HAYATI
Journal of Biosciences

Vol. 30 No. 2, March 2023 271-280
DOI:10.4308/hjb.30.2.271-280
ISSN: 1978-3019
EISSN: 2086-4094

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

Jepri Agung Priyanto[1*], Muhammad Eka Prastya[2], Gian Primahana[2], Ahmad Randy[2], Diyah Tri Utami[3]

[1]*Division of Microbiology, Department of Biology, Faculty of Mathematics and Natural Sciences, IPB University, Bogor 16680, Indonesia*
[2]*Research Center for Pharmaceutical Ingredients and Traditional Medicine, National Research, and Innovation Agency (BRIN), Kawasan PUSPIPTEK, Serpong, South Tangerang 15314, Indonesia*
[3]*Department of Pharmacy, Universitas Sebelas Maret, Surakarta 57126, Indonesia*

ARTICLE INFO

Article history:
Received August 29, 2022
Received in revised form September 26, 2022
Accepted October 31, 2022

KEYWORDS:
ABTS,
DPPH,
flavonoid,
MCF-7,
phenol

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 IC_{50} 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 IC_{50} values ranging from 410.24±0.30 to 831.57±6.91 µg/ml. F3 fractions showed the strongest cytotoxicity (IC_{50} 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.

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

* Corresponding Author
E-mail Address: jepriyanto@apps.ipb.ac.id

2020). 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).

2.4. Cytotoxicity Assay

The cytotoxicity of crude extract and fractions of *P. foetida* leaves was evaluated on MCF-7 cell lines (ATCC HTB-22). This assay was determined using AlamarBlue Resazurin Cell Viability Reagent (Gibco-Thermo Fisher Scientific). Cells were seeded in 96-well plates for 10^4 cells per well and incubated at 37°C and 5% CO_2. After 24 h incubation, the medium was washed with phosphate-buffered saline (PBS), and the growth media was changed to Dulbecco's-modified Eagle's medium (DMEM). Fractions and crude extract were added to the cells in a series of concentrations (500, 250, 125, 62.5, and 31.25 µg/ml). In addition, the blank was a group that contained medium only, whereas the positive control was a group that contained 5% dimethylsulfoxide (DMSO). After incubation for 24 h, the cells were washed with PBS, and the medium were changed with 100 µl AlamarBlue reagent (10%) in DMEM. After incubating for 3 h at 37°C, the absorbance (OD) was measured at 560/590 nm excitation/emission wavelength with a Varioskan Flash multimode reader (Thermo Fisher Scientific, USA). The obtained absorbance was reduced to a fluorescence signal of wells with no cells. The percentage of cell growth inhibition was determined using the following formula:

$$\text{Inhibition}(\%) = \frac{\text{OD of control cells - OD of treated cells}}{\text{OD of control cells}} \times 100\%$$

The cytotoxic property of the sample was presented as an IC_{50} value. The percentage of cell growth inhibition caused by each concentration of respective substances was then plotted to determine the IC_{50} value. Doxorubicin was applied as a positive control.

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 (R_f) 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.

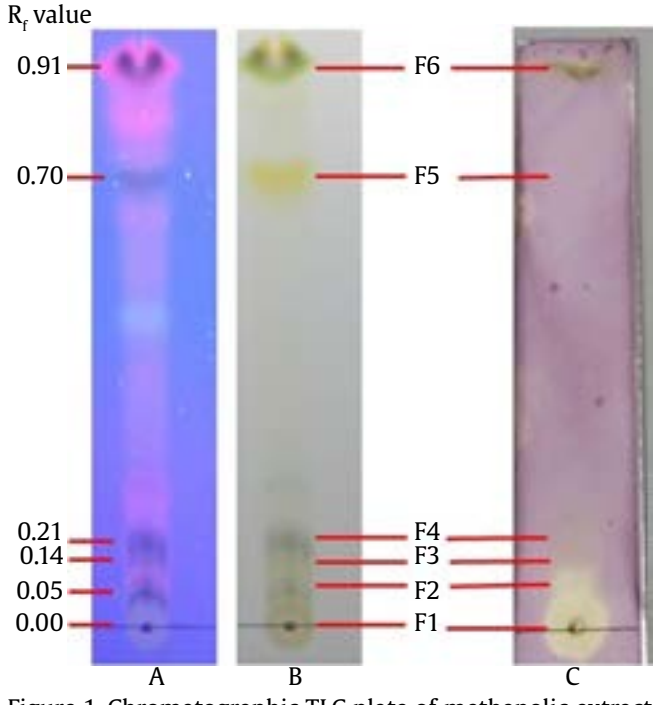

Figure 1. Chromatographic TLC plate of methanolic extract of *P. foetida* leaves using mobile phase from the mixture of ethyl acetate and *n*-hexane (7:3 v/v) visualized under (A) UV 366 nm, (B) visible light, and (C) after stained with DPPH reagent in visible light

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 IC_{50} values of this fraction were 10.94±2.67 µg/ml against DPPH and 50.04±0.48 µg/ml against ABTS. However, the IC_{50} 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 IC_{50} 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 IC_{50} 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 IC_{50} value of 16.71±4.01 µg/ml, which was considered higher cytotoxic activity than all fractions.

3.4. Total Phenolic and Flavonoid Content

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

Sample	Antioxidant activity (IC_{50} value ± standard deviation in µg/ml)	
	DPPH	ABTS
Crude extract	14.74±1.57^{d}	61.62±3.10^{c}
Fraction 1 (F1)	10.94±2.67^{c}	50.04±0.48^{d}
Fraction 2 (F2)	19.47±0.44^{e}	66.21±1.84^{e}
Fraction 3 (F3)	86.03±1.94^{f}	133.79±8.64^{f}
Fraction 4 (F4)	304.97±9.81^{h}	346.84±21.19^{g}
Fraction 5 (F5)	>800	>800
Fraction 6 (F6)	294.21±9.45^{g}	358.93±15.64^{h}
Ascorbic acid	5.84±0.40^{b}	18.40±0.13^{b}
Quercetin	3.08±0.24^{a}	9.59±0.78^{a}

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

Sample	IC_{50} value ± standard deviation (µg/ml)
Crude extract	550.12±5.14[d]
Fraction 1 (F1)	520.34±4.33[c]
Fraction 2 (F2)	750.11±6.36[e]
Fraction 3 (F3)	410.24±0.30[b]
Fraction 4 (F4)	831.57±6.91[g]
Fraction 5 (F5)	810.90±8.21[f]
Fraction 6 (F6)	633.13±5.60[h]
Doxorubicin	16.71±4.01[a]

All data presented as mean ±SD of triplicates (n = 3) test. Values with different superscript letters represent statistically different (P<0.01)

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

		Content±standard deviation (SD)
Total flavonoid (mgQE/g extract)	Crude extract	138.86±12.12
	Fraction 1	50.48±0.35
	Fraction 2	22.67±6.55
	Fraction 3	15.33±6.94
Total phenol (mgGAE/g extract)	Crude extract	154.76±3.37
	Fraction 1	57.45±1.88
	Fraction 2	26.41±1.16
	Fraction 3	17.05±3.55

All data presented as mean ±SD of triplicates (n = 3) test

Table 4. Chemical profiles of crude extracts and active fractions of *P. foetida* leaves

Sample	Proposed compounds	Retention time (min)	Molecular formula	Chemical class	Bioactivity	References
Crude extract	4-O-Caffeoylquinic acid-1	5.31	$C_{16}H_{18}O_9$	Phenol	Antioxidant; Antiinflam-matory	Ganzon *et al.* 2018; Motaal *et al.* 2016
	Quercimeritrin (quercetin-7-O-glucoside)	6.48	$C_{21}H_{20}O_{12}$	Flavonoid	Antioxidant; cytotoxic on MCF-7 cells; amylase se-cretion activity	Shin *et al.* 2010; Ahmed *et al.* 2016; Park *et al.* 2019
	Digiprolactone	8.10	$C_{11}H_{16}O_3$	Glycoside	unknown	
	Azedarachin C	10.58	$C_{32}H_{42}O_{10}$	Limonoid	Insecticidal	El-Ghany *et al.* 2012
	Stigmastan-3,6-dione	12.07	$C_{29}H_{48}O_2$	Steroid	Anti-inflammatory	Okoye *et al.* 2010
	Unknown	13.15	$C_{15}H_{18}O_{10}$			
	Unknown	10.64	$C_{34}H_{40}O_9$			
	Unknown	12.91	$C_{45}H_{84}O_{15}$			
	Unknown	13.26	$C_{45}H_{84}O_{14}$			
	Unknown	1.85	$C_{46}H_{86}O_{16}$			

Table 4. Continued

Sample	Proposed compounds	Retention time (min)	Molecular formula	Chemical class	Bioactivity	References
F1	Dihydrocaf-feic acid 3-O-glucuronide	0.48	$C_{15}H_{18}O_{10}$	Phenol	Antioxidant	Piazzon *et al.* 2012
	4-O-Caffeoylquinic acid-1	2.45	$C_{16}H_{18}O_9$	Phenol	Antioxidant; Antiinflammatory	Ganzon *et al.* 2018; Motaal *et al.* 2016
	Quercetin	3.19	$C_{15}H_{10}O_7$	Flavonoid	Antioxidant; antimicrobial; antiinflammatory; cy-totoxic; antibacterial and antibiofilm; antiviral	Ozgen *et al.* 2016; Nguyen and Bhattacharya 2022; Lesjak *et al.* 2018; Hong *et al.* 2021; Júnior *et al.* 2018; Johari *et al.* 2012
	Neohesperidin	3.86	$C_{28}H_{34}O_{15}$	Flavonoid	Antiaging; antioxidant; anticancer on human breast adenocarcinoma MDA-MB-231 cells	Guo *et al.* 2019; Masek *et al.* 2014; Xu *et al.* 2012
	Unknown	4.62	$C_{21}H_{28}N_2O$			
	Unknown	8.01	$C_{26}H_{45}N_3O_6$			
F2	Unknown	4.62	$C_{21}H_{28}N_2O$			
	Unkown	9.02	$C_{20}H_{34}O_8$			
	Unknown	9.81	$C_{35}H_{36}N_4O_7$			
	Azedarachin C	10.26	$C_{32}H_{42}O_{10}$	Limonoid	Insecticidal	El-Ghany *et al.* 2012
	Unknown	11.44	$C_{22}H_{43}NO$			
F3	Unknown	4.61	$C_{21}H_{28}N_2O$			
	Cuscohygrine	5.63	$C_{13}H_{24}N_2O$	Alkaloid	unknown	
	Unknown	9.02	$C_{20}H_{34}O_8$			
	Azedarachin C	9.71	$C_{32}H_{42}O_{10}$	Limonoid	Insecticidal	El-Ghany *et al.* 2012
	Pheophorbide A	10.20	$C_{35}H_{36}N_4O_5$	chlorophyll derivatives compound	Antioxidant; cytotoxic on human hepatocellular carcinoma; lymphatic vascular activator	Yoon *et al.* 2011; Tang *et al.* 2006; Kim *et al.* 2022

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_f 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_{50} 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_{50} values between 10.94±2.67 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_{50}/DPPH: 10.94±2.67 µg/ml; IC_{50}/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* ($IC_{50/DPPH}$: 287.52 µg/ml; $IC_{50/ABTS}$: 126.75 µg/ml) (Lahmadi *et al.* 2019), *Raphanus sativus* ($IC_{50/DPPH}$: 216.8 µg/ml; $IC_{50/ABTS}$: 359.7 µg/ml) (Noman *et al.* 2021), and *Cucumis africanus* ($IC_{50/DPPH}$: 955 µg/ml; $IC_{50/ABTS}$: 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).

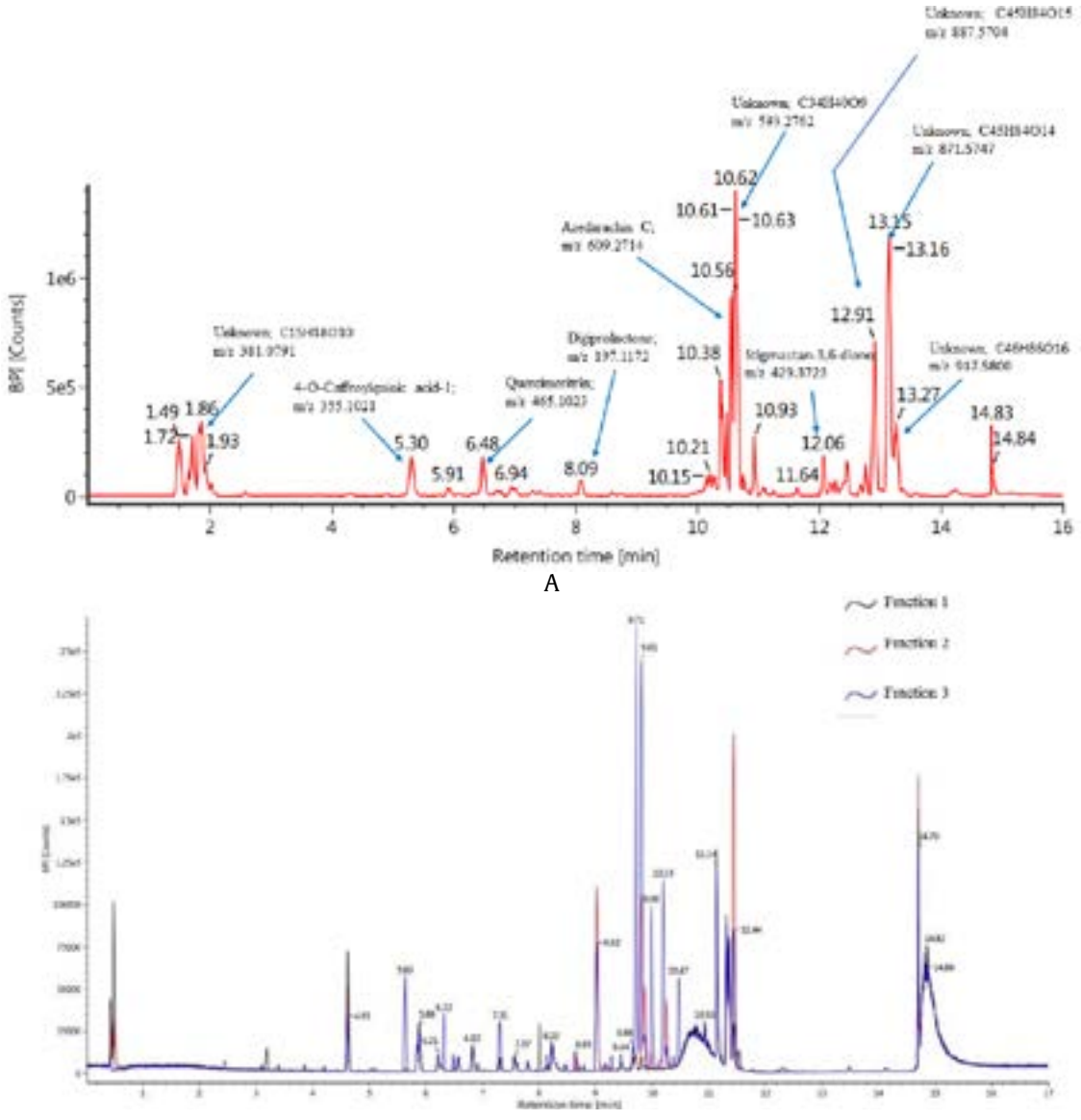

Figure 2. Chromatogram of (A) crude extract of methanolic extract of *P. foetida* leaves; (B) overlay of F1, F2, and F3 based on LC-MS/MS analysis (line color of black, red, and blue represent particular chromatogram of F1, F2, and F3 respectively)

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_{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_{30} values ranging from 15,000 to 20,000 µg/ml (Pradhan *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, antibiofilm, 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 allong 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.

Acknowledgements

This study was partially supported by The Institution of Research and Community Services of IPB University through the "Penelitian Dosen Muda" program under funding number: 2881/IT3.L1/PT.01.03/M/T/2022, and National Priority Program, Deputy for Engineering Science, Indonesian Institute of Sciences (LIPI), Indonesia, 2021 under funding number: 58/A/DT/2021. We are also grateful to Dr. Zetryana Puteri T. and Dr. Tjandrawati Mozef (Research Center for Pharmaceutical Ingredients and Traditional Medicine, BRIN) for the fruitful discussion related to the research.

References

Abotaleb, M., Liskova, A., Kubatka, P., Büsselberg, D., 2020. Therapeutic potential of plant phenolic acids in the treatment of cancer. *Biomol.* 10, 1-23. https://10.3390/biom10020221

Abifarin, T.O., Afolayan, A.J., Otunola, G.A., 2019. Phytochemical and antioxidant activities of *Cucumis africanus* L.f.: a wild vegetable of South Africa. *J Evid-Bas. Integr. Med.* 24, 1-8.

Ahmed, S.I., Hayat, M.Q., Tahir, M., Mansoor, Q, Ismail, M., Keck, K., Bates, R.B., 2016. Pharmacologically active flavonoids from the anticancer, antioxidant and antimicrobial extracts of *Cassia angustifolia* Vahl. *BMC Compl. Med Ther*. 16, 1-9. https://10.1186/s12906-016-1443-z

Chung, Y.C., Lee, J.N., Kim, B.S., Hyun, C.G., 2021. Anti-melanogenic effects of *Paederia foetida* L. extract via MAPK signaling-mediated mitf downregulation. *Cosmetics.* 22, 1-10. https://doi.org/10.3390/cosmetics8010022

El-Ghany, M.A.A., Farag, M., Yousef, H., Ahmed, M.H.M., El-badawey, S.S., Abdel-Rahman A.A.H., 2012. Insecticidal activity of *Melia azedarach* L. triterpeniods against *Spodoptera littoralis* (Boisd.). *J American Sci.* 8, 661-667.

Ganzon, J.G., Chen, L.G., Wang, C.C., 2018. 4-O-caffeoylquinic acid as an antioxidant marker for mulberry leaves rich in phenolic compounds. *J. Food Drug An.* 26, 985-993.

Guo, C., Zhang, H., Guan, X., Zhou, Z., 2019. The antiaging potential of neohesperidin and its synergistic effects with other citrus flavonoids in extending chronological lifespan of Saccharomyces Cerevisiae BY4742. *Molecules*. 24, 1-14. https://10.3390/molecules24224093

Hong, Y., Lee, J., Moon, H., Ryu, C.H., Seok, J., Jung, Y.S., Ryu, J., Baek, S.J., 2021. Quercetin induces anticancer activity by upregulating Pro-NAG-1/GDF15 in differentiated thyroid cancer cells. *Cancers.* 13, 1-14. https://doi.org/10.3390/cancers13123022

Johari, J., Kianmehr, A., Mustafa, M.R., Abubakar, S., Zandi, K., 2012. Antiviral activity of baicalein and quercetin against the japanese encephalitis virus. *Int. J. Mol. Sci.* 13, 16785-16795. https://10.3390/ijms131216785

Júnior, S.D.D.C., Santos, J.V.D.O., Campos, L.A.D.A., Pereira, M.A., Magalhães, N.S.S., Cavalcanti, I.M.F., 2018. Antibacterial and antibiofilm activities of quercetin against clinical isolates of *Staphyloccocus aureus* and *Staphylococcus saprophyticus* with resistance profile. *Int. J. Environt. Agricult. Biotech.* 3, 1948- 1958.

Kim, J., Gousopouulos, E., Faleschini, T.M., Hamburger, M., Potterat, O., Detmar, M., 2022. Pheophorbide a identified in an *Eupatorium perfoliatum* extract is a novel lymphatic vascular activator. *Biomed. Pharmacother*. 147, 1-6. https://doi.org/10.1016/j.biopha.2022.112664

Kumar, V., Anwar, F., Ahmed, D., Verma, A., Ahmed, A., Damanhouri, Z.A., Mishra, V., Ramteke, P.W., Bhatt, P.C., Mujeeb, M., 2014. *Paederia foetida* Linn. leaf extract: an antihyperlipidemic, antihyperglycaemic and antioxidant activity. *BMC Compl. Altern Med*. 14, 1-16. https://doi.org/10.1186/1472-6882-14-76.

Kurutas, E.B., 2015. The importance of antioxidants which play the role in cellular response against oxidative/nitrosative stress: current state. *Nutr. J.* 15, 1-22. https://doi.org/10.1186/s12937-016-0186-5

Lahmadi, S., Belhamra, M., Karoune, S., Kechebar, M.S.A., Bensouici, C., Kashi, I., Mizab, O.O., Ksouri, R., 2019. *In vitro* antioxidant capacity of *Euphorbia retusa* Forssk. from Algerian desert. *J. Pharm. Pharmacog. Res.* 7, 356-366.

Lesjak, M., Beara, I., Simin, N., Pintać, D., Majkić, T., Bekvalac, K., Orčić, D., Mimica-Dukić, N., 2018. Antioxidant and anti-inflammatory activities of quercetin and its derivatives. *J. Funct. Foods*. 40, 68-75.

Li, N., Zhou, H., Chen, X., Chen, H., Tang, H., Yao, Y., 2021. Potential chemoprotective effect of *Paederia foetida* Linn on benzyo[a]pyrene induced gastric cancer. *Latin American J. Pharm*. 40, 834-843.

Masek, A., Chrzescijanska, E., Zaborski, M., 2014. Antioxidative properties of silymarin, 7-aminoflavone, neohesperidin dihydrochalcone and trihydroxyethylenorutin studied by the electrochemical methods. *Int. J. Electrochem. Sci.* 9, 7875-7889.

Mazumder, K., Dey, Z.K., Dey, S., Hossain, M.D.S., Sajon, S.R., Hossain, K.M.D., 2018. Phytochemical screening and evaluation of ant diarrheal mode of action of leaves extracts of *Paederia foetida* Linn. I*nt. J. Complement. Altern. Med*. 11, 304–309. https://10.15406/ijcam.2018.11.00416

Morshed, H., Islam, Md, S., Parvin, S., Ahmed, M.U., Islam, M.S., Mustofa, A.G.M., Sayeed, M.S.B., 2012. Antimicrobial and cytotoxic activity of the methanol extract of *Paederia foetida* Linn (Rubiaceae). *J. Appl. Pharmaceut. Sci.* 2, 77-80.

Motaal, A.A., Ezzat, S.M., Tadros, M.G., El-Askary, H.I., 2016. *In vivo* anti-inflammatory activity of caffeoylquinic acid derivatives from *Solidago virgaurea* in rats. *Pharm. Biol.* 54, 2864–2870. https://10.1080/13880209.2016.1190381

Nguyen, T.L.A., Bhattacharya, D., 2022. Antimicrobial activity of quercetin: an approach to its mechanistic principle. *Molecules*. 27, 1-13. https://doi.org/10.3390/molecules27082494

Noman, O.M.M., Nasr, F.A., Alqahtani, A.S., Al-Zharani, M., Cordero, M.A.W., Alotaibi, A.A., Bepari, A., Alarifi, S., Daoud, A., 2021. Comparative study of antioxidant and anticancer activities and HPTLC quantification of rutin in white radish (*Raphanus sativus* L.) leaves and root extracts grown in Saudi Arabia. *Open Chem.* 19, 408-416.

Okoye, F.B.C., Osadebe, P.O., Edrada-ebel, R.A., Nworu, C.S., Esimone, C.O., 2010. Anti-inflammatory and membrane-stabilizing stigmastane steroids from *Alchornea floribunda* leaves. *Planta Med*. 76, 172–177.

Osman, H., Rahim, A.A., Isa, N.M., Bakhir, N.M., 2009. Antioxidant activity and phenolic content of *Paederia foetida* and *Syzygium aqueum. Molecules*. 14, 970-978. https://10.3390/molecules14030970

Ozgen, S., Kilinc, O.K., Selamoglu, Z., 2016. Antioxidant activity of quercetin: a mechanistic review. *Turkish J. Agricult.Food Sci. Tech*. 4, 1134-1138.

Panche, A.N., Diwan, A.D., Chandra, S.R., 2016. Flavonoids: an overview. *Journal of Nutritional Science*. 5 (e47), 1-15. https://10.1017/jns.2016.41

Park, S., Nhiem, N.X., Park, J.H., Bhattarai, K.R., Chae, H.J., Kiim, H.R., Kim, S.H., 2019. Isolation of amylase regulators from the leaves of Ixeridium dentatum. *Nat. Prod. Res*. 35, 1-6.

Patel, D.K., 2017. *Paederia foetida* Linn: a potential climbing medicinal herb in Central India. *Int. J. Environ. Sci. Nat. Resour.* 6, 118–24.

Piazzon, A., Vrhovsek, U., Masuero, D., Mattivi, F., Mandoj, F., Nardini, M., 2012. Antioxidant activity of phenolic acids and their metabolites: synthesis and antioxidant properties of the sulfate derivatives of ferulic and caffeic acids and of the acyl glucuronide of ferulic acid. *J. Agricult. Food Chem*. 60, 12312–12323. https://doi.org/10.1021/jf304076z

Pourreza, N., 2013. Phenolic compounds as potential antioxidant. *Jundishapur J. Nat. Pharmaceut. Prod.* 8, 149-150. https://10.17795/jjnpp-15380

Pradhan, N., Parbin, S., Kausar, C., Kar, S., Mawatwal, S., Das, L., Deb, M., Sengupta, D., Dhiman, R., Patra, S.K., 2019. *Paederia foetida* induces anticancer activity by modulating chromatin modification enzymes and altering pro-inflammatory cytokine gene expression in human prostate cancer cells. *Food Chem. Toxicol*. 130, 161-173. https://doi.org/10.1016/j.fct.2019.05.016

Prastya, M.E., Astuti, R.I., Batubara, I., Wahyudi, A.T., 2019. Antioxidant, antiglycation and *in vivo* antiaging effects of metabolite extracts from marine sponge-associated bacteria. *Ind. J. Pharm. Sci.* 81, 344-353.

Prastya, M.E., Astuti, R.I., Batubara, I., Takagi, H., Wahyudi, A.T., 2020. Natural extract and its fractions isolated from the marine bacterium *Pseudoalteromonas flavipulchra* STILL-33 have antioxidant and antiaging activities in *Schizosaccharomyces pombe. FEMS Yeast Res*. 20, 1-14.

Priyanto, J.A., Prastya, M.E., Sinarwadi, G.S., Datu'salamah, W., Avelina, T.Y., Yanuar, A.I.A., Azizah, E., Tachrim, Z.P., Mozef, T., 2022. The antibacterial and antibiofilm potential of *Paederia foetida* Linn. leaves extract. J. *Appl. Pharmaceut. Sci.* 12, 117-124.

Rani, K., 2017. Role of antioxidants in prevention of diseases. *J. Appl. Biotechnol. Bioeng*. 4, 495-496. https://10.15406/jabb.2017.04.00091

Rosli, N.H.M., Tajuddin, N.A., Shafie, S., 2013. Preliminary study: vitamin C in *Paederia foetida* leaves. *The Open Conf. Proceedings J*. 4, 9-11.

Satapathy, S., Pattnaik, G., 2020. Preliminary phytochemical screening and FTIR analysis of an Indian medicinal herb: *Paederia foetida* (Prasarini). *Indian J. Publ. Health Res. Dev.* 11, 641-646.

Shin, S., Lee, Y., Moon, S.R., Kooo, I.H., Hong, H., Shin, E., Lee, M., Park, J., Chung, H.S., 2010. Identification of secondary metabolites with antioxidant and antimicrobial activities from *Artemisia iwayomogi* and *Chrysanthemum zawadskii. J. Korean Soc. Appl. Biol. Chem*. 53, 716-723.

Tang, P.M.K., Chan, J.Y.W., Au, S.W.N., Kong, S.K., Tsui, S.K.W., Waye, M.M.Y., Mak, T.C.W., Fong, W.P., Fung, K.P., 2006. Pheophorbide a, an active compound isolated from *Scutellaria barbata*, possesses photodynamic activities by inducing apoptosis in human hepatocellular carcinoma. *Cancer Biol. Ther.* 5, 1111-1116.

Wang, L., Jiang, Y., Han, T., Zheng, C., Qin, L., 2014. A phytochemical, pharmacological and clinical profile of *Paederia foetida* and *P. scandens. Nat. Prod. Comm.* 9, 879-886.

Wong, I.O.L, Lam, Y.T., Lam, K.F., Cowling, B.J., Leung, G.M., 2021. Demographic and epidemiological contributions to recent trends in cancer incidence in Hong Kong. *Cancers.* 13, 1-11.

[WHO] World Health Organization, 2020. Available at: https://apps.who.int. [Date accessed: 1 August 2022]

Xu, F., Zang, J., Chen, D., Zhang, T., Zhan, H., Lu, M., Zhuge, H., 2012. N*eohesperidin induces* cellular apoptosis in human breast adenocarcinoma MDA-MB-231 cells via activating the Bcl-2/Bax-mediated signaling pathway. *Nat. Prod. Comm*. 7, 1475-1478.

Yoon, I., Park, H.S., Cui, B.C., Li, J.Z., Kim, J.H., Lkhagvadulam, B., Shim, Y.K., 2011. Photodynamic and antioxidant activities of divalent transition metal complexes of methyl pheophorbide-a. *Bull. Korean Chem. Soc.* 32, 2981- 2987. https://doi.org/10.5012/bkcs.2011.32.8.2981