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Research Article

Cytotoxicity and Antiproliferative Activities of Ethanolic Peel Extract of *Pyrus malus* L. on T47D Cells

Aktivitas Sitotoksik dan Antiproliferatif Ekstrak Etanol Kulit Pyrus malus L. *pada Sel T47D*

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ABSTRACT
Quercetin, a flavonoid compound in green apple peel (*Pyrus malus* L.), has shown anticancer potential in T47D cells. This study evaluated the cytotoxic effects and antiproliferative activities demonstrated by the Ethanolic Extract of Green Apple Peel (EEGAP) on T47D cells. The peel powder was extracted using 70% ethanol with ultrasonic waves. The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was performed for the cytotoxicity test using EEGAP concentrations of 31.25, 62.5, 125, 250, 500, and 1,000 µg/mL. The IC_{50} value was determined using probit analysis. Based on the IC_{50} results, an antiproliferative assay was conducted using the doubling time technique at concentrations of ½IC_{50} (404.5 µg/mL) and IC_{50} (809 µg/mL), which were observed at 0, 24, 48, and 72 hours. EEGAP has a moderate cytotoxic effect, with an IC_{50} value of 809 µg/mL. EEGAP showed significant antiproliferative activity, with a doubling time of 14 hours in control samples. EEGAP at a concentration of ½ IC50 showed a replication period of 21 hours, whereas the IC_{50} concentration replication period was 30 hours. These results suggest that EEGAP exhibits cytotoxic and antiproliferative properties, which may have potential as a natural compound for breast cancer therapy.

Keywords*:* Antiproliferative, Cytotoxicity, Green Apple Peel, T47D, Ultrasonic

ABSTRAK
Quersetin, senyawa flavonoid yang terdapat pada kulit apel hijau (Pyrus malus L.*), menunjukkan potensi antikanker terhadap sel T47D. Penelitian ini mengevaluasi efek sitotoksik dan aktivitas antiproliferasi yang ditunjukkan oleh Ekstrak Etanol dari Kulit Apel Hijau (EEGAP) terhadap pada sel T47D. Serbuk kulit diekstraksi menggunakan etanol 70% menggunakan gelombang ultrasonik. Metode 3-(4,5-dimetiltiazol-2-il)-2,5-difeniltetrazolium bromida (MTT) diaplikasikan untuk menguji sitotoksisitas, dengan ekstraksi sampel menggunakan bantuan ultrasonik pada berbagai konsentrasi EEGAP sebesar 31,25; 62,5; 125; 250; 500 dan 1.000 µg/mL. Nilai* IC_{50} *ditentukan menggunakan analisis probit. Berdasarkan hasil* IC_{50}*, uji antiproliferatif dengan teknik doubling time pada konsentrasi* ½IC_{50} *(404,5 µg/mL) dan* IC_{50} *(809 µg/mL), yang diamati pada jam ke-0, 24, 48 dan 72. EEGAP memiliki efek sitotoksik sedang, dengan nilai* IC_{50} *sebesar 809 µg/mL. EEGAP menunjukkan aktivitas antiproliferatif yang signifikan, dengan nilai doubling time 14 jam pada sel kontrol. EEGAP pada konsentrasi* ½IC_{50} *menunjukkan nilai waktu ganda adalah 21 jam, sementara konsentrasi* IC_{50} *sebesar 30 jam. Hasil ini menunjukkan bahwa EEGAP memiliki sifat sitotoksik dan antiproliferatif, sehingga berpotensi menjadi senyawa alami untuk terapi kanker payudara.*

Kata Kunci: *Antiproliferatif, Kulit Apel Hijau, Sitotoksisitas, T47D, Ultrasonik*

INTRODUCTION

Globally, cancer, particularly breast cancer, ranks among the most significant contributors to mortality (1). In 2022, as many as 11.6% of newly detected tumour cases in women were breast carcinoma, with a fatality rate of 6.9%. This malignancy remains the predominant form of neoplasm and the principal determinant of mortality from cancer among females (2). The occurrence of breast cancer is generally due to anomalies in the Oestrogen Receptor (ER) signalling pathway (3). The Human Breast Ductal Epithelial Carcinoma Cell Line (T47D), derived from human breast cancer cells expresses oestrogen and progesterone receptors, as well as a mutation in the p53 protein. Consequently, T47D cells are frequently used as an in vitro model for evaluating the efficacy of anticancer compounds (4).

Disease treatment can be categorised into modern and traditional medicine. Traditional medicine is chosen because it is safer, more practical, and has an empirical foundation (5). The green apple (*Pyrus malus* L.) is a plant that grows in tropical regions such as Indonesia. Green apples are widely utilised in processed products, such as canned fruit, juice, and fruit extract. However, this often results in apple peel waste, which is commonly used only as organic fertiliser. Green apple peel has many benefits, one of which is as an oncostatic compound (6).

Examination of the malignancy-suppressing activity of the *Malus* genus from various apple varieties has been extensively conducted. The 96% ethanol extract of Manalagi apple peel (*M. domestica* Mill.) contains chemical secondary metabolite groups including alkaloids, flavonoid compounds, phenolic substances, tannins, steroids, and triterpenoids (4). The peel of Rome Beauty apple (*M. sylvestris* Mill.) has a higher potential for inhibiting the proliferation of HepG2 cells compared to the flesh of the fruit (7).

Flavonoid compounds found in plants have already been proven to inhibit in both in vivo and in vitro settings. One such compound, quercetin, is a flavonoid that has a general anticancer effect, with a mechanism of action that involves inhibiting the cell replication process at the G1/S and G2/M phases in cancer cell cultures, leading to cell cycle arrest (8). Pure quercetin has potential as an anticancer agent that can inhibit cancer cell proliferation (9). Moreover, extracted Rome Beauty apples contain higher levels of quercetin compared to their fresh form, indicating that the extraction process can enhance quercetin content in apple extracts (10). Polar flavonoid compounds, including quercetin, can be extracted using 70% ethanol as a solvent (11).

Given the explanation above, the cytotoxic and antiproliferative properties of green apple peel against breast cancer cells have not yet been thoroughly documented. Accordingly, this research focuses on the assessment of the cytotoxic and antiproliferative activities of the Ethanolic Extract of Green Apple Peel (EEGAP) on T47D breast cancer cells.

Materials and Methods

Materials

Green apple peel was sourced from *Pyrus malus* L. T47D type breast cancer cells were retrieved from the Cell Culture Laboratory housed in the Faculty of Medicine and Health Sciences, Universitas Muhammadiyah Yogyakarta. The reagents used in this study included: Roswell Park Cancer Center Formula (RPMI) medium (from Gibco), at a proportion of 1% volume per volume penicillin-streptomycin (from Gibco), 0.5% volume for volume fungizone (from Gibco), a proportion of 10% volume per volume Fetal Bovine Serum (FBS, from Sigma-Aldrich), 100% Dimethyl Sulfoxide (DMSO, from Sigma-Aldrich), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) dye (from Sigma-Aldrich), a termination mix of 10% Sodium Dodecyl Sulphate in 0.01 N HCl (SDS, from Sigma-Aldrich), 0.25% trypsin-EDTA (from Sigma-Aldrich), and Phosphate Buffered Saline (PBS, from Biogear).

Methods

Collection of Green Apple Peel

Green apple peel was obtained from five-month-old green apples harvested in Batu City, Malang Regency, East Java. The botanical identification was conducted at the Faculty of Science and Mathematics, and was signed by the Head of the Ecology and Biosystematics Laboratory, Department of Biology, Diponegoro University, Semarang. The apples used were fresh, disease-free, green-coloured, and measured 3-5 cm in diameter. A total of 5.160 kg of green apples underwent wet sorting and were washed with running water. The peel was removed using a knife, yielding 770 g of peel, which was then dehydrated in a heating chamber at 40°C until completely dry. The dried peel (simplicia) was then ground into powder to produce 185 g of green apple peel powder.

Preparation of EEGAP

The ethanolic extract of green apple peel was prepared using Ultrasound-Assisted Extraction (UAE, Grant, Ultrasonic Bath XUB5, UK). Green apple peel powder weighing 185 grams was placed in a beaker, then amalgamated with 70% ethanol in a 1:10 proportion. The active substance withdrawal procedure was replicated

three times. The total volume of solvent used was 1,850 mL. The separation of compounds was carried out at 40°C with 40 kHz vibration for 30 minutes. After extraction, the solution was filtered, and the filtrate obtained was condensed via a rotating vacuum evaporation system (Heidolph, He-VAP, Germany) set to 40°C until a thick extract was obtained (12).

Preparation of T47D Cells

Cells preserved in ampoules were retrieved from liquid nitrogen storage and thawed at ambient temperature. A sample of cell suspension with a volume of 1,000 µL was transferred to a conical tube containing culture medium, then spun down at 2,500 rpm for 5 minutes at room temperature. Following removal of the supernatant, the cell pellet that developed was resuspended in 4 mL of RPMI medium. The fluid was afterwards distributed into two Tissue Culture Dishes (TCD), and each was supplemented with 5 mL of culture medium. The cultures were housed in a CO_2 incubator for the incubation process (Thermo Scientific, BB150, USA) to facilitate cell growth (13).

Preparation of Test Solutions

To conduct the cytotoxicity assay, the process of preparing the stock solution involved dissolving 10 mg of EEGAP in 100 µL of DMSO in a microtube, resulting in a final concentration of 1,000 µg/mL. Serial dilutions were then prepared to obtain concentrations of 500, 250, 125, 62.5, and 31.25 µg/mL using culture medium as the diluent. From the cytotoxicity test, the IC_{50} value was determined as the reference for antiproliferative testing. The ½IC_{50} (404.5 µg/mL) and IC_{50} (809 µg/mL) concentrations were reconstituted from the stock solution for antiproliferative assays.

Cell Harvesting

Cells at approximately 80% confluency were harvested by first removing the culture medium and washing the cells with PBS. After that, 300 µL of trypsin-EDTA with a concentration of 0.25% was added to the cell culture dish and incubated for 3 minutes. Subsequently, 3 mL of RPMI medium was added, and the cells were gently resuspended using a micropipette to avoid aggregation. After the cell suspension process was complete, the suspension was transferred to a sterilised conical tube for further analysis (13).

Cytotoxicity Assay

Harvested cells were counted, and 100 µL of a cell suspension containing $1x10^4$ cells per well was added to a 96-well plate. Three wells containing only RPMI medium were used as the control without cells. The 96-well plate was incubated in a CO_2 incubator for 24 hours. After this period, the culture medium was discarded, and the wells were rinsed with PBS. Serial dilutions of EEGAP (1,000; 500; 250; 125; 62.5; and 31.25 µg/mL) were then added to each well at a volume of 100 µL. The plate was subsequently incubated for a further 24 hours. Following incubation, cell morphology was examined under a microscope. The used medium was removed from the plate, and each well was washed with PBS solution. Add 100 µL of MTT dye (5 mg/mL) to each well. The plate was then incubated in a CO_2 incubator for 4 hours. After incubation, cells were inspected using an inverted microscope (Magnus, INVI, India), followed by the addition of 100 µL of halt reagent (SDS). The container holding the media was covered with paper or aluminium foil and kept under dark conditions at laboratory temperature overnight. The absorption reading of the specimen was assessed at 600 nm utilising an ELISA spectrophotometer (Tecan, Infinite F50, Switzerland). All tests were conducted in triplicate.

Antiproliferative Assay

Harvested and counted cells ($1x10^4$ cells per well) were plated at 100 µL per well in a 96-well plate, with three wells designated as medium controls. The cells were incubated in a CO_2 incubator for 24 hours. After the incubation process is complete, the cell growth medium is discarded, and the well is then washed with a PBS solution. EEGAP solutions at concentrations of ½IC_{50} (404.5 µg/mL) and IC_{50} (809 µg/mL) were added at 100 µL per well in triplicate. The plates were incubated in a CO_2 incubator and observed at 0, 24, 48, and 72 hours. Following each incubation period, the cell morphology was examined microscopically. After media removal, the wells were rinsed with PBS, and 100 µL of MTT reagent was added to the wells. The plate was then incubated in the CO_2 incubator for 4 hours. Subsequently, the cells were observed again under the microscope, and a total of 100 µL of termination solution (SDS) was added to the blend. The plate was then tightly covered with paper or aluminium foil and stored overnight at room temperature in the absence of light. At a wavelength of 600 nm, the sample's light absorption was quantified with an ELISA instrument (13).

Data Analysis

Cytotoxicity Test Analysis

Data analysis of the cytotoxicity test was performed to determine the proportion of viable cells and the IC_{50} value. The absorbance values obtained from ELISA reader measurements for each well were used to

calculate the cell viability percentage. This percentage was calculated by subtracting the absorbance value of the reference medium from the absorbance of the treatment, then dividing it by the difference in absorbance between the cell control group and the medium control group, and finally multiplying by 100%.

The IC_{50} value was obtained by comparing the EEGAP concentration with the percentage of cell viability using probit analysis, referring to a probability value of 0.5 (13). The IC_{50} value is a parameter in cytotoxicity testing that determines the cytotoxic potential of a compound. A higher IC_{50} value indicates lower cytotoxicity(14).

Antiproliferative Test Analysis

The analysis of the antiproliferative test was based on the count of viable cells at various concentrations measured at 0, 24, 48, and 72 hours, from which the doubling time of cell growth was calculated. To determine the doubling time, a linear regression analysis was performed between incubation time and the logarithm of cell viability at each concentration. If the calculated doubling time was longer than that of the control group, the sample was considered to have antiproliferative activity (15). The doubling time was calculated using the following formula:

$$\text{Doubling Time} = \frac{y-a}{b} \quad \text{.....(1)}$$

Where:
y = Log (2 x initial number of cells)
a = Intercept
b = Slope

RESULTS

A thick extract of green apple peel (**Figure 1**) was obtained in a quantity of 63.75 g, with a yield of 34.45%. The extraction employed UAE, a method that utilises ultrasonic waves to enhance extraction efficiency. Compared to conventional methods, UAE offers faster processing, improved compound recovery, reduced energy consumption, and lower operational costs (16). EEGAP exhibited a thick consistency, dark brown colour, and a sweet aroma characteristic of green apple.

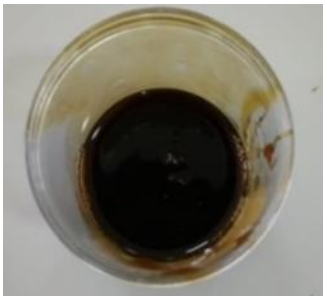


Figure 1. Ethanolic extract of green apple peel (EEGAP)

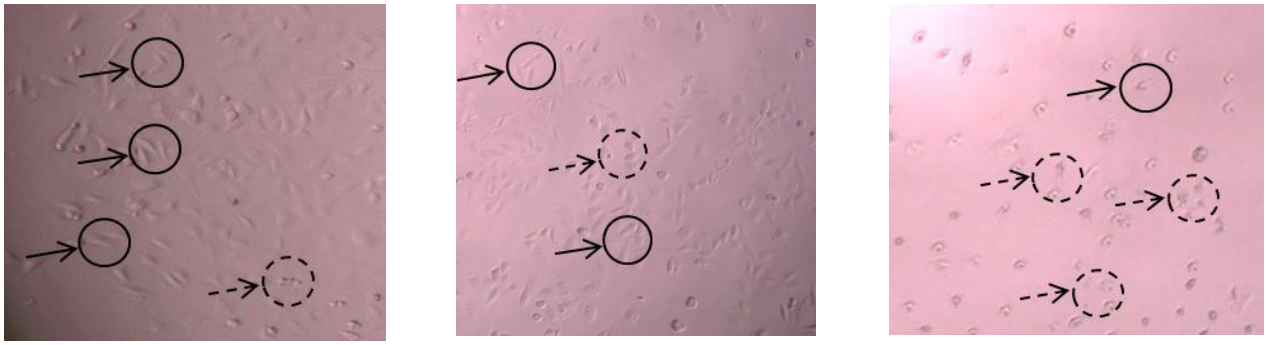
The cytotoxicity test method used in this study was the MTT assay. The principle of this method is based on measuring the formazan crystals formed. Formazan crystals are characterised by their purple colour, are insoluble in water, but soluble in 10% SDS (14).

Table 1. Cytotoxicity Test Results of EEGAP on T47D Cells

EEGAP Concentration (µg/mL)	Cell Viability (%)±SD
1,000	17.01±0.6
500	122.47±1.4
250	131.91±0.5
125	129.98±3.6
62.5	122.96±2.8
31.25	114.17±1.9

Description: EEGAP = Ethanolic Extract of Green Apple Peel; SD = Standard Deviation; T47D = Human ductal breast epithelial tumour cell line; Test performed in triplicate

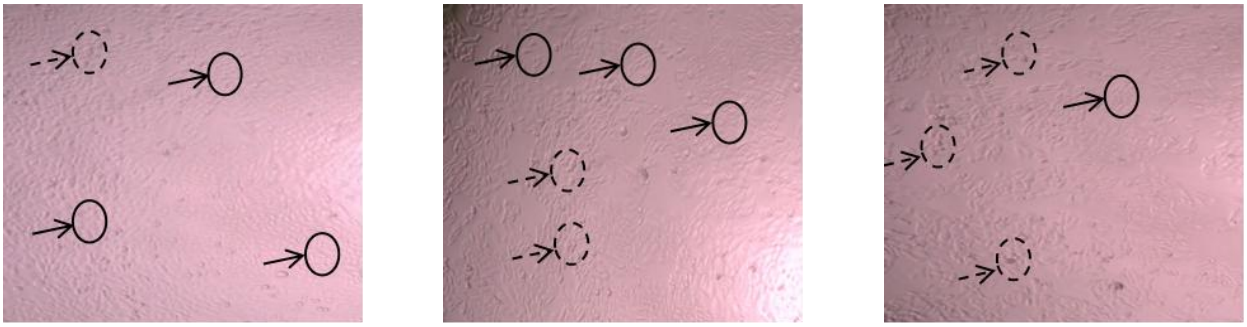
The morphology of T47D breast cancer cells after treatment with EEGAP (**Figure 2**) showed that viable cells appeared oval, whereas dead cells were round. This indicates that the intensified measure of EEGAP administered results in a diminished ratio of T47D breast cancer cell viability. The cytotoxicity test data are presented in **Table 1**. Interestingly, the percentage of cell viability (**Table 1**) at concentrations ranging from 31.25 to 500 µg/mL showed values above 100%. This is suspected to be because, at these concentrations, the extract was unable to inhibit the cancer cells, allowing them to continue replicating.



(A) (B) (C)

Figure 2. Morphology of T47D cells in the cytotoxicity test after treatment with a series of EEGAP concentrations. T47D cell control without treatment (A); EEGAP concentration 62.5 µg/mL (B); EEGAP concentration 1,000 µg/mL (C). Viable cells (→) and dead cells (--→)

The cytotoxic activity of EEGAP against T47D breast cancer cells was reflected in the IC_{50} value. The IC_{50} value of EEGAP was obtained from the analysis of cell viability percentage using probit analysis and was established at 809 µg/mL. This IC_{50} value was then used for the antiproliferative test. The objective of the following experiment was to evaluate the speed of cellular proliferation influenced by the administration of EEGAP based on incubation time. The parameter measured in this test was the time required for cells to double in number (doubling time). The concentrations used in this test were ½IC_{50} (404.5 µg/mL) and IC_{50} (809 µg/mL). The selection of concentrations for the antiproliferative assay was at or below the IC_{50} value so that cell growth and morphology could be observed (17). Administration of EEGAP in this study was found to reduce the number of viable cells over the incubation period. The morphology of T47D cells in the antiproliferative assay after 24 hours of incubation with EEGAP treatment is shown in **Figure 3**.



(A) (B) (C)

Figure 3. Morphology of T47D cells in the antiproliferative assay after 24 hours of incubation with EEGAP treatment. T47D cell control without treatment (A); EEGAP concentration of 404.5 µg/mL (B); EEGAP concentration of 809 µg/mL (C). Viable cells (--→) and dead cells (→)

Figure 3 illustrates the morphological differences observed among the treatment groups. In the control group, a significant number of viable cells were observed. In contrast, as the concentration increased, a noticeable rise in the number of dead (round) cells was observed. The antiproliferative effect of EEGAP on the survival rate of T47D breast carcinoma cells was analysed using the MTT assay. The results consisted of absorbance values representing viable cells at different concentrations. These absorbance values were then converted into viable cell counts (17). To quantify the viable cells for each treatment, a calibration curve was developed, resulting in a linear regression equation of $y = 0.000005x + 0.2526$, with a coefficient of determination (R^2) of 0.9653. The calibration curve data, along with the corresponding viable cell counts for each curve, are displayed in **Table 2**.

Table 2. Number of Viable Cells at Each Incubation Time for Different Treatments

Treatment	Hour 0		Hour 24		Hour 48		Hour 72	
	Abs	Cell Count	Abs	Cell Count	Abs	Cell Count	Abs	Cell Count
Control Cells	0.319	13,300	0.463	42,000	0.894	128,000	0.962	141,900
EEGAP 404.5 µg/mL	0.333	16,000	0.422	33,800	0.730	95,500	1.011	151,700
EEGAP 809 µg/mL	0.330	15,500	0.427	34,900	0.512	51,900	0.491	47,500

Description: EEGAP = Ethanolic Extract of Green Apple Peel; Abs = Absorbance

The antiproliferative effect of EEGAP on T47D breast cancer cells was evaluated by establishing a linear regression relationship between incubation time (in hours) and the logarithm of viable cell counts from each treatment, which allowed for the determination of the doubling time (15). The data on the number of viable cells obtained at 0, 24, 48, and 72 hours were used to determine the antiproliferative activity of the cells.

The slope values in this study for EEGAP treatments at concentrations of 404.5 µg/mL (½IC_{50}) and 809 µg/mL (IC_{50}) were lower than those of the untreated control cells. Consequently, the doubling time values obtained were 14±2.56 hours for the control, 21±1.87 hours for EEGAP 404.5 µg/mL, and 30±4.26 hours for EEGAP 809 µg/mL. The doubling times for each treatment are summarised in **Table 3**.

Table 3. Linear Regression Observations and Doubling Time Values of T47D Breast Cancer Cells Following EEGAP Treatment

Treatment	Regression Equation	A	B	R^2	Doubling Time (hours)±SD
Control Cells	y = 0.0148x + 4.2180	4.2180	0.0148	0.9128	14±2.56
EEGAP 404.5 µg/mL	y = 0.0141x + 4.2187	4.2187	0.0141	0.9812	21±1.87
EEGAP 809 µg/mL	y = 0.0068x + 4.2874	4.2874	0.0068	0.7770	30±4.26

Description: EEGAP = Ethanolic Extract of Green Apple Peel; A = Intercept; B = Slope; R^2 = Coefficient of Determination; SD = Standard Deviation; T47D = Human ductal breast epithelial tumour cell line; Test performed in triplicate

DISCUSSION

Cytotoxicity testing is an in vitro assay used as a preliminary testing method to examine the damaging effects of compounds present in EEGAP on T47D breast cancer cells. The T47D cell line is widely utilised in experimental studies as a model to investigate the specific role of progesterone in the luminal A subtype of breast cancer (4). The parameter observed in this test was cell viability, measured using the MTT assay. This method is based on the reaction between the MTT reagent and the succinate dehydrogenase enzyme in the mitochondria of viable cells (15). The IC_{50} value obtained indicates that EEGAP has moderate cytotoxic activity against T47D breast cancer cells and is categorised within the moderate cytotoxic group (200-1,000 µg/mL) (18). Cytotoxicity tests have been performed on both the flesh and peel of apples. Components from Annurca apples with multiple phenol rings exhibited an aptitude to target and eliminate triple-negative MDA-MB-231 breast cancer cells. This effect occurs through the mechanism of cell cycle arrest triggered by Reactive Oxygen Species (ROS) and activation of apoptosis (19).

Antiproliferative activity against cancer cells can be observed from a reduction in the quantity of vital cells following treatment during the incubation period (15). Antiproliferative activity can also be assessed by analysing the slope value, which is used to calculate the doubling time. A higher slope value corresponds to a shorter doubling time. The doubling time results indicated that EEGAP has antiproliferative effects on T47D breast cancer cells. Cells treated with EEGAP showed a longer doubling time compared to the control group, confirming the antiproliferative activity of EEGAP (17). The ethanol extract of Manalagi apple peel contains various chemical constituents including alkaloids, flavonoids, phenols, tannins, steroids, and triterpenoids (4). Phytochemical screening of apple peel (*Malus domestica* Bork.) revealed the presence of flavonoid compounds from the flavonol group based on tube tests. Further analysis using Thin Layer Chromatography (TLC), with quercetin as a reference, showed a Retention factor (Rf) of 0.85 for the sample and 0.87 for quercetin. These similar Rf values indicate that the apple peel extract contains quercetin compounds (11). Regulation of cell proliferation involves three classes of genes: proto-oncogenes, tumour suppressor genes, and genes related to apoptosis (20). Among these, the p53 tumour suppressor gene plays a critical role in preventing uncontrolled cell growth. When activated by genotoxic stress, p53 initiates transcriptional programmes that lead to apoptosis, cessation of the cell cycle, ageing process, and DNA repair. If p53 function is lost, this regulatory mechanism is impaired, enabling cells with damaged DNA to grow uncontrollably (21). Quercetin has been reported to induce expression of the CDK inhibitor p21, as well as simultaneously decrease the phosphorylation level of pRb,

thereby inhibiting the process of changing phases in the cell cycle from G1 to S by trapping the E2F1 protein (8).

Flavonoid compounds found in plants have been demonstrated to possess activity in inhibiting cancer cell growth, as demonstrated through both in vivo (within living organisms) and in vitro (outside living organisms) studies (8). Flavonoids are suspected to have anticancer activity, and the ethyl acetate fraction of moringa leaf extract shows greater ability to fight cancer in T47D breast cancer cells compared to its aqueous fraction (22). Extraction using 70% ethanol yields a higher flavonoid content compared with 50% and 96% ethanol. This shows that 70% ethanol is able to extract significantly more active compounds than 96% ethanol (23). Apple peel extract has demonstrated significant anticancer activity against Michigan Cancer Foundation-7 (MCF-7) and MDA-MB-231 breast cancer cells, with IC_{50} values of 78.5±2.3 µg/mL for MCF-7 cells and 85.2±3.1 µg/mL for MDA-MB-231 cells (24). The 70% EEGAP also shows cytotoxic activity and can induce MCF-7 breast cancer cells to undergo apoptosis (25). Apple peel contains phenolic compound concentrations two to four times higher, as well as higher total procyanidin and total flavonoid concentrations compared to the fruit flesh (26). Moreover, apple extracts rich in phloretin, a type of polyphenol, have been shown to suppress the growth of colorectal cancer cells by inhibiting Glucose Transporter Type 2 (GLUT2) and activating p53-mediated signalling pathways (27). Apples are known to have cancer-preventive effects, and their phytochemical profile strongly depends on the cultivar and fruit maturity stage (28). Therefore, the bioactive compounds such as quercetin, phenols, or procyanidins found in apple peel play a role in anticancer activity. Through various studies, it has been revealed that extracts from apple peel and flesh, particularly from green apple varieties, exhibit notable anticancer effects against various cancer cells, such as breast cancer cells, for example, T47D, MCF-7, and MDA-MB-231. These bioactive compounds help suppress and control the growth of cancer cells by inducing apoptosis and stopping cell turnover. Moreover, even a 50% ethanol extract of kitolod leaves containing flavonoid compounds has also been proven to have anticancer activity (29).

CONCLUSION

EEGAP shows cytotoxic effects on T47D breast carcinoma cells with an IC_{50} concentration of 809 µg/mL. It also displays antiproliferative properties at concentrations of 404.5 µg/mL (½IC_{50}) and 809 µg/mL (IC_{50}). Consequently, additional studies are needed to assess EEGAP's impact on cell cycle arrest to determine which specific phase of the cell cycle is influenced.

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