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Research Article

TOTAL PHENOLIC CONTENT AND FLAVONE BIOACTIVITY OF PEANUT HULLS AS ANTIOXIDANT AND ANTIPROLIFERATION TOWARD HENRIETTA LACKS CANCER CELLS

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ABSTRACT

Objective: This study aimed to determine the total phenolic content, correlation of it with antioxidant capacity, and peanut hulls as an antiproliferation on Henrietta Lacks (HeLa) cancer cells, which Indonesia has a serious problem in term of cervix cancer.

Methods: Peanut hulls were extracted by Soxhlet extraction, ultrasound vibration, and reflux boiling to obtain the best extraction method. The total phenolic content of the ethanol extract and the ethyl acetate fraction was determined using Folin–Ciocalteu method. The 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay was used to evaluated an antioxidant capacity of both samples. Identification of the isolated flavone was done by ultraviolet spectra and analyzed using liquid chromatography-mass spectrometer. Inhibition of proliferation of HeLa cancer cells was tested for the purified fraction using 3-(4,5-dimethyl thiazole-2-yl)-2, 5-diphenyltetrazolium bromide assay.

Results: The results of total phenolic content determination giving 262 (ethanol extract) and 532 (ethyl acetate fraction) mg gallic acid equivalent/g extract, respectively. DPPH assay resulted antioxidant capacity with value of inhibitory concentration 50% (IC_{50}) was 36.36 (ethanol extract) and 18.68 (ethyl acetate fraction) µg/mL, respectively. Identification of isolated flavone resulted an apigenin and indicated moderate potency in inhibiting the proliferation of HeLa cancer cells with IC_{50} value of 34 µg/mL.

Conclusions: There was a correlation between the total phenolics with antioxidant capacity of the peanut hulls. The isolated flavone is predominated by apigenin. This isolated compound is potential as antioxidant and inhibiting the proliferation of HeLa cancer cells moderately.

Keywords: Peanut hulls, Total phenolic, Antioxidant capacity, Flavone, Apigenin, Henrietta Lacks cancer cells.

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INTRODUCTION

Peanut plants are the largest crop production in Indonesia after soybeans. The seeds and the skin have been studied to have benefits in medicinal chemistry, such as for antioxidant and anticancer [1]. On the other hand, the peanut hulls contain eriodictyol, polyphenols, 5,7-dihydroxychromone, and luteolin [2-4]. Previous studied were reported that the phenolic compounds have pharmacological activity, such as antibacterial, anti-inflammatory, and larvacidal activities, as well as an antioxidant activity [5-7].

Antioxidant capacity correlates with the concentration of substances in a material [8]. The high of total phenolic content showed high antioxidant activity and can inhibit cancer cell growth effectively [9]. Cervical cancer is a serious problem for women. This cancer is the second biggest killer after breast cancers occur in women. In 2012, women who developed cervical cancer reached 84% of new cases occurred worldwide [10]. Flavones are one of the phenolic compounds that have been identified as an antioxidant and anticancer potential. Gao *et al*. [11] identified the presence of apigenin in the ethyl acetate fraction of *Cajanus cajan* and it exhibits activities as a strong antioxidant. Apigenin, as an antioxidant, has been proven in inhibiting the growth of cancer cells, such as lung (MRC-5), breast (MCF7), colon (HT-29), and cervical (Henrietta Lacks [HeLa]) cancer cell. Proliferation inhibition of this flavone was the highest against MCF7 and HeLa among cancer cells [12]. A chemical constituent in a material needs to be determined for the evaluation as an antioxidant and inhibitory activity against cancer cells. Therefore, this study aims to determine the total phenolic content, antioxidant activity, and antiproliferation of flavones isolated from peanut hulls toward HeLa cancer cells *in vitro*.

METHODS

Plant material

The peanut hulls were collected from Sukabumi area was verified as leguminous *Arachis hypogaea* L. by the Bogoriense Herbarium, Department of Botany Research Center for Biology LIPI, Bogor, Indonesia. The sample was cleaned and dried at room temperature, pulverized, and determined for the moisture content.

Chemicals and instrumentation

Gallic acid, Folin–Ciocalteu reagents, 2,2-diphenyl-1-picrylhydrazyl (DPPH), and ascorbic acid were purchased from a chemical vendor. Three different extraction apparatus were employed: Ultrasonic vibrator, reflux unit, and Soxhlet extractor. Vero cells (ATCC CCL 81), HeLa cancer cells (ATCC CCL 2), Dulbecco's Modified Eagle Medium (DMEM) (Gibco), 3-(4,5-dimethylthiazole-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) (Sigma), dimethyl sulfoxide (DMSO), and doxorubicin were provided by the Primate Center of Bogor Agricultural University. Ultraviolet-visible (UV-Vis) spectrophotometer (HITACHI UV/Vis Spectrophotometer U2800) and liquid chromatography-mass spectrometer (LC-MS) (Waters Acquity) were used for chemical structure identification that done in the Laboratorium Kesehatan Daerah Jakarta, Indonesia.

Phytochemical assays for phenolic and flavonoids

The assays for phenolic and flavonoids content in the powder form and in the extract of peanut hulls were based on Harborne method [13]. The existence of phenolic was characterized by purple, dark blue, and black or greenish color in the solution, whereas the presence of flavonoids was shown in red/yellow/orange on layers of amyl alcohol.

Preparation of ethanol extract

Dried powder (20 g) was extracted with ethanol 96% using three extraction methods (Soxhlet extraction, ultrasound vibration, and reflux boiling) to obtain the best extraction method [2,6,14]. Furthermore, the powder (900 g) was extracted using the best extraction method, which was boiling under reflux, for 210 minutes, then filtered. The filtrate was evaporated using a vacuum rotary evaporator (BUCHI Rotavapor R-114).

The ethanol extract was further treated with liquid-liquid extraction using n-hexane, giving n-hexane and ethanol fractions. The ethanol fraction was hydrolyzed using HCl 2 N. The acid hydrolyzate was fractionated using ethyl acetate. The ethyl acetate fraction was impregnated into silica gel until all of the material absorbed in the silica in a column chromatograph. The elution was started with n-hexane, followed by a gradient mixture of n-hexane: Dichloromethane (DCM), DCM, DCM: Methanol, and finally with methanol [15]. This process gave fractions that were collected and identified of isolated flavone based on thin layer chromatography (TLC), UV-Vis spectrophotometry, and LC-MS.

Determination of total phenolic content

The crude ethanol extracts from the Soxhlet, ultrasound, and reflux extractions were subjected to total phenolic determination. Each of the extract was dissolved in methanol, added with Folin–Ciocalteu reagent and incubated for 8 minutes at 25°C. Subsequently, NaOH 1% solution was added and the incubation was continued for another hour. The absorbance was measured at a wavelength of 730 nm using a UV-Vis spectrophotometer. The gallic acid solution with a concentration series was used as standards. This total phenolic assay was employed for ethyl acetate fraction as well.

Antioxidant activity by DPPH radical scavenging assay

The crude extract and ethyl acetate fraction solution were prepared at various concentrations (10-150 mg/mL), and ascorbic acid was used as a positive control. To each of solution, 10 mL of reagent DPPH was added. The blank solution was prepared by dissolving DPPH in ethanol without any additional test solution. The mixture was shaken, incubated for 30 minutes at room temperature, and the absorbance was read at a wavelength of 517 nm. The absorbance of DPPH was measured to obtain a percentage of DPPH radical scavenging. Antioxidant capacity of the sample was determined as the inhibitory concentration 50% (IC_{50}) value. Antioxidant capacity of the isolated flavone was carried out similarly.

Antiproliferation activity against HeLa cells using MTT assay

The crude ethanol extract, ethyl acetate fraction, and flavones isolated from peanut hulls were tested first to Vero (normal) cells to determine the concentrations that are required in cancer cells assay. Safe concentrations on testing against Vero cells were then selected to be tested on HeLa cancer cells. Both types of cells were cultured in a prepared medium. The medium of DMEM was added with Fetal Bovine Serum (FBS) 10% and penicillin-streptomycin 1%. The cell suspension in the medium was placed into 96-well microtiter plastic plates, and then incubated at 37°C for 24 hrs under 5% CO_2 atmosphere. The crude extract, ethyl acetate fraction, and the isolated flavone were each dissolved in DMSO in different concentrations that had been determined previously, and then added to the cell suspension on the plate. Subsequently, the plate was incubated at 37°C under 5% CO_2 atmosphere for 48 hrs. DMEM medium without test solution and Doxorubicin® were prepared as a negative and positive controls. In the following step, the medium in the plate was aspirated and MTT reagent was added. The samples in the plate were incubated for 4 hrs to form purple formazan in the living cells. Sodium Dedocyl Sulfate (SDS) solution (1%) was added to each well and further incubated for 24 hrs at room temperature. The absorbance was measured using an enzyme-linked immunosorbent assay reader spectrophotometer at a wavelength of 595 nm. The IC_{50} value was determined using the linear regression equation that states the relationship between the concentrations of the test sample and the inhibition, reported in percentage.

Statistical analysis

Correlation between total phenolic and antioxidant capacity was determined using Pearson procedure ($p<0.01$) through the application of SPSS version 23.

RESULTS AND DISCUSSION

Phenolics and flavonoids

The result of the phytochemical qualitative test showed that the dried powder and the ethanol extract of peanut hulls contained both phenolics and flavonoids (Table 1). The presence of phenolics was showed by yellow to green-black color, while the presence of flavonoids was highlighted in yellow in the amyl alcohol layer. Velu *et al*. [6] also reported that methanol, acetone, and aqueous extracts of peanut hull have a high content of phenolics and flavonoids based on phytochemical screening.

Flavone extract

The three different extraction methods gave different yields, being the boiling under reflux gave the highest in terms of crude extract (Table 2). The temperature and time of extraction at reflux boiling turns out to be the most effective in extracting flavonoids from the peanut hulls powder. Soxhlet extraction also uses boiling temperature, but only gives half of that obtained by refluxing, due to a minimum of contact between solvent and the sample. Ultrasound vibration is less efficient in extracting flavones from the peanut hull powder matrix. Although this extraction method uses ultrasonic waves, which helps in breaking the plant cell membrane, it needs longer time and higher temperature to obtain a higher yield of extracts. Extraction temperature below 80°C may also minimize the degradation of flavonoids [16].

Flavonoids were obtained from the ethanol extract by fractionation using ethyl acetate. First, the extract from the reflux boiling method was fractionated using n-hexane, giving n-hexane and ethanol fractions were hydrolyzed using HCl 2 N to break the glycoside bond. This study used HCl for hydrolysis because it is a strong catalyst that capable for breaking the glycosidic bond of a sugar group attached to the flavonoid [17]. The hydrolyzate was purified using ethyl acetate to collect the corresponding aglycone of the flavonoids. The yield of ethyl acetate fraction was slightly higher than that of the ethanol extract, i.e., 44.9% and 40.7%, respectively. The result indicated that the flavonoid aglycone is effectively extracted in ethyl acetate solvent.

The isolated flavone

The hydrolyzed ethyl acetate fraction was further purified to obtain flavones using column chromatography. The best eluent for this purpose was a mixture of DCM: Methanol (29:2 v/v). The fractions were collected in 589 vials and gave 14 subfractions. The highest yield (24.9%) was detected in subfraction L. On TLC plate, this particular subfraction showed phosphorescence blue (Rf=0.33) and yellow (Rf=0.23), observed under UV light at wavelengths of 254 and 366 nm, respectively. Again, the results on TLC using Forestal (HCl: HOAc:H_2O) eluent in 3:30:10 v/v ratio and butanol:acetic acid:water (4:1:5 v/v), the isolated flavone exhibited Rf values of 0.81 and 0.90, respectively. These Rfs indicated the presence of apigenin.

Absorption peaks appeared at 342, 297.5, and 220 nm wavelengths on the UV-Vis spectra. Absorption at 200-400 nm indicated the presence of chromophore group, which is one of the characteristics of flavones. The isolated material was assumed to contain flavonoids, as previously

Table 1: Phenolics and flavonoids in the peanut hulls

Compound	Peanut hulls	
	Dried powder	Ethanol extract
Phenolics	++	+++
Flavonoids	+	++

Table 2: The crude extract yield of three extraction methods (from 20 g dry mass of sample)

Extraction method	Time of extraction (minutes)	Extract mass (g)	Yield (%)
Reflux boiling	210	3.35±0.14	18.76
Soxhlet extraction	540	1.65±0.33	9.24
Ultrasound vibration	120	1.33±0.49	7.45

reported, namely by the appearance of the first peak at 310-350 nm and 297.5 nm of the second peak [18].

Analysis using LC-MS exhibited two peaks of high intensity at retention times of 1.93 and 10.8 minutes, being the highest peak at retention time of 10.8 minutes. Fragmentation of the molecular ion peak was found at m/z 271, and more fragments at m/z 153, 145, 119, 121, and 243. Once again, the fragmentation is similar to that of apigenin aglycone. The previous report suggested that the structure of the ion at m/z 271 is an aglycone of apigenin with fragmentation patterns of MS/MS 247, 229, 225, 171, 153, 145, and 119 [19]. However, there was another peak with low intensity at m/z 271, contributed by some impurities. Therefore, the isolated flavone was confirmed to be flavonoids, dominated by apigenin (Fig. 1).

Total phenolic content

Total phenolic content correlates with the bioactivity. In the assay, the reaction that occurred is marked by the formation of a blue color in solution after the addition of a base that can be measured an absorbance at 730 nm. The hydroxyl group of phenolic would reduce heteropoly acids (phosphomolybdate-phosphotungstate) contained in the Folin–Ciocalteu reagent into a complex of molybdenum-tungsten blue. The total phenolic content was determined on the three crude extracts (Table 3). The reflux boiling is more effective to extract phenolic and flavonoids; the heat treatment may liberate and activate the low molecular weight of polymeric molecule subunit [20].The total phenolic content of the crude extract was 262.3 mg gallic acid equivalent (GAE)/g extract. This value is much higher than that reported by others, using the same sample and the same reflux boiling, which is only 69.1 mg GAE/g. By ultrasound vibration and domestic microwave maceration they only get 5.94 and 7.79 mg GAE/g extract, respectively [14,21].

The total phenolic content in the ethyl acetate fraction was twice higher than that in ethanol extract (Table 4). This is understood due to the higher content of aglycone resulting from the hydrolysis, as the sugar moieties were removed. The total phenolics in this study is also higher that reported elsewhere, may be due to differences in the growth regions of peanut plants, as affected by temperature, soil, rainfall, humidity, and light intensity required by the plants [22]. These factors cause the chemical constituents of the plant to be diverse [23].

Antioxidant activity

The three samples from various extraction stages had different antioxidant activity in DPPH free radicals scavenging at the concentrations <80 µg/mL, until they reached approximately 95% scavenging activity (Table 5). On the other hand, pure ascorbic acid as a positive control gives the same activity at only <20 µg/mL. Based on this preliminary data, the values of IC_{50} were calculated. Antioxidant capacity of the ethyl acetate fraction is higher than that of the ethanol extract (Table 6).

The purified extract has caused the higher content of bioactive compounds than that of the crude extract where most components that interfere with activities such as fats and waxes are eliminated [24]. Flavonoids such as flavones, flavanols, and flavonols more attracted by the ethyl acetate solvent due to its low polarity. These compounds have hydroxyl groups which are able to supply hydrogen to be responsible in DPPH radical scavenging [25,26]. Many studies have found that phenolic functional group showed high antioxidant activity as explained by the ability to scavenge radicals. The released hydrogen atom of the hydroxyl group is bound by radicals and attains stable radical fenocyl [27].

Antioxidant activity of peel and skin of peanuts has been studied using different methods, i.e., DPPH radical scavenging, the ferrous ion chelating, reducing power, and $ABTS^{\bullet+}$ assay. According to the DPPH assay, acetone extract from the samples had antioxidant activity with a strong category on IC_{50} values of $50 \leq IC_{50} \leq 100$ (or between 70.51 and 52.18 µg/mL) [1]. The effect of acid hydrolysis in the extract which resulted aglycone form in ethyl acetate fraction was also clearly explained. Antioxidant capacity of the aglycone is higher than the glycoside as sugar can interfere with the radicals scavenging, so reducing the efficiency of its activity [17]. Meanwhile, the antioxidant capacity of the isolated flavone was similar with the ethyl acetate fraction that had IC_{50} value of 19.66 mg/mL.

The IC_{50} values were based on linear regression equation of samples, which were ($y=0.7819x + 21.566$, $R^2=0.9405$; $y=2.385x + 5.540$, $R^2=0.972$; and $y=2.4439x + 5.1178$, $R^2=0.9918$). There was a correlation between the level of total phenolic and antioxidant capacity (Table 7). This statement is based on an analysis using Pearson correlation ($p<0.01$, $r=-1$). There was a correlation between total phenolic content and antioxidant activity. The higher total phenolic content gave the higher the antioxidant activity. A strong and significant correlation indicated that phenolic compounds in the peanut hulls play an important role as a strong antioxidant and should be able to inhibit the growth of cancer cells.

Table 3: Total phenolic content of extract resulted from 3 extraction methods

Extraction method	Total phenolics (mg GAE/g extract)	Total phenolics (%)
Reflux boiling	496.84	49.7
Soxhlet	65.30	6.5
Ultrasound vibration	37.32	3.7

GAE: Gallic acid equivalent

Table 4: Total phenolic content of ethanol extract and ethyl acetate fraction

Sample	Total phenolics (mg GAE/g wet sample)
Ethanol extract	262.30
Ethyl acetate fraction	531.90

GAE: Gallic acid equivalent

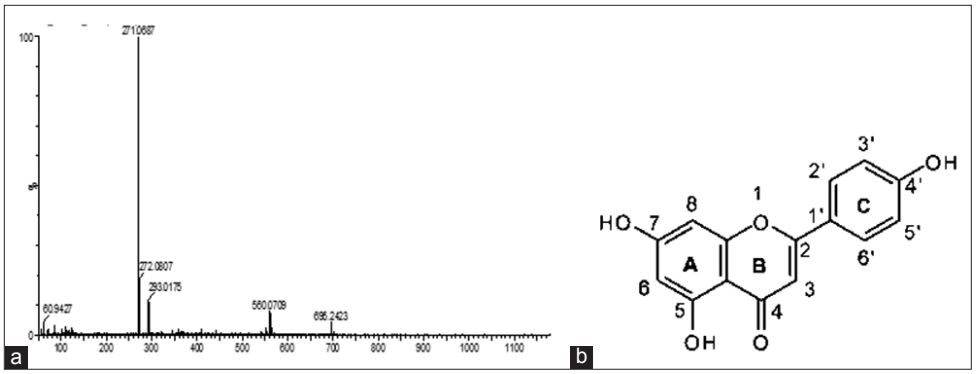

Fig. 1: Mass spectrum of the isolated flavone containing apigenin (a) and structure of apigenin (b)

Table 5: Percentage of DPPH radical scavenging activity (%)

Concentration of sample (µg/mL)	Sample (%)			Concentration of ascorbic acid (µg/mL)	Ascorbic acid (%)
	Ethanol extract	Ethyl acetate fraction	Isolated flavone		
140	92.5±0.17	96.5	93.0±0.12	20	95.8±0.25
120	92.8	96.0±0.37	92.7	10	93.5±0.50
100	91.7±0.85	95.8±0.12	92.6±0.25	5	54.3±1.52
80	88.7±1.10	95.3±0.12	92.7±0.12	2.5	27.0±1.52
60	75.7±1.61	93.5±1.12	92.1±0.12	1.25	13.8±1.27
40	55.5±1.70	93.1±1.50	91.1±0.12	0.62	8.1±0.76
20	31.9±0.34	77.8±2.87	86.7±2.00	0.31	4.3±0.50

DPPH: 2,2-diphenyl-1-picrylhydrazyl

Table 6: Antioxidant capacity of samples

Sample	IC_{50} (µg/mL)
Ethanol extract	36.36
Ethyl acetate fraction	18.68
Isolated flavone	19.66

IC_{50}: Inhibitory concentration 50%

Table 7: Pearson correlation between total phenolic content and antioxidant capacity

Antioxidant capacity of samples	Total phenolic content
Ethanol extract	−1.000**
Ethyl acetate fraction	−1.000**

**Correlation is significant at the 0.01 level

Antiproliferation activity on HeLa cancer cells

Flavonoids role as an impediment to the growth of cervix (HeLa) [28], breast (MCF7), colon (HT-29), and lung (MRC-5) cancer cells [12]. Any sample can be regarded as inhibiting the antiproliferation of cancer cells if it has properties of high toxicity to the cells. However, cytotoxicity to cancer cell from the tested sample must consider the effects on normal (Vero) cells in the body. The toxicity assay on Vero cells at the ethanol extract, the ethyl acetate fraction, and the isolated flavone showed different inhibitory actions. The sample was toxic to Vero cells at concentrations >150 µg/mL, as represented by percent mortality of 50.6%, 49.9%, and 64.2% with IC_{50} value of 254, 161, and 153 µg/mL, respectively. The concentration of sample up 150 µg/mL has been able to kill Vero cells resulting in damage to the membrane so that the Trypan blue binds to proteins in the cell that indicate dead cells that turn to blue under microscopic observation [29]. Therefore, concentrations of <150 µg/mL are safely used for Vero cells but should have the cytotoxic effect on HeLa cancer cells.

The ethanol extract, ethyl acetate fraction, and the isolated flavone were tested on HeLa cancer cells with different safe concentrations for Vero cells. Among the three samples, the isolated flavone exhibited the highest cytotoxicity as compared to the crude extract and the ethyl acetate fraction (Table 8). Flavonoids in the isolated flavone presumably serve as good antiproliferation agents for HeLa cancer cells. The inhibition percentage of this material toward HeLa cells reached 83% at 150 µg/mL. It is similar to giving Doxorubicin® at a concentration of 0.5 µg/mL. Doxorubicin was used as a positive control because it has a strong cytotoxic against HeLa cells.

According to Table 9, the isolated flavone demonstrates the highest toxicity as compared with the ethanol extract and the ethyl acetate fraction. It also has inhibition against cancer cells with a moderate category.

It can be said that all the three test samples from the peanut hulls had potential as an antioxidant and inhibited the proliferation of cancer cells as reported by Pushpa *et al.* [30], metanol extract of *Ganoderma applanatum basidiocarp* and *G. applanatum* mycelium had potential as an antioxidant to antitumor, which important for the development new therapeutic agents. Proliferation inhibition of the both was higher on HeLa cancer cells by IC_{50} value found to be 10±0.07 and 9.25±0.05 µg/mL than human liver (Hep G2) and human mammary gland; epithelial; ascites; ductal carcinoma (ZR-75-30) cancer cells.

Fig. 2 revealed the differences in proliferation inhibition of the samples in microscopic observation on the HeLa cancer cells. A plant extract predominated by flavonoids has been tested for cytotoxicity toward some cancer cells *in vitro*, namely HeLa cells, MCF7, HT-29, and MRC-5. The results proved that the extract was very toxic to HeLa and MCF7 cells at doses of 444 and 179 µg/mL, respectively [9]. The inhibition percentage of doxorubicin in HeLa cells can reach 83% after 72 hrs of incubation at certain doses [31]. In another study [32], methanol extract and ethyl acetate fraction of *Convolvulus arvensis* seeds showed high cytotoxicity against the same cancer cells with the IC_{50} values of 17.33±0.58 and 28.52±1.28 µg/mL, respectively. Those studies support the role of phenolics and flavonoids in the methanol extract and ethyl acetate fraction.

The isolated flavone was dominated by the presence of compound that has been identified as apigenin based on LC-MS analysis. The effectiveness of a material to inhibit the growth of cancer cells depend on the concentration, and types of cancer cell. Apigenin in our isolated flavone inhibits proliferation of HeLa cells strongly as well as demonstrated by chrysin and luteolin [33]. The structure of the apigenin which has double bonds C2-C3 relates to its cytotoxicity. Flavonoids (such as apigenin, luteolin, and quercetin) have higher toxicity than that of flavanones (such as taxifolin, naringenin, and eriodictiol) that only have a single bond at the C2-C3 based on the difference in effective concentration 50% values. The presence of other functional groups such as carbonyl groups at C4 affects the cytotoxicity level. Flavonoids that have a carbonyl group at the C4 has a higher cytotoxic effect on incubation time to 24 and 48 hrs as compared with the compound without. Apigenin does not have a hydroxyl group at C3 as well as luteolin and eriodictiol, but this gives the effect of increasing the cytotoxicity of compounds for up to 2-10 times compared to with the compound having a hydroxyl group at C3 [34]. Apigenin has strong potential in preventing the growth of cervical cancer cells (HeLa) with IC_{50} values of 36 µM. This proliferation inhibition process can be through various pathways including apoptosis cell through the process arrested the cell cycle that causes the growth of cancer cells, modulating the p53 pathway, and the arrangements made by Bcl-2. The inhibition pathways lead to this HeLa cell apoptosis. P53 tumor suppressor gene is regarded as a major factor in maintaining a balance between growth and death cell as well as providing an important role in inhibiting growth tumor by inducing apoptosis [35].

CONCLUSION

Based on Pearson correlation analysis ($p<0.01$, $r=-1$), there is a correlation between the total phenolics with antioxidant capacity of the peanut hulls. The total phenolics in the ethyl acetate fraction is higher than that of the ethanol extract so that the antioxidant capacity is higher

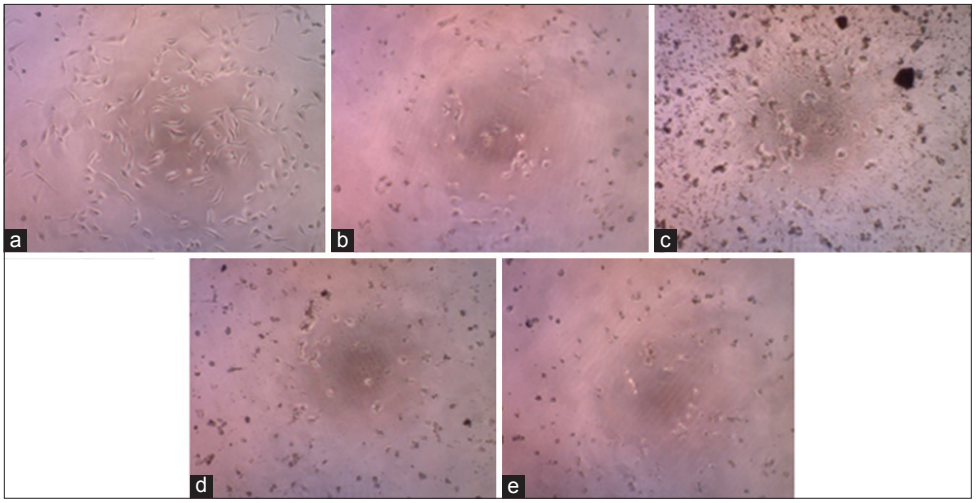

Fig. 2: Untreated Henrietta Lacks (HeLa) cancer cells (a); Proliferation inhibition by ethanol extract (b); by ethyl acetate fraction (c), by the isolated flavone (d), and by doxorubicin as positive control (e) on HeLa cancer cells

Table 8: Inhibition of sample on HeLa cancer cell proliferation

Concentration (µg/mL)	Sample (%)			Concentration (µg/mL)	Doxorubicin (%)
	Ethanol extract	Ethyl acetate fraction	Isolated flavone		
150	64.2±4.89	62.8±10.13	83.6±0.90	1	93.0±1.04
75	46.3±2.12	39.6±1.43	75.9±1.26	0.5	82.7±3.28
37.5	31.8±2.65	34.4±1.17	48.6±3.35	0.25	50.3±2.37
18.75	22.0±0.85	32.2±2.50	19.9±5.55	0.125	54.0±2.17
9.38	14.2±4.84	28.6±1.76	14.9±2.24	0.06	40.9±3.99
4.69	16.4±5.14	29.5±2.43	18.2±5.28	0.03	42.6±1.30

HeLa: Henrietta Lacks

Table 9: Cytotoxic effect of sample on HeLa cancer cell based on IC_{50} value

Samples	IC_{50} (µg/mL)
Ethanol extract	79.50
Ethyl acetate fraction	73.74
Isolated flavone	34.11

IC_{50}: Inhibitory concentration 50%

correspondingly with IC_{50} value approximately 20 µg/mL as compared with ethanol extract. The isolated flavone is predominated by apigenin. This isolated compound is potential as antioxidant and inhibiting the proliferation of HeLa cancer cells moderately with IC_{50} 34 µg/mL. Based on this study it is evident that peanut hulls should not be wasted but to be used as an herbal medicine instead. It is also important to test the isolated flavone toward other cancer cells before these materials can serve as a cancer drug.

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