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Investigating the Effects of the Ethyl Acetate Fraction of Mas Banana Bracts: Suppressing Cell Growth, Promoting Cell Death, and Targeting Specific Molecules in Hela Cells

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ABSTRACT

The leading cause of cancer-related deaths among women worldwide is cervical cancer, a prevalent malignant illness that mostly affects women. The development of plant-based natural medications has received particular attention in the field of complementary therapies in recent years. Phytochemicals obtained from plants are intriguing candidates for cancer treatment and prevention because they can impede the processes that cause cancer growth. The purpose of this investigation was to determine the anticancer activity of ethyl acetate fraction of mas banana bract (EAMB) against HeLa cervical cancer cells. The antproliferative, and apoptotic effects of EAMB was evaluated by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay, and Flow cytometry, respectively. The levels of Epidermal Growth Factor Receptor (EGFR), retinoblastoma protein (pRb), Ki-67, and Caspase-3 were measured by enzyme linked immunosorbent assay (ELISA). The MTT assay showed that EAMB significantly reduced HeLa cell viability, with a 59.12% decrease at 887.7 µl/mL EAMB combined with 41.6 µl/mL cisplatin after 72 hours of incubation. Flow cytometry revealed that EAMB increased late apoptosis up to 34.47% at 1775.4 µl/mL. EAMB treatment decreased EGFR levels, with a 462.9 pg/mL reduction in the combination treatment group, and elevated Caspase 3 levels, reaching 180.2 pg/mL at 443.8 µl/mL after 24 hours. These results demonstrate that EAMB inhibits cell proliferation, reduces EGFR and Ki-67 expression, and enhances apoptosis, suggesting its potential as a therapeutic agent for cervical cancer.

***Keywords*:** Anticancer, Apoptosis, Cervical cancer, Mas banana bracts, Proliferation.

Introduction

Cervical cancer is a prevalent form of cancer that affects women globally. In 2020, the WHO-International Agency for Research on Cancer (IARC) reported a significant number of new instances of cervical cancer, with 604,127 diagnoses, and an annual mortality rate of 341,831. Cervical cancer is a prominent cause of cancer-related mortality among women, with the majority of instances being recorded in underdeveloped nations[1]. Cervical cancer has a significant prevalence in Indonesia. Based on the IARC predictions in 2020, cervical cancer is the second most prevalent cancer in women, with an annual incidence of 36,633 new cases and a fatality rate of 21,033.[1,2] Cervical cancer is typically managed through a combination of surgical procedures, radiation therapy, and medications, including hormone therapy, chemotherapy, and targeted biological therapy. However, the rapid emergence of resistance to chemotherapy underscores the need for investigating alternative and effective treatments for this condition.

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Given the limited success in developing targeted anticancer medications, the use of natural substances in alternative treatments has gained significant importance.[3,4] Dysregulation of cell cycle progression is a hallmark of cancer, and targeting this process with plant-based compounds presents a promising treatment strategy. Flavonoids, polyphenols, and alkaloids have demonstrated the ability to inhibit cell cycle progression by modulating key regulatory proteins. These compounds can induce cell cycle arrest, inhibit proliferation, and promote apoptosis in cancer cells. Natural products are potential therapeutic agents for cancer due to their ready availability, low cost, and minimal harm to normal cells. Combining phytochemicals and their metabolites from plants with conventional anticancer drugs could offer a novel and highly effective therapeutic strategy for better managing and treating cancer.[5]

Mas Banana (*Musa acuminata colla*) is a highly cultivated and consumed food crop renowned for its delectable taste, rich nutritional content, and beneficial effects on health. However, banana bracts are frequently overlooked and seen as agricultural byproducts, typically used as animal fodder.[6,7] The banana bract contains a diverse range of bioactive chemicals that have considerable promise. Recent research suggests that banana bracts have biological advantages, including their

ability to act as antioxidants, antidiabetic agents, anti-inflammatory agents, and anticholesterolemic agents.[8–10]

The banana bract is a rich reservoir of bioactive chemicals. The banana bract extracts have exhibited antioxidant properties, and its anthocyanin concentration has been found to be an excellent source of natural food coloring derived from plants. The secondary compounds found in banana bracts include polyphenols, triterpenes, and sterols.[10] Studies suggest that the banana flower shows promise for use in pharmaceuticals, cosmetics, and food goods.[11,12]

The antioxidant properties of anthocyanins found in banana bracts can effectively prevent neoplastic processes. A study conducted by Roobha investigated the effects of anthocyanins from methanol extracts of banana bract on breast cancer cells (MCF-7). The results showed that these anthocyanins effectively inhibited the growth of MCF-7 cells.[13] The objective of this study is to investigate the potential of Mas banana (*Musa acuminata colla*) bracts in inhibiting the growth of cancer cells, inducing programmed cell death, and targeting specific molecular markers in HeLa cells.

Materials and Methods

Chemicals/Reagents

The materials used in the research included Annexin-V (BioLegend), dimethyl sulfoxide (Merck), ethanol (Merck), ethyl acetate (Merck), n-hexane (Merck), propidium iodide (BioLegend), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (Sigma), and ELISA kits (ABclonal).

Plant Collection and Identification

Mas banana bract was sourced from the Tanggamus region in the Lampung Province of Indonesia (104°18'-105°12' East Longitude and 5°05'-5°56' South Latitude). The harvesting of mas banana Bracts took place between December 2023 and January 2024. After a span of two weeks, the plants exhibited the emergence of flowers. The banana bract was identified by Dra. Yulianty, M.Si of the University of Lampung. The external purple-hued section of the mas banana bract was thereafter gathered, purified, fragmented, and subsequently dehydrated in a Memmert UN30 Universal Oven, produced by Memmert GmbH + Co. KG, Germany, at a temperature of 50ºC.

Preparation of fraction of mas banana bracts

The extraction process involved producing an extract from 1000 grams of powdered mas banana bracts through successive maceration steps, initially immersing the finely ground plant material in 5000 milliliters of ethanol for 72 hours. After this first 3-day maceration, the mixture was filtered using a vacuum-assisted Büchner funnel to separate the solid residue from the liquid phase. The liquid extract (macerate) was then concentrated by rotary evaporation to obtain a semi-thick extract. The residual ethanol from the evaporation process was reused to macerate the plant material for an additional 3 days. After the second maceration, the mixture was filtered again using the same vacuum filtration method, followed by further concentration of the macerate using a rotary evaporator to yield a moderately thick extract. The ethanol remaining after evaporation was utilized for a third round of maceration, again lasting 72 hours. Upon completion of the third maceration, the mixture was filtered, and the resulting macerate was evaporated using a rotary evaporator set at 50°C to obtain the final concentrated extract. The first, second, and third fairly dense extracts were combined in a porcelain dish and evaporated in a water bath to produce a concentrated extract. Subsequently, the highly concentrated extract was diluted in distilled water, subjected to extraction with n-hexane, and subsequently separated using a separating funnel. The fraction that was not soluble in n-hexane was subjected to fractionation using ethyl acetate and subsequently separated using a separating funnel. The ethyl acetate-soluble extract was evaporated using a rotary evaporator to obtain a somewhat viscous fraction. The moderately viscous portion was subjected to evaporation in a water bath to isolate the ethyl acetate component of the mas banana bract extract.

Cell Culture

HeLa cells were obtained from the Parasitology Laboratory of the Faculty of Medicine, Public Health, and Nursing at Gadjah Mada University, Yogyakarta. The HeLa cells were cultured in Roswell Park Memorial Institute (RPMI) medium with 10% fetal bovine serum, 1% non-essential amino acids, 1% sodium pyruvate, and 1% penicillin-streptomycin (Gibco, USA). The cells were kept in a 5% CO_2 incubator at 37°C.[14]

Cell proliferation assays

During the MTT experiment, the cells were seeded in 96-well plates at a density of 10^4 cells per well and then incubated. The cells of the same density were evenly divided among three 96-well microplates and left to incubate overnight at a temperature of 37°C in an incubator with 5% CO_2. The cells on the plate were exposed to different concentrations of EAMB and cisplatin for 24, 48, and 72 hours at a temperature of 37°C in a 5% CO_2 incubator. The media was extracted prior to the cells being re-incubated. The cells were allowed to attach and proliferate during the designated incubation period. Following the period of incubation, a solution containing 0.5 milligrams per milliliter of MTT was introduced into each well. Subsequently, the plate was incubated for a duration of 4 hours at a temperature of 37ºC. Following a four-hour period, the cells were subjected to a solution containing 10% SDS (Sigma) in 0.01N HCl (Merck) to break down the formazan crystals. The cells were incubated for 24 hours at room temperature in a dimly lit setting. Ultimately, the absorbance was quantified at a specific wavelength of 595 nm using a microplate reader (iMark manufactured by Bio-Rad Laboratories Inc, USA). The data collected from each well were converted into the ratio of viable cells.[15]

Cell apoptosis assay

The HeLa cells (500,000 cells/mL) were inoculated into a 6-well plate after being incubated at 37°C in 5% CO_2 for 24 hours. The media was placed in the conical tube and flushed with PBS (Phosphate Buffer of Standard). The cells were then incubated at 37°C in 5% CO_2 for 24 hours before being treated with EAMB and cisplatin at varying concentrations. The cells were collected into labelled conical tubes after being treated with 0.025% trypsin. rinsed with PBS and subsequently collected in the labelled conical tube. Sediment was collected from the media after centrifugation at 2.500 rpm for 5 minutes, and the supernatant was discarded. The cells were subsequently stained with PI and annexin V-FITC in the dark for 10 minutes. Thereafter, the cells were subjected to flow cytometry (FACSAria III manufactured by BD Biosciences, USA) at wavelengths of 488 and 530 nm.[16]

Measurements of EGFR, pRb, Ki-67, and Caspase-3 levels

The EAMB and cisplatin were incubated with the HeLa cells at variable concentrations for 24, 48, and 72 hours. A commercial reagent (ABclonan) and an ELISA reader (iMark manufactured by Bio-Rad Laboratories Inc, USA) were employed to measure the levels of EGFR (Cat. No: RK00274), pRb (Cat. No: RK11687), Ki-67 (Cat. No: RK04300), and Caspase-3 (Cat. No: RK04498) at 405 nm.

Statistical analysis

The SPSS software (IBM SPSS Statistics 26 manufactured by IBM Corporation, 2019) was utilized for data analysis. The findings are displayed as the mean ± the standard deviation (SD). The distinctions between various groups were assessed using Two-way analysis of variance (ANOVA), with a significance level of $p < 0.05$.

Results and Discussion

EAMB treatment inhibited Epidermal Growth Factor Receptor (EGFR)-stimulated proliferation in HeLa cells by suppressing Ki-67 and promoting Retinoblastoma protein (pRb) levels.

An optimal approach to managing intricate illnesses like cancer involves targeting multiple factors simultaneously. Discovering prospective natural chemicals with low toxicity in normal healthy cells that can effectively inhibit the growth and progression of cancer cells has become a crucial necessity in cancer treatment.[17] All plant extracts or fractions from plant elements have been employed as medicine,

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including isolates. In vitro and in vivo studies have demonstrated that a variety of alkaloids and flavonoids from medicinal plants have antiproliferative and anticancer properties in a variety of cancer types.[18] The antiproliferative activity of mas banana bracts fractions against HeLa cells were presented in Figure 1. Viable cells of HeLa were significantly lower after 72 hours incubation with 3550.8 µl/mL EAMB, 1775.4 µl/mL EAMB, 887.7 µl/mL EAMB, 443.8 µl/mL EAMB, 41.6 µl/mL Cisplatin, and combination 887.7 µl/mL EAMB with 41.6 µl/mL Cisplatin (16.45±2.63%, 35.83±8.11%, 24.57±4.14%, 66.75±1.49%, 3.39±2.31%, 2.14±2.49%, respectively) compared to HeLa cells after 24 hours incubation with the same concentration (18.11±0.51, 22.07±1.37, 83.01±3.71, 92.02±5.55, 45.25±4.24, 61.26±4.62, respectively), p-value 0.003 in all comparisons.

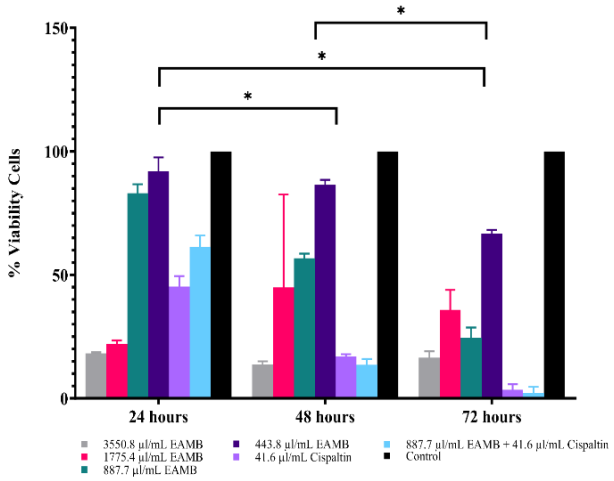

Figure 1: The proliferation analysis of HeLa cells after 24 ,48 and 72 hours incubation with mas banana bracts fractions. Data are presented as means ± SD. Two-way ANOVA were significant at p =0.001 among all incubation time groups; *p <0.05 between each incubation time group.

The most significant decrease in viable cells was observed after 72 hours incubation with combination 887.7 µl/mL EAMB with 41.6 µl/mL Cisplatin (59.12%), while the smallest decrease was observed after 72 hours incubation with 443.8 µl/mL EAMB (25.27%). Significantly lower viable cells after 72 hours incubation with a concentration of 887.7 µl/mL EAMB with 41.6 µl/mL Cisplatin were observed after treatment (2.14±2.49%) than the other concentration with p-value 0.001. The results of the two-way ANOVA analysis (Table 1) indicate a statistically significant difference between the treatment groups ($p < 0.001$), suggesting that both the variation in EAMB dosage and the combination treatment with Cisplatin have a significant impact on HeLa cell proliferation.

Table 1: The analysis of proliferation and EGFR levels after EAMB treatment in each group

Treatment	Proliferation Mean ± SD	EGFR Levels Mean ± SD
Control Cell	100 ± 0.00	837.6 ± 240.5
3550.8 µl/mL EAMB	16.10 ± 2.42	831.5 ± 226.2
1775.4 µl/mL EAMB	34.27 ± 21.67	1270.8 ± 513.9
887.7 µl/mL EAMB	54.76 ± 25.51	1887.5 ± 1072.5
443.8 µl/mL EAMB	81.73 ± 11.89	1697.6 ± 684.5
41.6 µl/mL Cisplatin	21.89 ± 18.65	968.1 ± 445.1
887.7 µl/mL EAMB with 41.6 µl/mL Cisplatin	25.65 ± 27.30	354.3 ± 84.8
Two-way ANOVA	**<0.001**	**<0.001**

EAMB at higher concentrations, specifically 1775.4 µl/mL and 3550.8 µl/mL, exhibited a strong antiproliferative effect, indicating its potential as an effective anticancer agent. Cisplatin, while effective, showed an antiproliferative effect comparable to that of high concentrations of EAMB, suggesting that EAMB could serve as a potential alternative or complementary agent. Furthermore, the combination of EAMB and Cisplatin may produce a synergistic effect, though the results show considerable variation (high SD). Therefore, further research is needed to ensure the consistency and effectiveness of this combination and to explore the potential for clearer synergy between these two agents. The Epidermal Growth Factor Receptor (EGFR) is a specific type of receptor for growth factors that plays a crucial role in regulating cell development and differentiation. In cancer, such as HeLa cells (which are human cervical cancer cells), the activity of EGFR is frequently elevated, resulting in unregulated cell proliferation. Measuring EGFR levels in HeLa cells in the context of cervical cancer research is crucial for assessing possible therapeutic targets that can impede EGFR function, therefore impeding the proliferation of cancer cells.[19,20] The ELISA approach was employed to ascertain the EGFR levels. The analysis of the three observation times revealed distinct patterns in the EGFR response to the administered therapies, as presented in Figure 2.

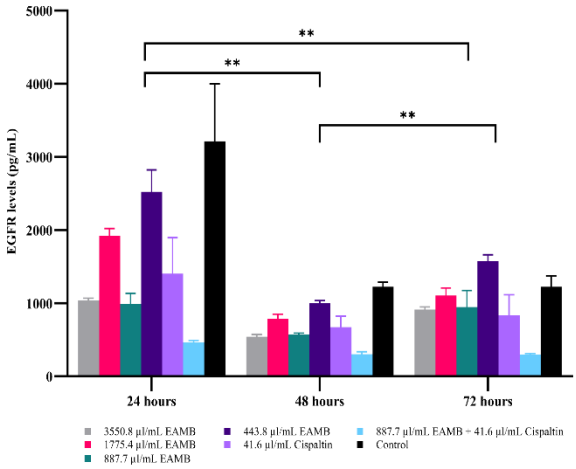

Figure 2: The EGFR levels of HeLa cells after incubation with mas banana bracts fractions for 24, 48, and 72 hours. Data are presented as means ± SD. Two-way ANOVA were significant at p <0.001 among all incubation time groups; **p <0.001 between each incubation time group.

After 24 hours, the control group had the highest average level of EGFR at 3213.6 pg/mL, while the treatment group with 443.8 µl/mL EAMB had an average of 2520.7 pg/mL. The combination treatment of 887.7 µl/mL EAMB with 41.6 µl/mL Cisplatin had the lowest average EGFR level, measuring at 462.9 pg/mL. Following a span of 48 hours, a decline in the mean EGFR level was detected in nearly all of the groups. The control group exhibited the highest mean value, however it declined to 1222.5 pg/mL. The combination treatment group, which previously had the lowest average, also observed a reduction in the average EGFR level. After 72 hours, noticeable variations were detected in the mean EGFR levels among all the groups. The group receiving the combination treatment demonstrated a remarkably low average of 297.6 pg/mL, which was statistically significant. Statistical analysis using the Two-way ANOVA test showed significant differences between the incubation time groups ($p < 0.001$). Additionally, significant differences

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were observed among the varying doses of EAMB and cisplatin when compared to each other after 24, 48, or 72 hours, with a p-value <0.001. The results of the two-way ANOVA analysis (Table 1) indicate a statistically significant difference between the treatment groups ($p < 0.001$), suggesting that both the variation in EAMB dosage and the combination treatment with Cisplatin significantly affect EGFR levels in HeLa cells. EAMB at the highest concentration (3550.8 µl/mL) showed EGFR levels similar to the control group (831.5 ± 226.2 vs. 837.6 ± 240.5), while lower concentrations of EAMB (1775.4 µl/mL, 887.7 µl/mL, and 443.8 µl/mL) resulted in a dose-dependent increase in EGFR levels. Notably, the combination of EAMB (887.7 µl/mL) with Cisplatin (41.6 µl/mL) produced the most pronounced reduction in EGFR levels (354.3 ± 84.8), suggesting a potential synergistic effect in downregulating EGFR expression. These findings highlight the potential of EAMB, particularly in combination with Cisplatin, to modulate EGFR levels, which may be critical in cancer cell signaling pathways. However, further investigation is required to confirm the consistency and therapeutic relevance of these effects. The ethyl acetate fraction of mas banana bracts had 1.0 ± 0.2 mg/g of total anthocyanins, according to a previous preliminary investigation. Further analysis revealed that the ethyl acetate fraction of mas banana bracts has a total flavonoid content of 74.2 ± 5.0 mg/g quercetin equivalent. Two flavonoid compounds were detected in the LCMS/MS QTOF chromatogram analysis: afzelechin and moracenin.[21] The research findings suggest that the ethyl acetate fraction derived from the bracts of the mas banana has a notable influence on the growth of HeLa cells. The ethyl acetate fraction derived from the mas banana bracts exhibited a notable inhibitory effect on the growth of HeLa cells in cell proliferation experiments. Roobha conducted an in vitro study on breast cancer cells (MCF-7) that demonstrated that anthocyanins from the methanol extract of the banana bracts suppressed MCF-7 cell proliferation. This is consistent with their findings.[13] Additionally, the ethyl acetate fraction of the mas banana bracts contains the compound afzelechin, which has the potential to act as an anti-cancer agent. Ragab conducted a study that showed the compound afzelechin from the leaves of *Ficus sprague*ana to inhibit the proliferative activity of liver cancer cells (HepG2).[22]

Epidermal Growth Factor Receptor is a protein that spans the cell membrane and is responsible for transmitting signals within the cell. It is engaged in multiple cellular pathways and has a vital function in controlling cell growth, specialization, replication, and viability. EGFR overexpression or activation has been linked to the formation of many malignancies, such as lung cancer, head and neck cancer, and cervical cancer.[23,24] Anthocyanins exert an anticancer impact by inhibiting the activity of EGFR through the disruption of the PI3K/Akt signaling pathway. This pathway plays a crucial role in the survival and proliferation of cancer cells.[25,26]

Elevated EGFR activity, a common occurrence in cancer, can disrupt the equilibrium of pRb activity. Upregulation of EGFR leads to increased phosphorylation of pRb, resulting in decreased inhibition of cell proliferation and enabling uncontrolled division of cancer cells.[27,28] Elevated EGFR levels and the deactivation of pRb can lead to the stimulation of Ki-67. Ki-67 is present in the active phases of the cell cycle, and its levels of expression indicate the rate at which cells are dividing. In the absence of functioning pRb, cancer cells can undergo uncontrolled cell division and enter the cell cycle, resulting in elevated production of Ki-67. Ki-67 activation indicates a significant increase in cell proliferation in cervical cancer. Cancer cells that divide quickly are typically more aggressive, which raises the likelihood of metastatic spread and suggests a negative prognosis.[29,30] In the study conducted by Bai, the expression of EGFR was markedly reduced in cells that were treated with Ki-67 siRNA, as compared to the control group. These findings demonstrate a direct relationship between Ki-67 and EGFR, implying that Ki-67 can control the growth of HeLa cells by influencing the EGFR signaling pathway. Table 2, Figure 3 and 4 shows the levels of pRb and Ki-67 activity in HeLa cells treated with different fractions of mas banana bracts.

The results of the two-way ANOVA analysis (Table 2) indicate a statistically significant difference between the treatment groups for both pRb levels ($p < 0.001$) and Ki-67 levels ($p < 0.023$), suggesting that the variation in EAMB dosage and its combination with Cisplatin affect these key markers in HeLa cells. For pRb levels, EAMB treatments across different concentrations (443.8 µl/mL to 3550.8 µl/mL) resulted in values that remained close to the control group (191.9 ± 0.9), with a slight increase at the highest concentration (193.2 ± 0.4) and the combination with Cisplatin showing the highest level (198.4 ± 2.1). This indicates minimal changes in pRb expression under most conditions, except when combined with Cisplatin, which showed a more noticeable increase. For Ki-67 levels, which reflect cell proliferation, the values across all EAMB concentrations were also similar to the control group (220.8 ± 1.6), with slight variations.

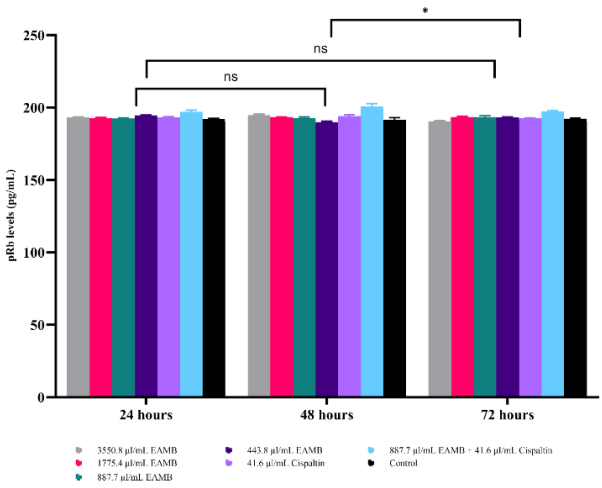

Figure 3: The pRb levels of HeLa cells after incubation with mas banana bracts fractions for 24, 48, and 72 hours. Data are presented as means ± SD. Two-way ANOVA were significant at $p = 0.030$ among all incubation time groups; *$p < 0.05$ compare between 48 hours and 72 hours incubation duration; ns $p \geq 0.05$.

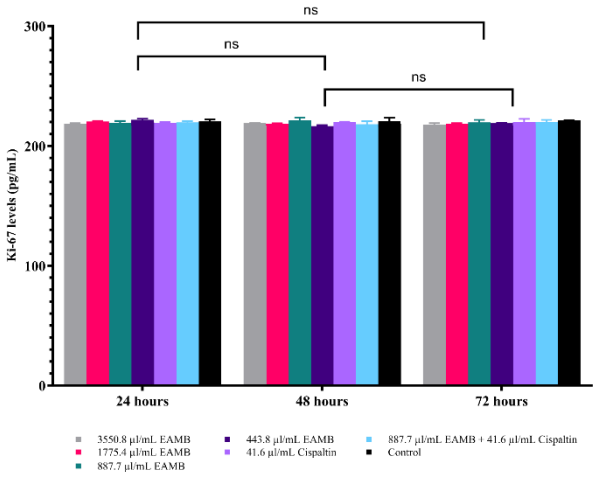

Figure 4: The Ki-67 levels of HeLa cells after incubation with mas banana bracts fractions for 24, 48, and 72 hours. Data are presented as means ± SD. Two-way ANOVA were not significant at $p > 0.05$ among all incubation time groups; ns $p \geq 0.05$ between each incubation time group.

However, the combination of EAMB (887.7 µl/mL) and Cisplatin showed no significant decrease in Ki-67 levels (219.3 ± 1.8), suggesting that EAMB alone or in combination has a limited impact on this particular proliferation marker. These findings suggest that while EAMB has some influence on pRb levels, particularly in combination with Cisplatin, its effect on Ki-67 remains minimal. Further studies may be needed to explore other markers or signaling pathways to fully understand the mechanisms through which EAMB exerts its potential anticancer effects.

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EAMB treatment induced apoptosis in HeLa cells by increasing caspase 3 levels.

The flow cytometry method was utilized to intentionally determine apoptosis in order to quickly calculate the number of living cells, cell necrosis, and apoptosis. In order to bind phosphatidylserine situated in the cell plasma membrane during fluorescence apoptosis, Annexin V was administered to cancer cells.[31] As seen in Table 3 and Figure 5, HeLa cells were administered with 1775.4 µl/mL EAMB, resulting in early apoptosis of 1.73%, late apoptosis of 34.47%, and cells necrosis

Table 2: The analysis of pRb and Ki-67 levels after EAMB treatment in each group

Treatment	pRb Levels Mean ± SD	Ki-67 Levels Mean ± SD
Control Cell	191.9 ± 0.9	220.8 ± 1.6
3550.8 µl/mL EAMB	192.9 ± 1.9	218.3 ± 1.0
1775.4 µl/mL EAMB	193.2 ± 0.4	219.1 ± 0.9
887.7 µl/mL EAMB	192.9 ± 0.7	220.1 ± 1.9
443.8 µl/mL EAMB	192.5 ± 2.0	219.1 ± 2.3
41.6 µl/mL Cisplatin	193.4 ± 08	219.7 ± 1.4
887.7 µl/mL EAMB with 41.6 µl/mL Cisplatin	198.4 ± 2.1	219.3 ± 1.8
Two-way ANOVA	**<0.001**	**<0.023**

of 11.83% compared to cell control, which was not treated with EAMB in the early apoptosis with 0.77%, which is lower than treated with EAMB. It indicates that the ethyl acetate fraction of mas banana bract can be used to promote apoptosis.

The ethyl acetate fraction of mas banana bracts has the potential to induce apoptosis. Based on our previous research, the phytochemical screening revealed that the ethyl acetate fraction contains antioxidants, anthocyanins, flavonoids, afzelechin, and moracin D. These bioactive compounds function as anticancer agents by initiating apoptosis pathways. El-Nashar discovered that the combination therapy of Pitaya extract with cisplatin on HeLa cells effectively enhances treatment efficacy by leveraging apoptosis using flow cytometry analysis. In that investigation, flavonoids, phenolic acids, anthocyanins, lignans, stilbenes, and coumarins were identified as compounds in the Pitaya extract.[32] Various varieties of plants, including grapes, blueberries, black rice, and purple yams, can be used to extract anthocyanins. In this investigation, anthocyanins were isolated from the ethyl acetate fraction of the mas banana bracts. The anticancer effect of anthocyanins on breast cancer cells is attributed to the reduction of cell viability through cytotoxic mechanisms, as demonstrated by numerous studies.[33,34]

Table 3: Apoptosis analysis of ethyl acetate fraction of mas banana bract (EAMB)

Treatment	LC[a]	EA[b]	LA[c]	CN[d]
Control Cell	94.23	0.77	3.30	0.40
3550.8 µl/mL EAMB	3.00	0.73	24.57	7.90
1775.4 µl/mL EAMB	4.40	1.73	34.47	11.83
887.7 µl/mL EAMB	45.17	2.07	12.23	5.67
443.8 µl/mL EAMB	72.67	1.73	6.77	4.33
41.6 µl/mL Cisplatin	81.27	3.53	2.80	0.73
887.7 µl/mL EAMB with 41.6 µl/mL Cisplatin	54.70	2.93	9.63	4.83

[a]Life Cell, [b]Early Apoptosis, [c]Late Apoptosis, [d]Cells Necrosis.

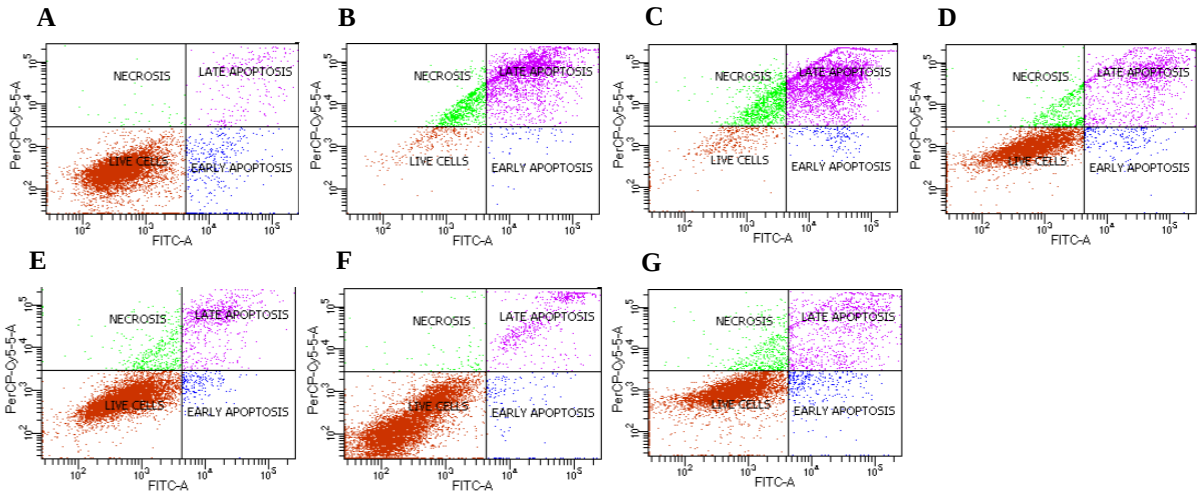

Figure 5: Flow cytometry analysis of apoptosis of HeLa cells: **A.** Control cell; **B.** Treatment with 3550.8 µl/mL EAMB; **C.** Treatment with 1775.4 µl/mL EAMB; **D.** Treatment with 887.7 µl/mL EAMB; **E.** Treatment with 443.8 µl/mL EAMB; **F.** Treatment with 41.6 µl/mL Cisplatin; **G.** Treatment combination of 887.7 µl/mL EAMB and 41.6 µl/mL Cisplatin

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Natural compounds demonstrate anticancer properties by inhibiting DNA damage and promoting DNA repair processes. It is well-established that the apoptosis pathway is the mechanism by which DNA damage induces cytotoxic effects for cell demise.[35]

Quantifying the amounts of Caspase 3 in HeLa cells can be used as a reliable measure of the activity of apoptosis. The ELISA approach was employed to quantify the levels of Caspase 3 in this study. According to Table 4 and Figure 6, after 24 hours of incubation, the concentration of caspase-3 was highest in the 443.8 µl/mL EAMB treatment, measuring at 180.2 pg/mL. The Caspase 3 levels were highest at 48 and 72 hours of incubation in the combined treatment of 887.7 µl/mL EAMB with 41.6 µl/mL Cisplatin, with values of 174.3 pg/mL and 170.3 pg/mL, respectively. The examination of caspase 3 levels in HeLa cells over the incubation periods of 24, 48, and 72 hours using Two-Way ANOVA revealed a notable disparity in the average Caspase 3 levels among all incubation time groups ($p = 0.022$). Furthermore, the pairwise comparison conducted using Two-Way ANOVA revealed a statistically significant p-value (0.035), showing a substantial disparity in the average Caspase 3 levels among the various treatment groups.

Table 4: The analysis of caspase-3 levels after EAMB treatment in each group

Treatment	Caspase-3 Levels Mean ± SD
Control Cell	168.5 ± 6.7
3550.8 µl/mL EAMB	170.9 ± 6.4
1775.4 µl/mL EAMB	166.6 ± 4.1
887.7 µl/mL EAMB	165.1 ± 3.6
443.8 µl/mL EAMB	168.2 ± 10.7
41.6 µl/mL Cisplatin	166.2 ± 1.8
887.7 µl/mL EAMB with 41.6 µl/mL Cisplatin	170.8 ± 4.4
Two-way ANOVA	**0.035**

The research findings suggest that the ethyl acetate fraction derived from the bracts of the Mas banana has a notable influence on the growth of HeLa cells. Research has demonstrated that the ethyl acetate fraction derived from the bracts of the Mas banana is capable of inducing apoptosis in HeLa cells. This is supported by the heightened activity of caspase-3, a protein that plays a role in the process of programmed cell death known as apoptosis. Based on our previous investigation, we have found that the ethyl acetate fraction has been proven to contain antioxidants, anthocyanins, flavonoids, afzelechin, and moracin D as determined by phytochemical screening. These bioactive compounds function as anticancer drugs by activating apoptotic pathways. In a study conducted Jang, black soybean extract containing isoflavones and anthocyanins was administered to a rat model with prostate hyperplasia. The study found that the extract led to a decrease in cell proliferation, levels of caspase-3, and reactive oxygen species (ROS).[36] In a study conducted by Natarajan the objective was to examine the anticancer properties of Idaein chloride (Cyanidin) on HeLa cells. The study revealed that Idaein chloride led to enhanced activation of the apoptotic pathway by reducing the expression of Bcl-2 and subsequently boosting the regulation of BAX and cytochrome C.[37] Furthermore, this also resulted in an elevation in the levels of caspase-3 and caspase-9 expression. Anthocyanins have the ability to trigger programmed cell death, known as apoptosis, in cancer cells by activating caspases through the involvement of reactive oxygen species (ROS) and JNK/p38-MAPK pathways. Anthocyanins have the ability to trigger apoptosis in cancer cells by activating AMPK and inhibiting the Akt protein, both of which play a vital role in the apoptosis process. Activation of AMPK and inhibition of Akt can result in the increase of pro-apoptotic proteins and the suppression of anti-apoptotic proteins. This leads to the liberation of cytochrome C from the mitochondria, leading to the fragmentation of caspase-3 and initiation of apoptosis in cancer cells.[25,38] The discovery has significant ramifications for the advancement of cervical cancer treatment. The ethyl acetate fraction derived from the bracts of the mas banana exhibits promise as a strong anticancer drug, acting through processes that involve suppressing cell growth and promoting programmed cell death.

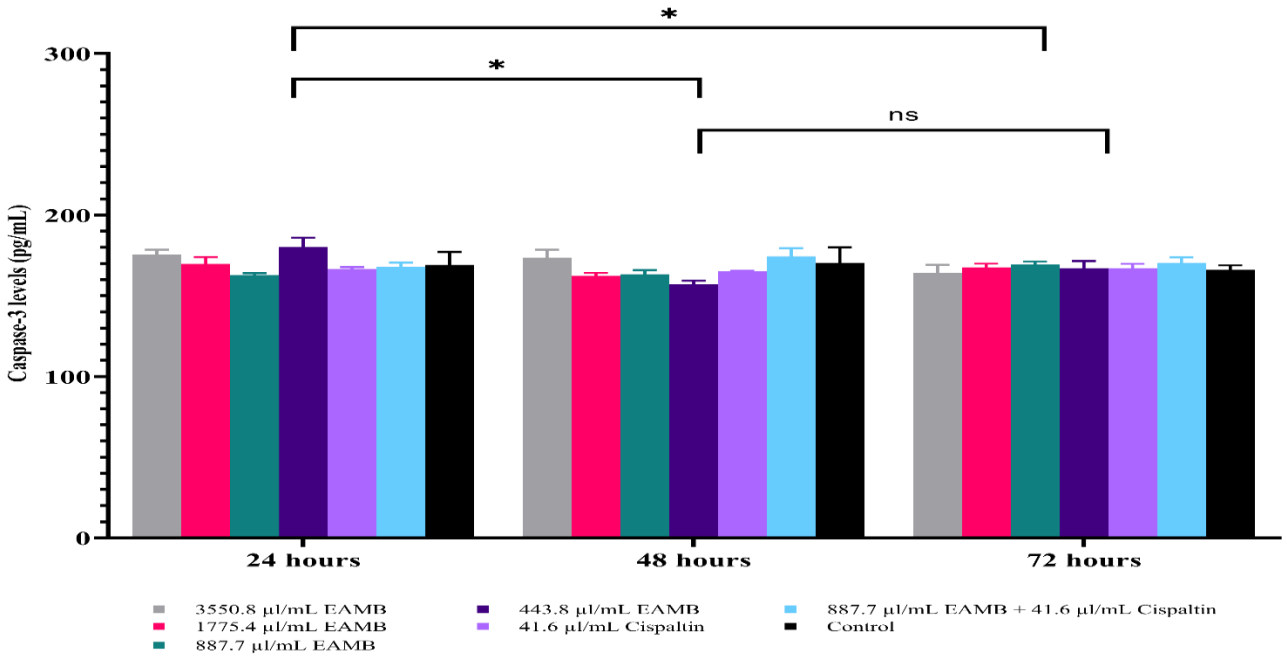

Figure 6: The caspase-3 levels of HeLa cells after incubation with mas banana bracts fractions for 24, 48, and 72 hours. Data are presented as means ± SD. Two-way ANOVA were significant at $p = 0.022$ among all incubation time groups; *$p < 0.05$ when comparing the incubation durations of 24 hours to 48 hours and 24 hours to 72 hours; ns $p \geq 0.05$.

Conclusion

The study findings demonstrate that the ethyl acetate fraction derived from the bracts of the mas banana possesses anticancer properties. This fraction effectively lowers the levels of EGFR and Ki-67, while increasing the levels of pRb and Caspase 3. Consequently, it inhibits cancer cell growth and promotes cancer cell death.

Conflict of Interest

The authors stated that there were no conflicts of interest in relation to the publication of this paper.

Authors' Declaration

The authors hereby declare that the work presented in this article is original, and any liability for claims relating to the content of this article will be borne by them.

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