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Pea Seed (*Pisum sativum* L.) Extract Exhibits Cytotoxic Activity against T47D Cancer Cells

Failasufia M. Chasani[1], Devi N. Hidayati[1*], and Indah Hartati[2]
[1]Faculty of Pharmacy, Wahid Hasyim University, Semarang, Central Java, Indonesia
[2]Department of Chemical Engineering, Faculty of Engineering, Wahid Hasyim University, Semarang, Central Java, Indonesia

Abstract
Pea (*Pisum sativum* L.) contain flavonoids with potential anticancer activity against breast cancer cells. Yet, the influence of extraction methods and solvent choice on flavonoid yield and bioactivity remains insufficiently studied. This study evaluated the effect of solvent type and extraction method on the flavonoid content and cytotoxic activity of Pisum sativum L. seed extract on T47D breast cancer cells. Extraction was performed using reflux and infusion methods with 96% ethanol, 70% ethanol, and water solvents. Flavonoid content was determined using the $AlCl_3$ colorimetric test, while cytotoxic activity was tested using the MTT method. The results showed that the 96% ethanol extract had the highest flavonoid content (9.88 ± 0.05 mg QE/g), followed by 70% ethanol (5.91 ± 0.26 mg QE/g) and water extract (2.63 ± 0.06 mg QE/g). Cytotoxic activity was demonstrated by 96% and 70% ethanol extracts with IC_{50} values of 147.12 ± 8.81 µg/mL and 90.59 ± 5.75 µg/mL, respectively, while the water extract was inactive. These findings confirm that solvent polarity plays an important role in optimizing flavonoid content and cytotoxic potential. However, high flavonoid content does not always correlate with cytotoxic effects, so a comprehensive approach is needed in the development of natural-based anticancer agents.

Keywords: cytotoxic, ethanol extracts, Pisum sativum L., total flavonoid content, T47D cells

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*Corresponding author:
devinisahidayati@unwahas.ac.id

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Ekstrak Biji Kacang Polong (*Pisum sativum* L.) Menunjukkan Aktivitas Sitotoksik terhadap sel kanker T47D

Abstrak
Kacang polong (*Pisum sativum* L.) mengandung flavonoid yang memiliki potensi aktivitas antikanker terhadap sel kanker payudara. Namun, pengaruh metode ekstraksi dan pilihan pelarut terhadap hasil flavonoid dan aktivitas biologisnya masih belum cukup diteliti. Penelitian ini mengevaluasi pengaruh jenis pelarut dan metode ekstraksi terhadap kandungan flavonoid serta aktivitas sitotoksik ekstrak biji *Pisum sativum* L. pada sel kanker payudara T47D. Ekstraksi dilakukan dengan metode refluks dan infus menggunakan pelarut etanol 96%, etanol 70%, serta air. Kandungan flavonoid ditentukan dengan uji kolorimetri $AlCl_3$, sedangkan aktivitas sitotoksik diuji dengan metode MTT. Hasil menunjukkan ekstrak etanol 96% memiliki kandungan flavonoid tertinggi (9,88 ± 0,05 mg QE/g), diikuti etanol 70% (5,91 ± 0,26 mg QE/g), dan ekstrak air (2,63 ± 0,06 mg QE/g). Aktivitas sitotoksik ditunjukkan oleh ekstrak etanol 96% dan 70% dengan nilai IC_{50} masing-masing 147,12 ± 8,81 µg/mL dan 90,59 ± 5,75 µg/mL, sedangkan ekstrak air tidak aktif. Temuan ini menegaskan bahwa polaritas pelarut berperan penting dalam optimasi kandungan flavonoid sekaligus potensi sitotoksik. Namun, tingginya kandungan flavonoid tidak selalu berbanding lurus dengan efek sitotoksik, sehingga diperlukan pendekatan komprehensif dalam pengembangan agen antikanker berbasis bahan alam.

Kata Kunci: flavonoid total, ekstrak etanol, *Pisum sativum* L., sel T47D, sitotoksik

1. Introduction

The cancer with the highest number of new cases and deaths every year in the world is breast cancer. Around 2.3 million (11.5%) new cases and 666.1 thousand (6.8%) deaths. Breast cancer is the highest cancer case in Indonesia, with 66.3 thousand (16.2%) new cases and 22.6 thousand (9.3%) deaths. Breast cancer cases are around 151.3 per 100,000 population.[1] The high prevalence of breast cancer in Indonesia encourages the development of natural medicines (herbs) as an anticancer to prevent the development of cancer cells.[2]

Breast cancer is one of the most common and deadly cancers worldwide, with a significant impact on public health and clinical treatment approaches. Despite advances in breast cancer therapy, the development of more effective and less toxic treatment options remains a high priority. T47D cancer cells, which are a human breast cancer cell line often used in research, are a relevant model for evaluating the anticancer potential of natural products. T47D cells belong to the luminal A classification with an ER^{+}, $PR^{+/-}$, $HER2^{-}$ immune profile characterized by low Ki-67, endocrine responsiveness, and often responsive to cancer chemotherapy.[3] These cells have a response to estrogen and exhibit similar characteristics to many female breast cancers, making them an appropriate model to study the cytotoxic effects of compounds from plant sources.

Pisum sativum L. (pea) is a leguminous plant that is widely consumed due to its nutritional value and has recently attracted attention for its pharmacological potential, particularly as an anticancer agent.[4] Several studies have reported that peas contain various secondary metabolites such as flavonoids, isoflavonoids, phenolic acids, and phytoalexins.[5] Flavonoids are well known for their cytotoxic effects, exerting anticancer activity by inducing apoptosis, suppressing cell proliferation, and modulating signal transduction pathways in malignant cells.[6,7] Peas (*Pisum sativum* L.) contain secondary metabolites such as flavonoids, isoflavonoids, phenolic acids, and phytoalexins.[8]

Peas contain flavonoid compounds that are thought to have cytotoxic potency.[9] Flavonoid compounds have a mechanism of action that can induce cell death and inhibit the life cycle of cancer cells.[10] Flavonoid compounds contained in pea seeds are: quercetin compounds (flavonol group), luteolin (flavone group), and genistein (isoflavonoid group).[11] Among the flavonoids identified in pea seeds are quercetin (flavonol group), luteolin (flavone group), and genistein (isoflavonoid group), which have been shown to exhibit anticancer activity in various in vitro models.[12] However, previous studies have generally focused on identifying these compounds or evaluating their biological activity individually, without addressing the impact of extraction methods or solvent polarity on the cytotoxic potential of the whole extract.

Pea seeds exhibit cytotoxic effects on MDA-MB-453 breast cancer cells.[13] Compounds of quercetin found in peas possess a mechanism that hinders growth and triggers apoptosis in T47D breast cancer cells.[14] Luteolin compounds demonstrate significant antiproliferative properties against breast cancer cells MDA-MB-231 and MCF-7.[15] Genistein acts by suppressing cell proliferation (decreasing ERα signaling) in breast cancer cell lines like MCF-7 and MDA-MB-231.[16] The T47D, MCF-7, and MDA-MB breast cancer cell lines exhibit nearly identical immune profiles, particularly being ER^{+}, $PR^{+/-}$, and $HER2^{-}$. The distinction lies in the mutation of the P53 gene that is present in T47D cells.[3]

Solvents commonly used for extraction are ethanol, ethanol-water mixture, and water.[17] The 96% ethanol extract from the telang flower plant, scientifically known as *Clitoria ternatea* L. from the Fabaceae family, comprises flavonoids, alkaloids, terpenoids.[18] The 70% ethanol extract of the telang flower plant contains phenolic compounds, flavonoids, alkaloids, terpenoids, tannins, and saponins.[19] Water extracts of telang flower plants contain flavonoids, saponins, tannins, and terpenoids.[20] Plants from the same family often contain the same metabolite compounds, so these plants likely have the same pharmacological activity.[21]

The 96% ethanol extract of telang flowers or *Clitoria ternatea* L. (Fabaceae family) has a total flavonoid content of 7.2188 mgQE/mg or 7218.8 mgQE/gram.[22] The 70% ethanol extract of telang flowers has a total flavonoid content of 5.74 mgQE/100 mg or 57.4 mgQE/gram.[23] Telang flower water extract has a total flavonoid content of 0.88 mgQE/gram.[24] Based on this study, the total flavonoid content of the 96% ethanol extract is higher than that of the 70% ethanol and water extracts.

The type of solvent and extraction technique are critical factors that influence the efficiency of phytochemical recovery and the biological activity of the resulting extract. Polar solvents such as ethanol and water are commonly used in natural product extraction, but their effectiveness in extracting cytotoxic flavonoids from *P. sativum* has not been directly compared.[6] Furthermore, the correlation between total flavonoid content and cytotoxic activity remains underexplored in this context.

Therefore, this study aimed to evaluate the effects of different extraction solvents (96% ethanol, 70%

ethanol, and water) on the flavonoid content and cytotoxic activity of *P. sativum* seed extracts against T47D breast cancer cells. Extraction was conducted using reflux and infusion methods, total flavonoid content was measured using the $AlCl_3$ colorimetric assay with quercetin as a standard, and cytotoxicity was assessed via the MTT assay at various concentrations to determine IC_{50} values.The results of this study are expected to provide insight into the optimal extraction conditions for maximizing the cytotoxic potential of *P. sativum*, contributing to the development of plant-based anticancer agents.

2. Materials and Method

2.1. Tools

The tools used include: Electric balance (Hanherr®), analytical balance (Ohaus®), oven (Irastar®), reflux set (Iwaki®), infundation set, vacuum rotary evaporator (Heidolph®), freezedry (Christ®), UV-Vis spectrophotometry (Shimadzu UV1800®), CO2 incubator (Thermoscience®), culture discs (Nunclon®), Biosafety Cabinet level 2 (Esco Airstream®), vortex (Cleaver®), inverted microscope (Magnus®), hemocytometer (Neubauer®), 96well-plate (Nunc®), ELISA reader (Bio-Rad®).

2.2. Materials

The pea plants (*Pisum sativum* L.) originated from Sumowono Village, Sumowono District, Semarang Regency, Central Java Province. Harvesting of pea seeds was done when the peas were 70 days old. At this age, the pea seeds were green-brown to brown. The collected pea seeds were then sorted to obtain green-brown or brown seeds that had a hard texture. Green-brown or brown colored pea seeds have been reported to exhibit potential as chemopreventive and chemotherapeutic agents for cancer.[13] Pea plants had been determined with the number B/588/UN23.6.10/TA.00.01/2024 at the Environmental Laboratory of the Faculty of Biology, Universitas Jenderal Soedirman, Purwokerto.

The materials used include: T47D breast cancer cells obtained from the Cell Culture Laboratory of the Faculty of Medicine, Gadjah Mada University. Ethanol 96% (technical), ethanol 70% (technical), aquadest (technical). Ethanol pro analysis (Supelco®), quercetin, $AlCl_3$ (aluminum chloride) (Sigma-Aldrich®), CH_3COOK (potassium acetate) (Himedia®), DMSO 100% (Dimethyl Sulfoxide) (Merck®), DMEM medium (Gibco), trypsin EDTA 0.25% (Sigma®), PBS (Phosphate Buffer Saline) (Merck®), FBS (Fetal Bovine Serum) 10% (Sigma®), Penicillin-Streptomycin 1% (Gibco), Fungizon 0.5% v/v (Gibco®), MTT reagent 5 mg/ml (Sigma®), SDS 10% in HCl 0.01 N (Merck®).

2.3. Methods

2.3.1. Powder Manufacturing

In the first stage of the harvesting and seed selection process, the selected seeds were 70 days old and either brownish green or brown, with a hard texture. After wet sorting, the pea seeds had a wet weight of 2000 grams. In the second stage of the drying process, fresh pea seeds were processed into simplisia using an oven at 45°C and achieved a moisture content of 6%, which is below the requirement for simplisia moisture content of <10%.[25] Dry simplisia were pollinated.

2.3.2. Reflux Method Extraction

Extraction with 96% ethanol and 70% ethanol solvents using the reflux extraction method, 100 grams of pea seed powder with the addition of solvent up to ¾ of a round-bottom flask, then extracted for 3 hours, with repetition 3 times at ethanol boiling point temperature (±78.32ºC).The filtrate was filtered using a Buchner funnel and then concentrated with a rotary evaporator at 50°C until the extract was thick.

2.3.3. Infundation Method Extraction

Extraction with a water solvent using the infusion method, with 100 grams of pea seed powder and a 1:15 solvent ratio (1500 mL), was performed for 15 minutes at 90°C. The filtrate is filtered using a Buchner funnel and then dried by freeze drying with a temperature range of -20°C until the extract is dry.[26]

2.3.4. Total Flavonoid Content

Preparation of Quercetin Mother Liquor (100 ppm), 10% $AlCl_3$ Mother Liquor and 1M Potassium Acetate Mother Liquor

Quercetin was weighed at 100 mg and dissolved in ethanol p.a. until completely dissolved, then transferred into a 100 mL flask. Ethanol p.a. was added until the limit mark, and dilution was carried out 10 times by taking 1 mL of quercetin solution and placing it into a 10 mL flask, then ethanol p.a. was added until the limit mark. Preparation of $AlCl_3$ parent solution by weighing as much as 100 1 gram dissolved in 10 mL of ethanol p.a in a measuring flask until the limit mark. Preparation of parent solution of potassium acetate, as much as 1 gram dissolved into 10 mL of ethanol p.a in a measuring flask until the limit mark.[27]

Preparation of Parent Solution of 96% Ethanol Extract, 70% Ethanol and Water

Each weighed 1 gram and was then dissolved with ethanol p.a. using a magnetic stirrer in a 100 mL glass beaker at 300 ppm until completely dissolved. The solution was filtered using filter paper into a 100 mL measuring flask and then ethanol p.a. was added until the limit mark.[27]

Determination of Quercetin Standard Curve

A series of quercetin solutions (2, 4, 6, 8, 10, and 12 ppm; 1000 µL each) was prepared, followed by the addition of 200 µL of 10% $AlCl_3$ and 200 µL of 1 M potassium acetate. Absorbance was measured at the maximum wavelength using a UV–Vis spectrophotometer, and the optimal operating time was recorded. Each treatment was conducted in triplicate.[27]

Determination of Maximum Wavelength

Briefly, 1000 µL of 6 ppm concentration series was added with 200 µL of $AlCl_3$ 10% and 200 µL potassium acetate 1M, read on UV-Vis spectrophotometer from 400-500 nm range with absorbance value of the solution between 0.2-0.8.[27]

Determination of Operating Time

A total of 1000 µL of 6 ppm concentration series was added 200 µL $AlCl_3$ 10% and 200 µL potassium acetate 1M, read using a spectrophotometer at the maximum wavelength and operating time that has been determined at minutes 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, and 60.[27]

Determination of Total Flavonoid Content

Each sample of pea seed extract was pipetted with 1000 µL, followed by the addition of 200 µL of 10% AlCl3 and 200 µL of 1 M potassium acetate. The absorbance was read using a spectrophotometer at the maximum wavelength, and the operating time was obtained. The treatment was carried out 3 times in replication.[27]

2.3.5. Cytotoxic Assay

The 96-well plate wells each contained 100 µL of T47D breast cancer cells. Testing the cytotoxic activity of pea seed extract (Pisum sativum L.) with a concentration series of 1000, 500, 250, 125, 62.5, and 31.25 µg/mL, the concentration series was added to the wells as much as 100 µL (replication was done 3 times). Plates containing T47D cells and pea seed extract concentrations were incubated in a CO2 incubator for 24 hours at 37°C. A plate containing T47D cells and pea seed extract was added 100 µL of MTT reagent, then incubated in a CO2 incubator for 2-4 hours at 37°C. Cell condition was examined using an inverted microscope. When formazan crystals (violet crystals) had formed clearly, 100 µL of stopper reagent was added to each well. The plate was wrapped in aluminum foil and then stored in a dark place at room temperature for one night. The plates that had been left overnight were then analyzed using an ELISA reader at a wavelength of λ 595 nm.[28]

2.3.6. Data Analysis

Analysis of Flavonoid Determination Results

Determination of total flavonoid content was analyzed based on the absorbance data of the results from analyzed samples using the UV-Vis spectrophotometric method at a 340 nm wavelength.[29] The percentage of live cells was analyzed using the linear regression method, resulting in the regression equation Y = bx+a.[28]

$$\text{Total flavonoid content} = \frac{sample\ conc.\left(\mu\frac{g}{mL}\right) \times sample\ vol.(mL) \times diluting\ factor}{sample\ weight(g)}$$

Total flavonoid levels were also analyzed statistically using SPSS software. SPSS software to determine the significance of differences in total flavonoid levels quantitatively between different types of solvents, 96% ethanol, 70% ethanol, and distilled water. Parametric requirements typically involved distributed data (p> 0.05) and homogeneous distribution (p> 0.05). 30 Data on the total flavonoid content of pea seed extracts with 96% ethanol, 70% ethanol, and distilled water solvents were not normally distributed and not homogeneous. The data were not normally distributed and not homogeneous, so a non-parametric difference test was performed using the Kruskal-Wallis method.[31]

Analysis of Cytotoxic Results

The cytotoxic test was analyzed based on absorbance data from analyzed samples using the ELISA reader method at a wavelength of 595 nm.[28]

$$\text{Percentage of live cells (\%)} = \frac{(\text{abs. treatment} - \text{abs. media control})}{(\text{abs. cell control} - \text{abs. media control})} \times 100\%$$

The percentage of live cells was analyzed using the linear regression method.[28] The IC_{50} value is the concentration that can kill 50% of the cell population. The value of IC_{50} values was also analyzed statistically using SPSS software to determine the quantitative significance differences between different types of solvents and IC_{50} values against T47D breast cancer cells. IC_{50} value against T47D breast cancer cells.

Parametric requirements typically involve normally distributed data (p-value > 0.05) and homogeneous distribution (p-value > 0.05).[30] Data on the IC_{50}value of pea seed extracts with 96% ethanol and 70% ethanol

were normally distributed and homogeneous, so a parametric difference test was carried out with the one-way anova method.[31] Meanwhile, pea seed extracts with water solvent were not analyzed statistically because the extracts did not have cytotoxic activity. The compound cytotoxicity criteria are divided into four criteria, namely potential cytotoxic criteria when the IC_{50} value is <20 µg/mL, moderate cytotoxic criteria when the IC50 value is >20-100 µg/mL, weak cytotoxic criteria when the IC_{50} value is >100-1000 µg/mL and non-toxic criteria when the IC_{50} value is >1000 µg/mL.[32]

2.3.1. Ethical Approval

The cytotoxic test with T47D breast cancer cells was approved with number 647/B.1-KEPK/SA-FKG/XI/2024 from the Health Research Ethics Commission of the Faculty of Dentistry, Sultan Agung Islamic University.

3. Result

3.1. Determination of peas

Plant determination aims to ensure that the type of plant used as research material is valid, to avoid errors in taking research materials.[33] The pea determination test obtained results that were in accordance with the research material. The morphology of pea seeds is illustrated in Figure 1A.

3.2. Extract of pea seeds using 96% ethanol, extract using 70% ethanol, and water extract.

The drying shrinkage obtained was 3% and the yield was 97%. The third stage of the extraction process, using 96% ethanol and 70% ethanol extracts of pea seeds, resulted in an extract yield of 13.60%. Meanwhile, the water extract of pea seeds obtained an extract yield of 14.40%. Macroscopically, the 96% extract was light brown, slightly liquid (containing oil), and had a characteristic aroma. The 70% ethanol extract is dark brown, thick, and has a distinctive aroma. Meanwhile, the water extract is a brownish-white, flake-shaped substance with a distinctive aroma. The macroscopic appearance of the pea seed extract is shown in Figure 1B-1D.

3.3. Total flavonoid content

The quercetin standard curve yielded a linear regression equation of Y = 0.05068x + 0.11882, with a correlation coefficient of r = 0.99645. The results of determining the flavonoid content of pea seed extract with 96% ethanol solvent obtained flavonoid content of 9.88 ± 0.05 mg QE/gram, 70% ethanol extract obtained flavonoid content of 5.91 ± 0.26mg QE/gram, and water extract obtained

flavonoid content of 2.63 ± 0.06 mg QE/gram. The total flavonoid content in the pea seed extract is presented in Table 1. Data on the total flavonoid content of pea seed extracts with 96% ethanol, 70% ethanol, and distilled water solvents were not normally distributed and not homogeneous. The results of the Kruskal-Wallis analysis showed significant differences in all extracts with a significance value of 0.027.

3.4. Cytotoxic activity

The results of the cytotoxic activity test based on cell morphology in Figures 2B-2G showed that the pea seed extract with 96% ethanol solvent obtained an IC_{50} value of 147.12 ± 8.81 µg/mL which means that the extract has moderate cytotoxic activity against T47D breast cancer cells (<1000 µg/mL),

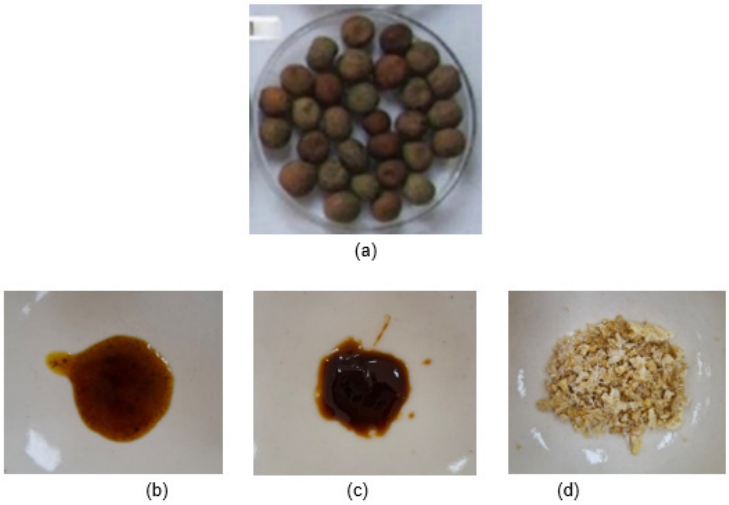

Figure 1. Morphology of pea seeds (a), Macroscopic view of pea seed extract (b) 96% ethanol extract (c) 70% ethanol extract (d) water extract

Table 1. Total flavonoid content of pea seed extract.

Extract Solvent	Average Flavonoid Content ± SD (mg QE/gram)
96% ethanol	9.88 ± 0.05
70% ethanol	5.91 ± 0.26
Water	2.63 ± 0.06

Table 2. The IC_{50} value in pea seed extract.

Extract Solvent	Average IC50 Value ± SD (µg/mL)
96% ethanol	147.12 ± 8.81
70% ethanol	90,59 ± 5.75
Water	>1000

70% ethanol obtained an IC_{50} value of 90.59 ± 5.75 µg/mL which means the extract has strong cytotoxic activity againstT47D breast cancer cells (<100 µg/mL), while the water extract obtained IC_{50} value >1000 µg/mL which means the extract has no cytotoxic activity against T47D breast cancer cells.[34] The IC_{50} value results are presented in Table 2. Data on IC_{50} values of 96% ethanol extract and 70% ethanol extract of pea seeds were normally distributed and homogeneous. One-way ANOVA analysis showed significant differences in the IC_{50} values of 96% ethanol extract and 70% ethanol extract, with a significance value of 0.001.

4. Discussion

The type of solvent and extraction technique are critical factors that influence the efficiency of phytochemical recovery and the biological activity of the resulting extract. Polar solvents, such as ethanol and water, are commonly used in natural product extraction; however, their effectiveness in extracting cytotoxic flavonoids from *P. sativum* has not been directly compared.[6] The choice of solvent type for *P. sativum* extraction is based on the research of Ramdhini,[18] Hidayati19 and Purwaniati,[20] which states that 96% ethanol, 70% ethanol, and water solvents can extract flavonoid compounds that are thought to have cytotoxic activity.[10] Phytochemical screening tests of pea seed extracts using 96% ethanol, 70% ethanol, and water as solvents have not been carried out. However, Phytochemical screening tests on plants of the Fabaceae family, such as Telang flowers or *Clitoria ternatea* L., reveal that the 96% ethanol extract contains phenolic compounds, flavonoids, alkaloids, steroids, terpenoids, saponins, and tannins.[18]

The 70% ethanol extract contains phenol compounds, flavonoids, alkaloids, terpenoids, tannins, and saponins.[19] The water extract contains flavonoids, saponins, tannins, and terpenoids.[20] Plants with the same family or genus often contain the same metabolite compounds, so the plants likely have the same pharmacological activity.[35]

Determination of total flavonoid content using quercetin standard. Flavonoids possess a structural framework of 15 carbons recognized as flavone, characterized by a C6-C3-C6 arrangement, which features two benzene rings linked by three carbon atoms from a pyran ring.[36] Quercetin is a flavonoid compound contained in pea seeds.[11] The results of the quercetin standard curve

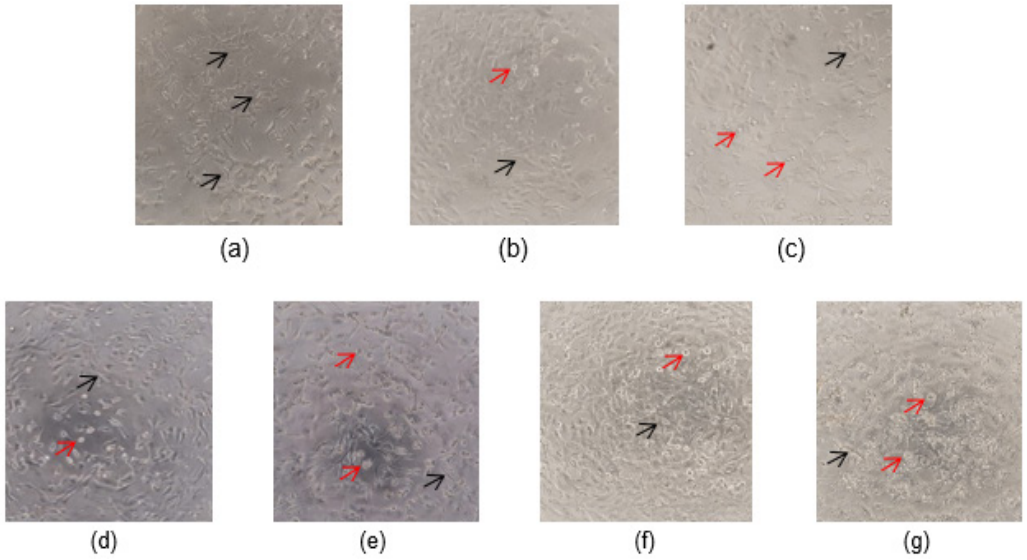

Figure 2. Effect of pea seed extract treatment on T47D cell morphology after 24 hours of incubation at 40× magnification. Control T47D cells concentration 100 µL (a), 96% ethanol extract concentration 125 µg/mL (b), 96% ethanol extract concentration 250 µg/mL (c), 70% ethanol extract concentration 125 µg/mL (d), 96% ethanol extract concentration 250 µg/mL (e), water extract concentration 125 µg/mL (f), water extract concentration 250 µg/mL (g). Live cells were indicated as oval form (→), and dead cells were round-like in morphology (→).

obtained a linear regression equation Y = 0.05068 x + 0.11882 with a value of r = 0.99645. The quercetin standard curve shows a strong relationship between concentration and absorbance value and shows a linear relationship.[29]

The flavonoid content of bean seeds extracted using 96% ethanol was higher than that of seeds extracted with 70% ethanol and water. The total flavonoid content in the pea seed extract is presented in Table 1. Research related to the determination of total flavonoid content in pea seed extract using different solvents, including 96% ethanol, 70% ethanol, and distilled water, has not been conducted before. However, determination of total flavonoid levels in plants of the fabaceae family such as telang flowers or *Clitoria ternatea* L., 96% ethanol extract has a total flavonoid content of 7.22 mg QE/mg or 72,20 mg QE/gram.[22] 70% ethanol extract has a total flavonoid content of 5.74 mg QE/100 mg or 57.40 mg QE/gram.[23] water extract has a total flavonoid content of 0.88 mg QE/gram.[24] This is because differences in solvents or differences in the polarity of solvents used for extraction can affect the withdrawal of compounds and compound content.

Cytotoxic activity is expressed as the IC_{50} value, representing the concentration required to kill 50% of the cell population. In T47D breast cancer cells, pea seed extract in 96% ethanol showed weak cytotoxicity (IC_{50} >100–1000 µg/mL), 70% ethanol extract demonstrated moderate cytotoxicity (IC_{50} >20–100 µg/mL), and the water extract showed no activity (IC_{50} >1000 µg/mL).[32] For pea leaves, the 70% ethanol extract exhibited stronger cytotoxicity than either the 96% ethanol or water extracts (Table 2). Total flavonoid content alone was not a reliable predictor of cytotoxicity, likely due to solvent-specific extraction of different bioactive compounds. Quercetin, being less polar, was more abundant in the 96% ethanol extract,[37] whereas the more polar genistein[38] and semipolar luteolin were enriched in the 70% ethanol extract.[39]

Quercetin selectively induces breast cancer cell death[40] by binding to the active site of uridine 5-monophosphate synthase, forming hydrogen bonds with Tyr432 and Gly450, and hydrophobic interactions with Asn312, Met371, and Pro417.[41] Genistein modulates the cell cycle, apoptosis, angiogenesis, and metastasis through targets such as caspases, Bcl-2/Bax, NF-κB, PI3K/Akt, ERK½, MAPK, and Wnt/β-catenin pathways.[42] Luteolin influences inflammation, cell cycle arrest, apoptosis, angiogenesis, and signaling via PI3K/Akt, STAT3, EGFR, and autophagy pathways.[43] Further investigations, particularly apoptosis and cell cycle assays, are warranted for the 70% ethanol pea seed extract, given its superior cytotoxicity against T47D cells.

5. Conclusion

The flavonoid content of pea seed extract with 96% ethanol, 70% ethanol, and water solvents, respectively, was 9.88 ± 0.05 mg QE/gram, 5.91 ± 0.26 mg QE/gram, and 2.63 ± 0.06 mg QE/gram. The cytotoxic activity of pea seed extract with 96% ethanol solvent, 70% ethanol, respectively, has an IC_{50} value of 147.12 ± 8.81 µg/mL, 70% ethanol obtained an IC_{50} value of 90.59 ± 5.75 µg/mL. While the water extract obtained an IC_{50} value greater than 1000 µg/mL, indicating that the extract has no cytotoxic activity on T47D breast cancer cells. Based on the results of the cytotoxic activity test, the high and low levels of total flavonoids cannot be used as a definitive measure of the high and low cytotoxic effects of a plant.

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Conflict of Interest

The authors declare no conflicts of interest.

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