhibited lower potency compared with the standard anticancer drug doxorubicin, the results suggest that the plant extract contains promising bioactive constituents that could serve as lead molecules for the development of novel anticancer agents.

Crude extracts often contain complex mixtures of compounds that may act synergistically to produce biological effects. Therefore, further studies involving bioassay-guided fractionation and isolation of active compounds are necessary to identify the specific constituents responsible for the observed cytotoxic activity.

The findings of the present study demonstrate that the methanolic extract of *Muntingia calabura* root bark possesses significant cytotoxic activity against MCF-7 breast cancer cells and induces morphological changes associated with apoptotic cell death. These results support the traditional medicinal value of the plant and highlight its potential as a source of natural anticancer compounds.

5.0 CONCLUSION

The present study demonstrated that the methanolic extract of *Muntingia calabura* root bark exhibits significant cytotoxic activity against MCF-7 breast cancer cells, as evidenced by the MTT assay. The extract produced a dose-dependent reduction in cell viability and a corresponding increase in cell inhibition, indicating its ability to suppress cancer cell proliferation. Microscopic examination further revealed characteristic morphological changes associated with apoptotic cell death, including cell shrinkage, rounding of cells, membrane blebbing, and detachment from the culture surface.

The calculated IC₅₀ value of the methanolic extract suggests moderate anticancer potential, although its activity was comparatively lower than that of the standard anticancer drug doxorubicin. These findings indicate that the root bark of *Muntingia calabura* contains bioactive phytoconstituents, possibly flavonoids and phenolic compounds, which may contribute to its cytotoxic activity.

The results of the present investigation support the potential of *Muntingia calabura* as a promising natural source of anticancer agents. However, further studies involving bioassay-guided fractionation, isolation of active compounds, and mechanistic investigations are required to identify the specific constituents responsible for the observed anticancer activity and to evaluate their therapeutic

potential.

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CONFLICT OF INTEREST:

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

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