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FULL PAPER

Cytotoxic activity of the purified extracts from duku (*Lansium domesticum* Corr.) Leaf against MCF-7 and HTB-183 cell lines, and the correlation with antioxidant activity

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One of the plants that potential to develop as an anticancer agent is Duku leaf (*Lansium domesticum* Corr.). From previous studies, Duku leaf extract had cytotoxic activity against several cancerous cell lines. The aim of this study was to determine the cytotoxic activity of crude and purified extracts of Duku leaf against MCF-7 and HTB-183 cell lines. The crude extract of Duku leaf was obtained using maceration in ethanol absolute. The vacuum liquid chromatography with a gradient mobile phase was performed to obtain the purified extracts. The crude and purified extracts were observed to inhibit MCF-7 and HTB-183 cells using 3-(4,5- dimethyl thiazol 2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay. The antioxidant properties of samples were determined using radical DPPH scavenging activity. The data was collected and analyzed to provide the inhibitory concentration 50 (IC_{50}) and Pearson's correlation was analyzed to describe the correlation between cytotoxic and antioxidant properties. Five purified extracts were obtained and tested against MCF-7 and HTB-183. The purified extract D has stronger anticancer and antioxidant activities than other samples with IC_{50} of 56.26 ± 3.11 µg/mL, 70.94 ± 2.92 µg/mL, and 53.65 ± 1.55 µg/mL, respectively ($p < 0.05$). We confirmed that cytotoxic activity has a strong correlation with antioxidant properties. Furthermore, the active compounds of purified extract D needed to be investigated and tested against MCF-7 and HTB-183 to explore the possible anticancer mechanisms.

KEYWORDS

Lansium domesticum corr; vacuum liquid chromatography; cytotoxic; antioxidant.

Introduction

According to the World Health Organization (WHO), cancer is the primary cause of mortality on a global scale. In 2020, breast and lung cancer exhibited the highest incidence rates, as reported by the WHO [1]. According to the American Society, it is projected that around 43,700 women will succumb to breast cancer in the United States in the year 2023 [2]. In addition, the estimated number of deaths from lung cancer is approximately

127,070, with 67,160 occurring in men and 59,910 in women [3]. China, as the largest and most densely populated upper-middle-income nation, has witnessed a notable increase in the incidence of breast cancer [4]. Breast cancer is the prevailing form of cancer, accounting for 16.6% of all cancer cases in Indonesia [5]. It stands as the second leading cause of cancer-related mortality, with a rate of 9.6%, following lung cancer, which holds a mortality rate of 13.2% [6].

The utilization of natural ingredients in cancer treatment has experienced a notable rise [7-9]. Herbal remedies have a strong historical and cultural significance in various societies and traditional practices [10-13]. There have been reports indicating that compounds derived from natural components had anticancer properties [14-17]. *Lansium domesticum* Corr., also referred to as Duku is a tropical fruit tree belonging to the Meliaceae family. It is extensively farmed primarily for local use and is indigenous to Southeast Asia, including Malaysia, Thailand, Indonesia, Vietnam, and the Philippines [18]. Multiple investigations have documented the potential medicinal qualities of Duku, including its anticancer [19], antidiarrheal [20], antipyretic [21], and anthelmintic [22] effects. The presence of alkaloids, flavonoids, tannins, triterpenoids/steroids, and saponins has been detected in the leaves of the Duku plant [18]. The primary objective of this investigation was to assess the anticancer properties of crude and purified extract from Duku leaves against MCF-7 and HTB-183 cell lines.

The MCF-7 cell line holds the distinction of being extensively investigated in human breast cancer research, making it the subject of several studies worldwide. The findings derived from this cell line have played a pivotal role in advancing our understanding of breast cancer and improving the prognosis and treatment options available to patients. Approximately 25,000 scientific articles have utilized this particular cell line. The MCF-7 cell line plays a significant role in elucidating the efficacy of pharmaceutical agents for the treatment of breast cancer, particularly in the transition from non-metastatic to metastatic stages. Hence, MCF-7 cells continue to be employed for cancer medication development in contemporary research [23,24]. Meanwhile, similar to the exploration of anti-breast cancer agents, an *in vitro* study was conducted to find anti-lung cancer agents. Commonly, lung cancer is classified into two main groups, these are non-small cell lung cancer (NSCLC) and small cell lung cancer. The three major subtypes of NSCLC are squamous cell carcinoma, adenocarcinoma, and large cell carcinoma. These cancer types account for nearly 80% of all lung cancer cases. Therefore, to develop an anti-lung cancer drug approach, HTB-183 cells which are part of large cell carcinoma can be used because they are considered to reflect a frequently occurring lung cancer condition [25]. However, molecular characteristics of cancer cells regarding survival are a main factor that needs to be considered [26].

Several parts of Duku were used to assess the anticancer activity against various cancer cell lines. As reported by Manosroi *et al.* (2012), the parts like ripe fruits, stalks, and young fruits from Duku have activity to inhibit HT-29 and KB cell lines [19]. While, Khalili *et al.* (2017) were successful to compared the anticancer activity of several fruit extracts from Duku against HT-29 [21]. On the other hand, the focus on the anti-breast cancer activity of Duku parts, Fadhilah *et al.* (2020) explain the fruit peels of Duku exhibit cytotoxicity against the T47D cell line [27]. Recently, Fadhilah *et al.* (2020) reported that the fruit peels of Duku have anticancer activity against HepG2 [28]. A similar activity was reported by Lubis *et al.* (2022), but utilizated the leaf part of Duku against HepG2 [29]. The utilization of Duku leaf as an anti-cancer not only against HepG2 cells. As reported by Lubis *et al.* (2023), the extract of Duku leaf has anti-pancreatic cancer against PANC-1 cells [30]. However, the study of Duku leaf extract and its

derived or fraction against MCF-7 and HTB-183 cells is not identified. This study was conducted to identify the anti-cancer activity of crude and purified extracts of Duku leaf against MCF-7 and HTB-183 cells. The process of purifying the extract involved the utilization of vacuum liquid chromatography. In addition, the mechanism by which purified extracts function as anticancer agents was elucidated through the assessment of their antioxidant activity utilizing the radical DPPH scavenging method.

Experimental

Materials

Dimethyl sulfoxide (DMSO), DMEM and RPMI medium, 0.25% trypsin EDTA, Fetal bovine serum, Fungizone® (Gibco, USA), 0.4% trypan blue, Penicillin-streptomycin, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT reagent) (Sigma, USA), Ethanol, Ethyl acetate, and n-hexane (Merck, USA), silica gel 60 for column (0.063-0.200 mm) and 2,2-Diphenyl-1-picrylhydrazyl (DPPH) (Sigma, USA). The MCF-7 and HTB-183 cell lines were collected from the Parasitology Laboratory, Universitas Gadjah Mada, Yogyakarta, Indonesia.

Extract preparation and purification

Duku leaf (*Lansium domesticum* Corr.) was obtained in Medan, North Sumatera, Indonesia. The plant species were verified and identified by botanical practice in the Herbarium Medanense, Universitas Sumatera Utara, Medan, Indonesia (Voucher ID: 2084/HM-USU/12/2023). The leaves were washed, dried in a drying cabinet at 40-50 °C, and ground using the conventional blender. Extraction of dry powder was finished using maceration. The 180 g dry powder was put into a container, soaked with 1800 mL of solvent (ethanol absolute), and kept out for 24 h at room temperature. Afterward, the macerate was obtained after filtration using Whatman No. 1. The macerate was evaporated using a rotary evaporator (Heidolph, Germany) to obtain a crude extract [31]. The crude extract was purified using vacuum liquid chromatography with silica gel 60 as an adsorbent and a combination of n-hexane: ethyl acetate in gradient (100:0 up to 10:90) and ethanol absolute for finishing the purification. The purified extracts were collected and categorized by Retention factor (Rf) values using thin-layer chromatography with sulfuric acid 50% as a visualization reagent. All purified extracts with the same kind of spotted profiles were grouped and kept in a temperature room before use [28,32].

Physicochemical analysis and phytochemical screening

Physicochemical analysis of dry powder and crude extract of Duku leaf include water content, total ash, acid insoluble ash content, water-soluble content, and ethanol soluble content [33]. Alkaloids were identified using color reagents such as Mayer, Bouchard, and Dragendroff. Shinoda and cyanidin test for flavonoids, tannin with $FeCl_3$ solution, and steroid/triterpenoids with Liebermann bourchard reagent [34].

Cytotoxic activity of crude and purified extract

The cytotoxicity activity was carried out using the MTT method. It is a calorimetric, non-radioactive, and quick test that converts 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), which is produced when the mitochondrial enzymes in a healthy cell convert the yellow MTT substrate to the insoluble purple formazan [35]. MCF-7 and HTB-183 cells were planted into each well of 96-well plates. Afterward, varied concentrations of crude and purified extract including 500, 250, 125, 62,5, and 31,25 $g.mL^{-1}$, were applied to the planted cells, and incubated for 24 h at 37 °C. Excess media

was discarded and fresh media was added, then 10 µL of tetrazolium dye was added and incubated for 4 h. After incubation, a color change from yellow to purple was observed. MTT reaction stopped with stopper reagent (SDS 10% in 0.1 N HCl). In addition, the absorbances were detected with a microplate reader at 595 nm wavelength [36]. The % viability cell was measured based on control absorbance and sample absorbance.

Antioxidant activity of crude and purified extract

The measurement of free radical scavenging activity was conducted using the DPPH technique. A 0.2 mM solution of DPPH in methanol was produced. Subsequently, 100 µL of this solution was added to a solution containing extracts at a concentration of 100 µg/mL. After 60 minutes, the measurement of absorbance was conducted at a wavelength of 516 nm. The calculation of the percentage of inhibition was performed by comparing the absorbance values obtained from the control group with those obtained from the samples [37].

Statistical analysis

Statistical analyses were run on SPSS software version 22. The Kolmogorov-Smirnov test was used to check and confirm the normality of the data distribution. Using the factorial ANOVA test, we evaluated the impact of the tested samples. Tukey's HSD post-hoc test was used to determine significance, and $p < 0.05$ was chosen as the threshold for statistical significance. Meanwhile, the correlation of cytotoxic and antioxidant activity was described using Pearson's correlation test.

Results and discussion

The determination of water content in the sample was set to maintain the quality of the sample. The less water content in dry powder and crude extract, the less possibility of contamination by mold growth. The water content should not be more than 10% for dry powder [38]. Determination of ash content was carried out to measure the total amount of external and internal minerals in a sample from the initial process to the end of manufacture. Determination of water and ethanol-soluble content was carried out to see the number of soluble compounds in water and ethanol solvents [39]. The results of the determination of dry powder and crude extract show that the results meet the requirements and are guaranteed quality based on Materia Medika Indonesia (MMI) [40] (Table 1).

TABLE 1 Physicochemical analysis of dry powder and crude extract of Duku leaf

Parameters	Dry powder	Crude extract
Water content (%)	6.62	10.55
Total ash content (%)	7.95	4.70
Acid-insoluble ash content (%)	4.45	2.52
Water soluble content (%)	18.40	NI
Ethanol soluble content (%)	12.60	NI

NI = Not Identified.

Based on the result of the phytochemical screening, Duku leaf has identified the presence of bioactive compounds and contains metabolites of alkaloid, flavonoid, saponin, steroid/triterpenoid, tannin, and glycoside. Dry powder and ethanol extract of Duku leaf showed positive results containing alkaloid compounds in 3 tests characterized by the formation of a white residue after adding the Mayer reagent, a brown residue after the addition of Bouchardat reagent and no red-orange residue formed when the

Dragendorff reagent added [41]. From the results of the examination of flavonoids, the addition of concentrated hydrochloric acid to Mg powder and amyl alcohol formed a yellow-orange color layer on the amyl alcohol layer [42]. Compounds of the saponin group were declared to contain saponins due to the presence of stable foam after administration of hydrochloric acid [43]. Steroid/ triterpenoid examination showed positive steroid was indicated by the appearance of a green color. In the tannin test, a positive result is indicated by a change in the color of the filtrate to blackish-blue [44]. The result was supported by research that mentioned crude extract of Duku leaf obtained alkaloid, flavonoid, steroid/ triterpenoid, and tannin (Table 2) [18].

TABLE 2 Phytochemical screening of dry powder and crude extract of Duku leaf

Secondary metabolites	Dry powder	Crude extract
Alkaloids	Positive	Positive
Flavonoids	Positive	Positive
Saponin	Positive	Positive
Steroids/triterpenoids	Positive	Positive
Tannin	Positive	Positive
Glycoside	Positive	Positive

The crude extract was purified using vacuum liquid chromatography, yielding 10 fractions. Each fraction was grouped according to the profile of spotted after visualized using 50% sulfuric acid (Figure 1). The Rf values of spotted were identified and found in 5 purified extracts with different Rf values and color of the spot (Table 3). Two purified extracts, A and B come from one fraction each. Two spots were determined from purified extract A with Rf of 0.8125 (blue) and 0.875 (blue), and then three spots were identified from purified extract B with Rf 0.6875 (red), 0.8125 (red), and 0.875 (red), respectively. These results described the purified extracts A and B as almost similar based on the identified spots. The purified extract C was a purified extract from the merger of many fractions. Each fraction has 7 spots with Rf of 0.3125 (red), 0.4375 (red), 0.5625 (purple), 0.6875 (red), 0.8125 (red), and 0.875 (blue), respectively. In addition, the purified extract D was generated from a merger of two fractions. Fractions 8 and 9 contained 7 spots with Rf of 0.125 (blue), 0.3125 (red), 0.4375 (red), 0.5625 (purple), 0.6875 (red), 0.8125 (red), and 0.875 (blue), respectively. Similar to purified extract D, the purified extract E was produced from two fractions with six spots. The Rf values of each spot were 0.125 (blue), 0.3125 (red), 0.4375 (red), 0.5625 (purple), 0.6875 (red), and 0.875 (blue). Afterward, the steroid/triterpenoids compound of each purified extract was identified with Liebermann-Burchard reagent. This method was used to detect and analyze compounds in the sample [45]. Lieberman-Burchard reagent for detection of steroid and triterpenoid compounds, with positive tests for the appearance of green-blue for steroid and the appearance of red, brown, or purple for triterpenoid [46]. At the chromatogram, each purified extract had an appearance of green, brown, and purple which indicates the presence of steroid and triterpenoid compounds (Figure 2).

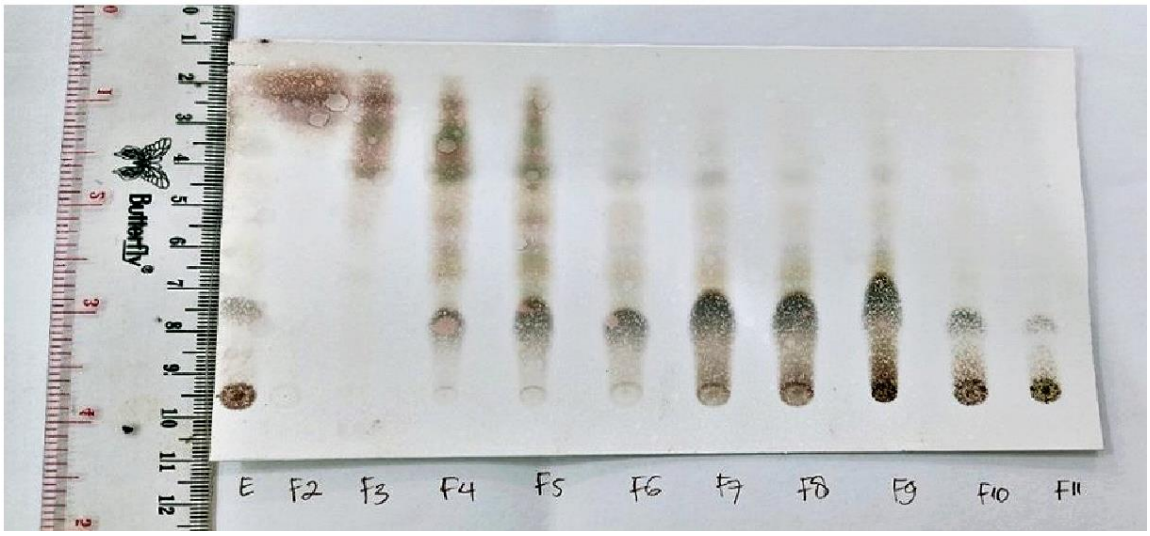

FIGURE 1 Thin layer chromatography (TLC) profile with gradient mobile phase (n-hexane: ethyl acetate) in silica gel F254 after being sprayed with 50% sulfuric acid

TABLE 3 The Retention factor (Rf) values of chromatograms after purification using vacuum liquid chromatography

Fraction Composition	weight (g)	Yield (%)	Many stains	Rf	Stain color	Purified extracts
Fraction 1	0	0	-	-	-	
Fraction 2	0.0537	1.79	2	0.8125; 0.875	Blue; Blue	A
Fraction 3	0.16	5.33	3	0.6875; 0.8125; 0.875	Red; Red; Red	B
Fraction 4	0.2316	7.72	6	0.3125; 0.4375; 0.5625; 0.6875; 0.8125; 0.875	Red; Red; Red purple; Red; Red; Blue	C
Fraction 5	0.2317	7.72	6	0.3125; 0.4375; 0.5625; 0.6875; 0.8125; 0.875	Red; Red; Red purple; Red; Red; Blue	
Fraction 6	0.1944	6.48	7	0.125; 0.3125; 0.4375; 0.5625; 0.6875; 0.8125; 0.875	Blue; Red; Red; Red purple; Red; Red; Blue	
Fraction 7	0.1523	5.07	7	0.125; 0.3125; 0.4375; 0.5625; 0.6875; 0.8125; 0.875	Blue; Red; Red; Red purple; Red; Red; Blue	
Fraction 8	0.1193	3.97	7	0.125; 0.3125; 0.4375; 0.5625; 0.6875; 0.8125; 0.875	Blue; Red; Red; Red purple; Red; Red; Blue	D
Fraction 9	0.0927	3.09	7	0.125; 0.3125; 0.4375; 0.5625; 0.6875; 0.8125; 0.875	Blue; Red; Red; Red purple; Red; Red; Blue	
Fraction 10	0.0732	2.44	7	0.125; 0.3125; 0.4375; 0.5625; 0.6875; 0.8125; 0.875	Blue; Red; Red; Red purple; Red; Red; Blue	
Fraction 11	0.2103	7.01	6	0.125; 0.1875; 0.3125; 0.375; 0.6875; 0.75	Blue; Red; Red; Red purple; Red; Red; Blue	E

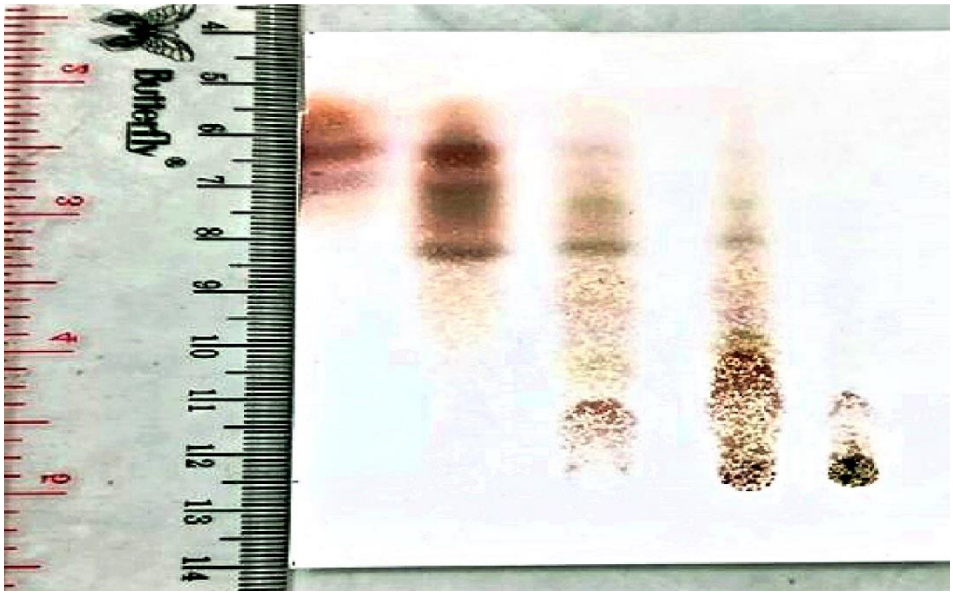

FIGURE 2 Detection of steroids/triterpenoids compound of purified extract using thin layer chromatography (TLC) with Liebermann-Bourchard

Cytotoxic activity test from crude and purified extracts of Duku leaf was carried out against MCF-7 and HTB-183 cells using the MTT method. The absorbance was measured at 595 nm using a microplate reader. Absorbance obtained from the test results is used to calculate cell viability. The cancer cells were treated with various concentrations of samples ranging from 31.25 µg/mL to 500.00 µg/mL. This study showed that the higher concentration gave the smaller % viability in living cancer cells (Figure 3). The 500.00 µg/mL of samples have the strongest activity to kill cancer cells, even the % viability of MCF-7 and HTB-183 cells after treatment with the crude, purified extracts C, D, and E in this concentration is 0.00% ± 0.00, respectively. After the concentration was decreased to 125.00 µg/mL, the purified extract D had a superior activity compared to the other samples in inhibiting the MCF-7 and HTB-183 cells of 0.00% ± 0.00 and 4.67% ± 1.89 with $p < 0.05$, respectively (Figure 3A and 3B). The inhibitory concentration 50 (IC_{50}) of each sample supported the purified extract D as the strongest sample than the other of 56.26 ± 3.11 µg/ mL against MCF-7 cells and 70.94 ± 2.92 µg/ mL against HTB-183 cells (Table 4). The lowest IC_{50} showed the best cytotoxic activity [47]. This phenomenon is not applied in all purified extracts. The IC_{50} showed that purified extracts have different cytotoxic activity against MCF-7 and HTB-183 cell lines. In these cases, only purified extract D has an IC_{50} value under 100 µg/mL against two both cancer cells. Other purified extract was identified as similar to crude extract with IC_{50} in a range of 100-200 µg/mL. However, this study showed the purified extract A has IC_{50} more than 200 µg/mL against MCF-7 and HTB-183 cell lines. This situation illustrated that the active compound from Duku leaf was contained in purified extract D more than other purified extract even the crude extract.

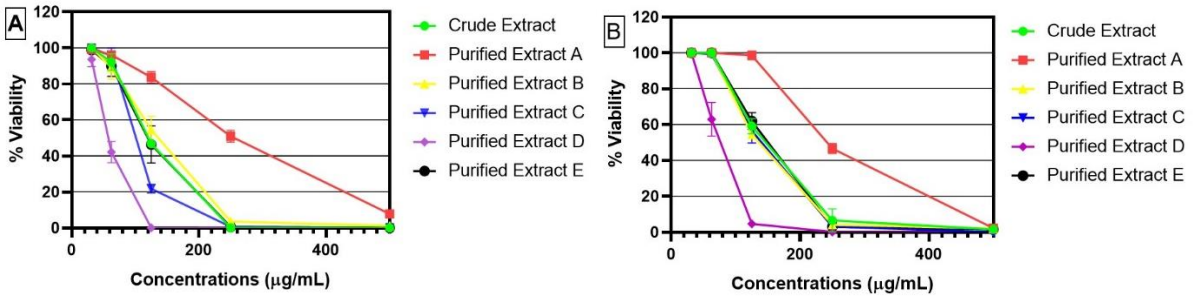

FIGURE 3 The % viability of MCF-7 and HTB-183 cells after treatment using crude and purified extracts for 24 hours. All data are described in mean ± SD, n = 3. A: % viability of MCF-7 cells, B: % viability of HTB-183 cells

This study described the cytotoxic activity of crude and purified extracts against MCF-7 and HTB-183 cells, and the result is similar to the previous study. The ethanolic extract of Duku leaf was reported to have cytotoxic activity against $HepG_2$ with IC_{50} of 19.93 ± 0.93 µg/mL. That was reported the extract has a mechanism to inhibit PI3K/Akt/mTOR pathways [29]. On the other hand, the ethanolic extract of Duku leaf identified a strong agent to inhibit the PANC-1 cells with IC_{50} of 28.61 ± 0.13 µg/mL [30]. One of the secondary metabolites that exerts anticancer activity is the terpenes group [48]. The mechanism of action of terpenoids as anticancer is to inhibit the cell cycle [49], inhibit cell proliferation [50], induce apoptosis [51], block the transition of epithelial cells to mesenchymal cells [52], inhibit migration and invasion of cancer cells [53], and modulate the immune system PD-L1 [54].

The crude and purified extracts showed antioxidant properties. Several concentrations were created from the crude and purified extracts ranging from 31.25 µg/mL to 500.00 µg/mL. The purified extract D showed the highest % scavenging DPPH activity compared to the other samples in 125.00 µg/mL with the values of 72.99% ± 2.37, significantly different with $p < 0.05$ (Figure 4). After IC_{50} calculation, the purified extract D has stronger antioxidant properties than other samples of 53.65 ± 1.55 µg/mL, with $p < 0.05$ (Table 4). The antioxidant properties of purified extract D describe a kind of reason for the strongest cytotoxic activity between samples and prove a strong correlation between antioxidant properties and cytotoxic activity (Table 5). Many studies have illustrated the correlation of antioxidant properties with cytotoxic activity [55]. The presence of antioxidant compounds increases the activity to kill cancer cells [56]. Several studies show that antioxidant compounds like phenolic and flavonoid have a role in inducing some proteins to lead the cell cycle inhibition and apoptosis induction [57,58].

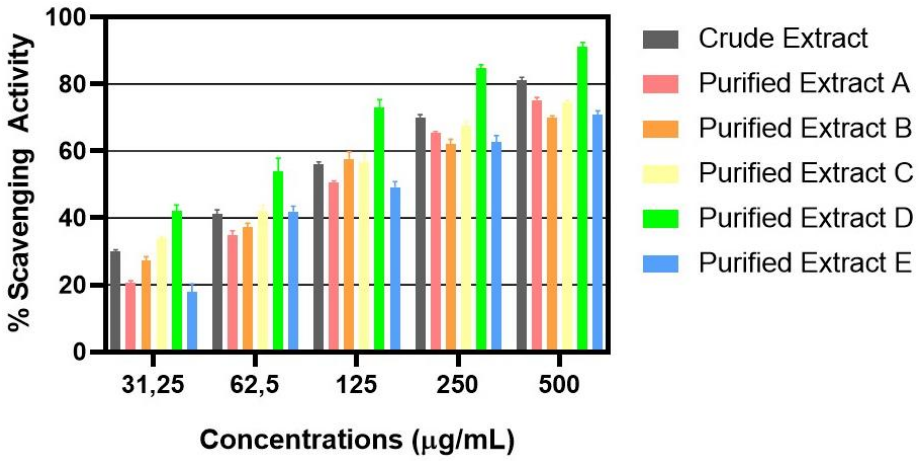

FIGURE 4 The % DPPH scavenging activity after treatment using crude and purified extracts. All data are described in mean ± SD, n = 3**.**

TABLE 4 The inhibitory concentration 50 (IC_{50}) values of crude and purified extracts (All data described in mean ± SD, n =3)

Samples	IC_{50} (µg/mL) MCF-7	HTB-183	DPPH Scavenging
Crude extract	114.56 ± 2.80	142.68 ± 9.97	110.24 ± 1.80
Purified extract A	229.14 ± 4.48	246.96 ± 10.43	126.34 ± 1.35
Purified extract B	123.44 ± 6.74	142.77 ± 8.91	117.87 ± 2.05
Purified extract C	101.58 ± 3.70	133.45 ± 6.32	112.46 ± 2.21
Purified extract D	56.26 ± 3.11	70.94 ± 2.92	53.65 ± 1.55
Purified extract E	112.23 ± 11.95	141.66 ± 7.46	151.23 ± 1.67

TABLE 5 Pearson's correlation and significant values between cytotoxic and antioxidant activities

	Antioxidant properties	Cytotoxic activity (MCF-7)	Cytotoxic activity (HTB-183)
Antioxidant properties	-	R = 0.986 $p < 0.05$	R = 0.952 $p < 0.05$
Cytotoxic activity (MCF-7)	R = 0.986 $p < 0.05$	-	-
Cytotoxic activity (HTB-183)	R = 0.952 $p < 0.05$	-	-

Conclusion

The present study described the cytotoxic activity of crude and purified extracts from Duku leaf against MCF-7 and HTB-183 cell lines. Based on the findings, the purified extract D has better cytotoxic and antioxidant activities than the crude extract and other purified extracts. These results described the opportunity to develop anti-breast and lung cancer agents. Further investigations will be needed to describe the active compounds in purified extract D and provide an explanation or scientific reason for the anti-cancer mechanism of purified extract D through several tests such as cell cycle arrest inhibition, apoptosis induction, and protein expression test.

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Conflict of interest

All authors declare to have no conflict of interest.

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