**Paederia foetida** Linn Leaves-Derived Extract Showed Antioxidant and Cytotoxic Properties Against Breast Carcinoma Cell

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**ABSTRACT**

*Paederia foetida* Linn. is a rich source of active compounds, yet its antioxidant and cytotoxic properties remain poorly studied. This current research aimed to investigate the chemical profiles of a crude extract derived from *P. foetida* leaves, its fractionated substances, and their antioxidant and cytotoxic properties on MCF-7 cells, a breast carcinoma cell. Six fractionated substances have been separated in thin layer chromatography (TLC) plate. Five fractions showed antioxidant properties, as indicated by the formation of the yellowish band on the TLC plate after spraying with the DPPH reagent. Furthermore, crude extract of *P. foetida* leaves and its fractions are also characterized by antioxidant properties against 2, 2'-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radicals. F1 fraction exhibited the most potent antioxidant activity with IC₅₀ values of 10.94±2.67 and 50.04±0.48 µg/ml, respectively, toward DPPH and ABTS radicals. Crude extract and all six fractions also varied in their cytotoxic properties against MCF-7 cells, with IC₅₀ values ranging from 410.24±0.30 to 831.57±6.91 µg/ml. F3 fractions showed the strongest cytotoxicity (IC₅₀ value: 410.24±0.30 µg/ml). Methanolic extract and active plant leaf fractions contained flavonoid and phenolic compounds, as measured by aluminum chloride and Folin-Ciocalteu reagent, respectively. Liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis showed that phenol, flavonoid, alkaloid, limonoid, and steroid were the major compound found in the crude extract and fractions. In conclusion, the present study proved that crude extract of *P. foetida* leaves and its fractionated substances were potential as antioxidant and cytotoxic agents.

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**1. Introduction**

Oxidants and free radicals are generally produced during the physiological processes of aerobics metabolism of human cells. A high number of these oxidizing agents in the body could be the internal factor for causing oxidative stress at the cellular and tissue level because of their capability to damage DNA and other cellular molecules. Therefore, oxidants and free radicals could damage healthy cells, disrupt cell structure and function, and develop many degenerative diseases, such as diabetes, cancer, cataracts, cardiovascular disease, neurodegeneration, and immune system weakening (Rani 2017). However, the human body has a defensive mechanism against free radicals by producing superoxide dismutase, glutathione peroxidase, and catalase (Kurutas 2015). However, supplementation of natural antioxidants is still necessary to protect the body from the deleterious properties of oxidants and free radicals.

Cancer is one of the critical public health problems across the globe. Among various cancers, lung, breast, and colorectum cancer are of great concern globally, responsible for 11.6%, 11.6%, and 10.2% of all new cancer incidences, respectively, in 2018. In females, breast cancer is one of the most frequently diagnosed cancers and is responsible for 6.6% mortality of 9.6 million deaths (WHO...
Nevertheless, new cases and mortalities remain rising because of increasing life expectancy, demographic, and epidemiological transitions (Wong et al. 2021). Natural products derived from plant provides an endless source of new drugs, mainly antioxidant and anticancer agents. Paederia foetida Linn, locally known in Indonesia as sembukan, is an aromatic perennial climbing plant commonly used as a leafy vegetable that can be consumed raw or steamed. This plant contain several phytochemicals, including steroid, saponin, alkaloids, flavonoids, vitamin C, and volatile oils (Mazumder et al. 2018; Patel 2017; Rosli et al. 2013). Moreover, extract originating from this plant is also well studied to have some biological activities, such as antibacterial and antibiofilm (Priyanto et al. 2022), antifungal (Morshed et al. 2012), antihyperlipidemic, antihyperglycemic, antioxidant (Kumar et al. 2014), anti-melanogenic (Chung et al. 2021), chemoprotective (Li et al. 2021), and cytotoxic activities on human prostate cancer cells (Pradhan et al. 2019). In the earlier study, fresh and dried P. foetida leaf extracts (collected from Malaysia) were reported to have promising antioxidant activity up to 68% and 76%, respectively, correlated with their total phenolic content (Osman et al. 2009). In addition, its methanolic extract also showed antioxidant activity with IC$_{50}$ ranging from 538.97-859.20 µg/ml. However, different plant growth areas and extraction techniques may result in different content and solubility of active components.

Plant extract obtained from different sources and extraction techniques may have different antioxidant activities. Nonetheless, there is no comprehensive study about the purified compounds of P. foetida leaves related to their antioxidant and anticancer activities, especially P. foetida leaves collected from Indonesia. This research is also the first study investigating the cytotoxic property of P. foetida leaves extract on human breast cancer cells. This recent study aimed to evaluate breast carcinoma cells antioxidant and anticancer potentials of the crude extract and fractionated substances derived from P. foetida leaves. In addition, the total flavonoids and phenolic compounds of its whole extract and active fractions were also studied.

2. Materials and Methods

2.1. Sample Sources and Extraction

Paederia foetida Linn fresh leaves obtained from Tasikmalaya, West Java, Indonesia, were washed with water and dried at 60°C. Powder plant materials (100 g) were extracted in 1,000 ml methanol (1:10 w/v) for 24 h at room temperature on an orbital shaker with an agitation of 120 rpm. Subsequently, the mixture was then filtered through Whatman no.1 paper. The filtrate was evaporated using an evaporator at 40°C. This crude extract was stored at 4°C prior to bioactivity analysis.

2.2. Thin Layer Chromatography (TLC) Analysis and Antioxidant Bioautography

TLC analysis was conducted using TLC silica gel 60 F254 pre-coated aluminum-backed TLC plates (Merck, Germany) as the stationary phase. Briefly, 10 µl of crude extract (0.032 g/ml) was applied to the TLC plate. After the spots were dried, the TLC plates were developed using the mobile phase (ethyl acetate and hexane (7:3 v/v)) (Priyanto et al. 2022). The corresponding TLC plate was then eliminated from the solvents and dried at ±28°C, followed by visualization under visible light and UV 366 nm. Subsequently, the TLC plates were immersed in DPPH solution (5 mM) for 1 minute for their bioautography derivatization. Furthermore, the active bands associated with antioxidant activity indicated by a yellowish band against a purple DPPH background were isolated in a preparative-TLC Silica Gel GF 500 micron 20 × 20 cm (Uniplate Miles Scientific, USA).

2.3. Antioxidant Activity Assay

The antioxidant activity of P. foetida leaf extract and its fractionated constituents were evaluated using different radicals, including 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2, 2’-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS). In the DPPH assay, 100 µl DPPH solution (125 µM) was mixed with 100 µl of extract or fractions and incubated for 30 minutes prior to be observed. In the ABTS assay, ABTS radicals were produced by reacting potassium persulfate (2.45 mM) (1:1) and ABTS solution (7 mM), followed by incubation at ±28°C for 12-14 hours in the dark. Further, 180 µl ABTS radical was reacted with
20 µl samples and incubated for 30 minutes at room temperature. The absorbance of both DPPH and ABTS assays was determined at 515 nm and 734 nm using Varioskan Flash (ThermoFischer). Ascorbic acid and quercetin were used as a positive control. All assays were performed in triplicates. The inhibition values of each sample were calculated using the following formula:

\[
\text{Inhibition value(%) = } \frac{A_0 - A_1}{A_0} \times 100%
\]

\(A_0\) represents the absorbance of radicals without samples (blank), and \(A_1\) = the absorbance of samples. The data are presented as the inhibitory concentration of 50% (IC\(_{50}\)) following a particular method (Prastya et al. 2020).

### 2.5. Determination of Total Flavonoid and Phenolic Compounds

The total flavonoid content (TFC) of crude extract and selected fractions was estimated according to the aluminum chloride (AlCl\(_3\)) colorimetric methods. Shortly, 2.45 ml of distilled water was mixed with 500 µl sample (1,000 µg/ml) and 150 µl of 5% NaNO\(_2\), followed by homogenized and incubated for 2 minutes at ±28°C. The mixtures were further reacted with 150 µl of 10% aluminum chloride and incubated for 8 minutes at ±28°C. Subsequently, 2 ml of NaOH (1 M) was added, and the absorbance was read at 510 nm using an ELISA reader Thermo Scientific Varioskan Flash (ThermoFischer). The amount of total flavonoid was presented as mg quercetin equivalent (QE)/g dry extract weight as derived from a standard curve \((Y = 0.0007X + 0.0384, R^2 = 0.9559)\) of quercetin (0–100 µg/ml). On the other hand, total phenolic content (TPC) was carried out using the Folin-Ciocalteu colorimetric method. Briefly, 250 µl of the Folin-Ciocalteu reagent was reacted with 500 µl of sample solution (1,000 µg/ml), and 3.5 ml distilled water along with the incubation for 8 min at ±28°C. Next, 750 µl of 20% sodium bicarbonate solution was supplemented and incubated for 2 hours at ±28°C. The absorbance was further detected at 765 nm. The TPC was expressed in mg gallic acid equivalent (GAE)/mg extract as calculated from a calibration curve \((Y = 0.0441X + 0.2525, R^2 = 0.9795)\) of gallic acid (0–100 µg/ml). TPC and TFC were performed in triplicates (Prastya et al. 2019).

### 2.6. Liquid Chromatography Quadrupole-Mass Spectrometry (LC-MS/MS) Analysis

\(P.\ foetida\) leaf extract and its three selected fractions (F1, F2, and F3) attributed to the best antioxidant and cytotoxic properties were analyzed using LC-MS/MS (liquid chromatography-tandem mass spectrometry) Xevo G2-XS QToF (Quadrupole Time-of-Flight) instrument (Waters Milford, Massachusetts, USA). Separation was conducted on Waters BEH C18 column 1.8 µm (50 mm) in MSn positive mode. The gradient was set from 5% B (solvent B: Acetonitrile + 0.1% formic acid; solvent A: Ultrapure water + 0.1% formic acid) held for 1 minute and increased to 100% B for 10 minutes, held in 100% in 3 minutes followed by re-conditioning to
initial gradient in 3 minutes with the flowrate 0.3 ml/min and injection volume 1 µl (concentration 1 mg/ml). Post-processing analysis was performed on UNIFI (version 1.5) software and compared to the instrument’s built-in library (Waters Traditional Natural Product Library version 1.8), including the accurate mass, isotopic pattern, and mass fragmentation (Priyanto et al. 2022).

2.7. Statistical Analysis
All experimental data were presented as mean ± SEMs (n = 3). Furthermore, data obtained from cytotoxicity and the antioxidant assay was analyzed using one-way ANOVA followed by multiple Duncan test ranges. A p-value of less than 0.05 was considered statistically significant.

3. Results

3.1. TLC Antioxidant Bioautography
*P. foetida* leaves crude extract was successfully separated in TLC plates. Six different fractions with various retention factor (Rf) values have been found and coded as F6, F5, F4, F3, F2, and F1 (Figure 1). After spraying with DPPH solution, 5 (F1, F2, F3, F4, F6) out of 6 fractions exhibited yellowish bands, considered active bands characterized by antioxidant properties. All active fractions were then subjected to preparative TLC and then scratched out for further analysis. These selected fractions were further diluted with 96% ethanol, followed by evaporation until obtaining these fractions before further analysis.

3.2. Antioxidant Activity
In this study, crude extract and fractionated substances derived from *P. foetida* leaves showed various antioxidant capacities against DPPH and ABTS free radicals. As shown in Table 1, fraction 1 showed the best inhibitory potential against these two free radicals compared to other samples, followed by crude extract, F2, and F3 fractions. The IC50 values of this fraction were 10.94±2.67 µg/ml against DPPH and 50.04±0.48 µg/ml against ABTS. However, the IC50 values of all samples tested are still higher than ascorbic acid and quercetin as the positive control.

3.3. Cytotoxic Property
The crude extract of *P. foetida* leaves and its fractions were found to have different cytotoxic properties on MCF-7 cells, with IC50 values ranging from 410.24±0.30 to 831.57±6.91 µg/ml (Table 2). F3 fraction showed the greatest potential of cytotoxic property on MCF-7 cells compared to other samples, as indicated by its IC50 value of 410.24±0.30 µg/ml, followed by crude extract (550.12±5.14 µg/ml) and F1 fraction (520.34±4.33 µg/ml). Doxorubicin as positive control has an IC50 value of 16.71±4.01 µg/ml, which was considered higher cytotoxic activity than all fractions.

3.4. Total Phenolic and Flavonoid Content

![Figure 1. Chromatographic TLC plate of methanolic extract of *P. foetida* leaves using mobile phase from the mixture of ethyl acetate and n-hexane (7:3 v/v) visualized under (A) UV 366 nm, (B) visible light, and (C) after stained with DPPH reagent in visible light](image)

Table 1. Antioxidant activity of *P. foetida* leaf extract and fractions against DPPH and ABTS free radicals

<table>
<thead>
<tr>
<th>Sample</th>
<th>Antioxidant activity (IC50 value ± standard deviation in µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DPPH</td>
</tr>
<tr>
<td>Crude extract</td>
<td>14.74±1.57d</td>
</tr>
<tr>
<td>Fraction 1 (F1)</td>
<td>10.94±2.67e</td>
</tr>
<tr>
<td>Fraction 2 (F2)</td>
<td>19.47±0.44e</td>
</tr>
<tr>
<td>Fraction 3 (F3)</td>
<td>86.03±1.94e</td>
</tr>
<tr>
<td>Fraction 4 (F4)</td>
<td>304.97±9.81h</td>
</tr>
<tr>
<td>Fraction 5 (F5)</td>
<td>&gt;800</td>
</tr>
<tr>
<td>Fraction 6 (F6)</td>
<td>294.21±9.45f</td>
</tr>
<tr>
<td>Ascorbic acid</td>
<td>5.84±0.40b</td>
</tr>
<tr>
<td>Quercetin</td>
<td>3.08±0.24a</td>
</tr>
</tbody>
</table>

All data presented as mean ±SD of triplicates (n = 3) test. Values with different superscript letters in the same column represent significantly different (P<0.01).
The total flavonoid and phenolic contents in *P. foetida* leaf methanolic extract and its active fractions are provided in Table 3. The total phenolic of the crude extract was higher than the total flavonoid compound. The extract's total phenol and flavonoid were 154.76±3.37 mgGAE/g extract and 138.86±12.12 mgQE/g extract, respectively. The total flavonoid and phenolic compounds of crude extracts are also higher than those selected fractions (Fraction 1, fraction 2, and fraction 3). Fraction 1 contained the highest total phenolic and flavonoid compounds compared to other fractions. Total phenolic and flavonoid compounds of fraction 1 were 57.45±1.88 mgGAE/g extract and 50.48±0.35 mgQE/g extract, respectively.

### 3.5. Chemical Profiles

The chemical composition of *P. foetida* leaf-derived extract and three active fractions attributed to antioxidant and cytotoxic properties have been identified through LC-MS/MS approach (Table 4). The major components with diverse retention times and abundance found in the crude extract were 4-O-caffeoylquinic acid-1, quercimeritrin, digiprolactone, azedarachin C, stigmastan-3,6-dione, and other unknown compounds (Figure 2). The more purified component was identified in fractionated substances. F1 fraction contained dihydrocaffeic acid 3-O-glucuronide, 4-O-caffeoylquinic acid-1, quercetin, and neohesperidin. F2 fraction contained azedarachin C. F3 fraction contained cuscohygrine, azedarachin C, and pheophorbide A. Unfortunately, this method could not identify several compounds in the crude extract and fractions.

### 4. Discussion

Plant materials are well known as rich sources of secondary metabolites with various biological activities. This study evaluated the antioxidant and cytotoxic properties of crude extracts and fractions from *P. foetida* leaves. A TLC bioautography method

### Table 2. Cytotoxic property of *P. foetida* leaf extract and its selected active fractions against MCF-7 cell line

<table>
<thead>
<tr>
<th>Sample</th>
<th>IC$_{50}$ value (µg/ml)</th>
<th>Retention time (min)</th>
<th>Molecular formula</th>
<th>Chemical class</th>
<th>Bioactivity</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude extract</td>
<td>550.12±5.14</td>
<td>5.31</td>
<td>C$<em>{35}$H$</em>{38}$O$_9$</td>
<td>Phenol</td>
<td>Antioxidant; Anti-inflam-matory; Antiinflam-matory</td>
<td>Ganzon et al. 2018; Motaal et al. 2016</td>
</tr>
<tr>
<td>Fraction 1 (F1)</td>
<td>520.34±4.33</td>
<td>6.48</td>
<td>C$<em>{21}$H$</em>{20}$O$_{12}$</td>
<td>Flavonoid</td>
<td>Antioxidant; cytotoxic on MCF-7 cells; amylose secretion activity</td>
<td>Shin et al. 2010; Ahmed et al. 2016; Park et al. 2019</td>
</tr>
<tr>
<td>Fraction 2 (F2)</td>
<td>750.11±6.36</td>
<td>8.10</td>
<td>C$<em>{14}$H$</em>{14}$O$_3$</td>
<td>Glycoside</td>
<td>Insecticidal</td>
<td>El-Ghany et al. 2012</td>
</tr>
<tr>
<td>Fraction 3 (F3)</td>
<td>410.24±0.30</td>
<td>10.58</td>
<td>C$<em>{29}$H$</em>{48}$O$_{2}$</td>
<td>Steroid</td>
<td>Anti-inflammatory</td>
<td>Okoye et al. 2010</td>
</tr>
</tbody>
</table>

### Table 3. Total flavonoid and phenolic contents of *P. foetida* leaf extract and its active fractions

<table>
<thead>
<tr>
<th>Sample</th>
<th>Total flavonoid (mgQE/g extract)</th>
<th>Retention time (min)</th>
<th>Molecular formula</th>
<th>Chemical class</th>
<th>Bioactivity</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude extract</td>
<td>138.86±12.12</td>
<td>138.86±12.12</td>
<td>C$<em>{35}$H$</em>{38}$O$_9$</td>
<td>Phenol</td>
<td>Antioxidant; Anti-inflam-matory; Antiinflam-matory</td>
<td>Ganzon et al. 2018; Motaal et al. 2016</td>
</tr>
<tr>
<td>Fraction 1 (F1)</td>
<td>50.48±0.35</td>
<td>50.48±0.35</td>
<td>C$<em>{8}$H$</em>{10}$O$_2$</td>
<td>Phenol</td>
<td>Antioxidant; Anti-inflam-matory; Antiinflam-matory</td>
<td>Shin et al. 2010; Ahmed et al. 2016; Park et al. 2019</td>
</tr>
<tr>
<td>Fraction 2 (F2)</td>
<td>22.67±6.55</td>
<td>22.67±6.55</td>
<td>C$<em>{12}$H$</em>{30}$O$_5$</td>
<td>Phenol</td>
<td>Anti-inflammatory</td>
<td>El-Ghany et al. 2012</td>
</tr>
<tr>
<td>Fraction 3 (F3)</td>
<td>17.05±3.55</td>
<td>17.05±3.55</td>
<td>C$<em>{34}$H$</em>{40}$O$_9$</td>
<td>Phenol</td>
<td>Anti-inflammatory</td>
<td>Okoye et al. 2010</td>
</tr>
</tbody>
</table>

All data presented as mean ±SD of triplicates (n = 3) test. Values with different superscript letters represent statistically different (P<0.01)
was done to purify the chemical components of *P. foetida* leaf extract and preliminary screen their antioxidant activity. After separation on TLC plates, six fractions with diverse *R*<sub>f</sub> values have been found. Five fractions (F1, F2, F3, F4, and F6) out of six fractions exhibited yellowish bands, indicating that these fractions have antioxidant potential. Furthermore, all fractions and crude extracts were subjected to an antioxidant analysis against DPPH and ABTS free radicals to determine and verify their antioxidant activity. The lower IC<sub>50</sub> value indicates the higher antioxidant property of the tested materials. The DPPH radical scavenging activity of *P. foetida* leaf crude extract and fractions varied widely, with IC<sub>50</sub> values between 10.94±2.67 µg/ml and >800 µg/ml. F1 fraction displayed the strongest scavenging activity against DPPH radical (10.94±2.67 µg/ml), followed by crude extract (14.74±1.57 µg/ml), F2 fraction (19.47±0.44 µg/ml), F3 fraction (86.03±1.94 µg/ml), and other samples. In line with this result, the antioxidant activity of all samples against ABTS free radicals also showed a similar pattern. The F1, F2, F3 fractions and crude extract consistently exhibited greater antioxidant activity against ABTS free radicals than other fractions. The best radical scavenging activity both against ABTS and DPPH free radicals was showed by F1 Fraction (IC<sub>50</sub>/DPPH: 10.94±2.67 µg/ml; IC<sub>50</sub>/ABTS: 50.04±0.48 µg/ml). However, scavenging activity of crude extract and fractions (F1, F2, F3) against both DPPH and ABTS is considered a high antioxidant activity compared to the earlier studies by employing other plant leaf samples, such as *Euphorbia retusa* (IC<sub>50</sub>/DPPH: 287.52 µg/ml; IC<sub>50</sub>/ABTS: 126.75 µg/ml) (Lahmadi et al. 2019), *Raphanus sativus* (IC<sub>50</sub>/DPPH: 216.8 µg/ml; IC<sub>50</sub>/ABTS: 359.7 µg/ml) (Noman et al. 2021), and *Cucumis africanus* (IC<sub>50</sub>/DPPH: 955 µg/ml; IC<sub>50</sub>/ABTS: 69 µg/ml) (Abifarin et al. 2019). However, F5 and F6 fractions of *P. foetida* leaf extract are more active in antibacterial activity against *Escherichia coli* and *Mycobacterium smegmatis* which was evaluated in the previous study (Priyanto et al. 2022).
The present study reported the cytotoxic property of crude extract of *P. foetida* leaves and its fractions on MCF-7 cells. The extract and fractions suppressed the cell viability in a concentration-dependent manner. The best cytotoxic property was performed by F3 fraction with an IC\textsubscript{50} value of 410.24±0.30 µg/ml. In the earlier study, the crude extract of *P. foetida* leaves and its purified compounds also showed the cytotoxic property to prostate cancer cells (PC3 and DU145) with higher IC\textsubscript{30} values ranging from 15,000 to 20,000 µg/ml (Pradhan \textit{et al.} 2019). However, the inhibition of cancer cell growth and proliferation may influence by the resistance of each cancer cell and the concentration of the active substances.

Phenolic and flavonoid compounds are the largest active components found in plants and are considered to have positive effects on human health, including antioxidant and cytotoxic agents. In the present study, the methanolic extract of *P. foetida* leaves contained the highest phenolic (154.76±3.37 mgGAE/g extract) and flavonoid compounds (138.86±12.12 mgQE/g extract) compared to its fractions. Furthermore, compared to other active fractions, fraction 1 showed the highest flavonoid...
and phenolic content of 50.48±0.35 mgQE/g extract and 57.45±1.88 mgGAE/g extract, respectively. The earlier study also reported that phenolic compounds are found as the most dominant compound on _P. foetida_ leaves, either extracted with methanol or other solvents (Satapathy and Pattnaik 2020). A high content of flavonoid and phenolic compounds may be responsible for this extract’s antioxidant and cytotoxic activities. The hydroxyl (OH) groups in both those compounds are responsible for redox properties in free radical scavenging activity (Pourreza 2013; Panche _et al._ 2016). Many phenolic compounds also showed cytotoxic effect on cancer cells by reducing cancer cells proliferation capability, arresting the cell cycle, and causing apoptosis (Abotaleb _et al._ 2020).

LC-MS/MS analysis was conducted to investigate the chemical profile of _P. foetida_ leaf crude extract and its fractionated substances that may contribute to their antioxidant and cytotoxic properties. Crude extract and three selected fractions of this plant leaf contained several chemical classes, such as phenol, flavonoid, glycoside, limonoid, steroid, alkaloid, and chlorophyll derivatives. This study identified several compounds, such as quercetin, stigmasterol, and caffeic acid, commonly found in the _Paederia_ genus (Wang _et al._ 2014). These compounds possibly play a vital role in the antioxidant and anticancer activities of the extract and fractions. As reported by earlier studies, some compounds in _P. foetida_ leaves were well known to have various pharmaceutical properties, including antioxidant, anticancer, anti-inflammatory, antimicrobial, insecticidal, antibiotic, antiviral, and antiaging (Table 4). Interestingly, the most active fraction (fraction 1) contained phenolic (dihydrocaffeic acid 3-O-glucuronide and 4-O-caffeoylquinic acid-1) and flavonoid compounds (quercetin and neohesperidin). These compounds are well investigated to act as antioxidant and anticancer activities. Unfortunately, several compounds in the extract and fractions could not identify through this approach. Therefore, another method, especially HPLC, is recommended for more isolation and structure elucidation of novel molecules from _P. foetida_ leaves.

In conclusion, our study has clearly indicated that _P. foetida_ leaf extract and its fractionated substances demonstrated antioxidant and cytotoxic properties on MCF-7 cells. Both crude extract and selected fractions of this plant leaves exhibited great antioxidant activity against DPPH and ABTS free radicals. MCF-7 cell viability was remarkably reduced in a dose-dependent response after treatment with crude extracts and fractions of _P. foetida_ leaves. Phenol, flavonoid, alkaloid, limonoid, chlorophyll derivatives compound, and glycoside were identified as the major component of the extract and fractions. Phenolic and flavonoid compounds were found in the extract and active fractions. This result provides new knowledge on the possible pharmaceutical benefits of _P. foetida_ leaves collected from Indonesia, particularly as antioxidant and cytotoxic agents along with its chemical profile. However, future studies are still necessary to further identification active phenolic or flavonoid components or other compounds behind its antioxidant and cytotoxic properties.

**Conflict of Interest**

All authors declared there is no conflict of interest.

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