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ORIGINAL ARTICLE

# Pharmaceutical Properties and Phytochemical Profile of Extract Derived from Purple Leaf *Graptophyllum pictum* (L.) Griff

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## ABSTRACT

**Objective:** *Graptophyllum pictum* (L.) Griff is a medicinal shrub belonging to the Acanthaceae family and is traditionally used to treat various diseases. Therefore, this study aimed to evaluate the pharmaceutical properties and phytochemical profiles of the methanolic extract of *G. pictum*.

**Materials and Methods:** *G. pictum* leaves was extracted using methanol. Antioxidant, cytotoxic on Michigan Cancer Foundation-7 (MCF-7) and HepG2, antidiabetic, and antibacterial properties were evaluated *in vitro*. Chemical profile of the extract was identified through qualitative (for phytochemicals), quantitative (for phenolic and flavonoid content), and gas chromatography-mass spectrometry (GC-MS) analysis.

**Results:** The results showed that the extract had potent antioxidant activity against 2,2-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) and 2,2-diphenyl-1-picrylhydrazyl radicals with $IC_{50}$ values of 49.00 ± 3.20 µg/mL and 70.18 ± 3.27 µg/mL, respectively. It also exhibited cytotoxic effects on human breast (MCF-7) and liver (HepG2) carcinoma cells with growth inhibition percentages of 74.29 ± 1.53% and 64.90 ± 1.94%, respectively. The antidiabetic assay showed that the extract had inhibitory effects on α-glucosidase activity with $IC_{50}$ value 194.59 ± 15.59 µg/mL, indicating its potential to be developed as an antidiabetic agent. Furthermore, it had antibacterial properties against four test strains, and the highest activity was found against *Bacillus subtilis* American Type Culture Collection 19659, with minimum inhibitory concentration and minimum bactericidal concentration values of 625 µg/mL and 1250 µg/mL, respectively. Phytochemical tests indicated the presence of alkaloids, flavonoid and terpenoids in the extract, with total phenolic content and total flavonoid content of 41.17 ± 2.38 mg gallic acid equivalents/g and 26.52 ± 0.61 mg quercetin equivalent/g, respectively. GC-MS analysis revealed that it contained several active compounds, including eicosane, 2,4-Di-*tert*-butylphenol, hentriacontane, tetracosane, octacosane, sulfurous acid, 2-methylhexacosane, docosane, heneicosane, 1-propene-1,2,3-tricarboxylic acid, tributyl ester, and pentacosane.

**Conclusion:** The extract derived from *G. pictum* leaves was a potential source of therapeutic compounds, particularly for antioxidant, antidiabetic, anticancer, and antibacterial agents.

**Keywords:** Antioxidant, cytotoxic, flavonoids, phytochemical, phenols, α-glucosidase inhibitor

## INTRODUCTION

Several plants have gained recognition for their potential as primary sources of medicine in drug discovery. These natural sources of herbal medicine offer an alternative to synthetic and modern drugs because of their lower potential to have side effects. An estimated 70.000 species have been studied for their therapeutic functions,[1] and more than 50% of commercially available drugs are derived from medicinal plants, which act as analgesics, anticancer agents, antidiabetics, and antioxidants.[2] Indonesia is a tropical country with the second largest potential for medicinal plants, following Brazil, with a minimum of 30.000 species spread across various regions.[3]

*G. pictum* Griff, locally known as daun ungu, daun wungu, and handeuleum, is an herbal shrub of the Acanthaceae family.

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The plant is native to New Guinea and has spread widely to various countries, including the United States, Mexico, Ghana, Bolivia, India, and Indonesia.[4,5] Furthermore, it has brownish-purple leaves due to its high anthocyanin, chlorophyll, and carotenoid content.[6] *G. pictum* leaf has long been used as a traditional drug to treat various diseases, including hemorrhoid, analgesic, antipyretic, menstrual problems, and wound healing.[7] Several studies have investigated the therapeutic values of *G. pictum* leaf, which have been shown to possess *in vitro* anti-inflammatory, antibacterial, and antioxidant.[8,5,9] *In vivo* studies also revealed that it can decrease blood glucose levels, as well as act as an antihemorrhoid, antioxidant, and anti-inflammatory agents.[10,11,12] These biological activities have been linked to its phytochemical content, namely phenols, flavonoids, tannins, alkaloids, saponins, terpenoids, and steroids.[8]

Although several studies have reported the biological properties and metabolite profiles of *G. pictum*, the use of different geographical plant origins, extraction techniques, and solvents can lead to varying chemical profiles and bioactivities.[13,14] The majority of reports on this species used plants growing in Thailand, India, as well as East and Central Java-Indonesia, but there is no information on the pharmaceutical values of those cultivated in Cirebon, West Java-Indonesia.[9,4,10] Therefore, this study aims to evaluate the chemical profile, as well as the antibacterial, antioxidant, antidiabetic, and cytotoxic properties of *G. pictum* leaf methanolic extract obtained from Cirebon, Indonesia.

## MATERIALS AND METHODS

*Plant materials and extraction*

Fresh leaves of *G. pictum* were harvested from Cirebon, West Java, Indonesia, at the coordinates 6°36'15.7"S 108°21'23.0"E. The obtained leaves were then air-dried and crushed into powder for further procedures. Subsequently, 100 g of the powder was extracted in 1000 mL methanol (1:10, *w/v*) and shaken continuously in a rotary shaker (100 rpm) at room temperature for 24 h. The mixture was filtered using filter paper (Whatman no. 1), and the filtrate was collected, followed by evaporation at 40 °C using a rotary evaporator.[15]

*Antioxidant assay*

The free radical scavenging activity of the extract was measured using 2,2 diphenylpicrylhydrazyl (DPPH) and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assay.[16] A total of 100 µL of 250 µM DPPH radical solution was added to 100 µL extract solutions, ranging from 2500 to 20 µg/mL. The reaction was allowed to proceed for 30 min at room temperature, and the absorbance was measured at 515 nm using a Thermo Scientific Varioskan Flash (Thermo Fischer), followed by the calculation of the percentage inhibition (%). For the ABTS assay, radicals were produced by reacting 7 mM ABTS solution with 2.45 mM potassium persulfate (1:1) and incubating for 12-14 h at room temperature in dark conditions. Furthermore, 170 µL of the radicals was mixed with 30 µL extract and incubated for 30 min, with the determination of absorbance at 734 nm. The inhibition of both assays was calculated using the formula: % = $[(A_1-A_2)/A_1] \times 100\%$. $A_1$ represents the absorbance of the DPPH/ABTS blank (without samples), and $A_2$ = the absorbance of the samples. The concentration of the sample required to scavenge 50% free radicals ($IC_{50}$ value) was calculated from the plotted graph of radical scavenging activity against each extract concentration. In this study, ascorbic acid and quercetin were used as positive controls.

*Cytotoxicity assay*

This study used human breast adenocarcinoma Michigan Cancer Foundation-7 (MCF-7) and liver carcinoma HepG2 cell lines [American Type Culture Collection (ATCC); Rockville, MD, USA], which were obtained from the Laboratory of Biochemical and Natural Product Isolation, Research Centre for Pharmaceutical Ingredients and Traditional Medicine, KST BJ. Habibie, BRIN, Serpong, Banten, Indonesia. Cells were cultured in Dulbecco's Modified Eagle Medium high glucose medium (Sigma), which was supplemented with 10% fetal bovine serum and 1% antibiotics (penicillin/streptomycin) (Sigma) in a humidified atmosphere containing 5% $CO_2$ and 95% air at 37 °C. The cytotoxic assay was then performed by seeding MCF-7 and HepG2 cells on a 96-well microplate at a concentration of $1 \times 10^4$ cells *per* well, followed by incubation for 24 h to maximize attachment. Subsequently, the media were replaced with fresh samples containing 100 µg/mL of extract (diluted on DMSO) and incubated for 48 h. A total of 10 µL of 3- (4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide) stock solution (0.5 mg/mL) was added and incubated for 3 h at 37 °C, leading to the dissolution of the crystals in 99% DMSO. After the complete dissolution of formazan blue, cell proliferation was measured at 570 nm using a Thermo Scientific Varioskan Flash (Thermo Fischer). The inhibition percentage was then calculated using the formula: $[1- (Abs._{Sample}-Abs._{DMSO\ control})] \times 100\%$. DMSO at a final concentration of 0.05% and 100 µg/mL cisplatin (Sigma) were used as negative and positive controls, respectively.[17]

*Antidiabetics assay*

Antidiabetic activity was measured based on the method proposed by a previous study.[18] The extract was diluted in 99% DMSO to prepare various concentrations, ranging from 12.5 to 200 µg/mL, while quercetin was used as the positive control, ranging from 35 to 70 µg/mL. A total of 495 µL of 100 mM phosphate buffer with pH 7 and 250 µL substrate (20 mM, *p*-nitrophenyl-α-glucopyranoside) were added, and the mixture was incubated at 37°C for 5 min. Subsequently, 250 µL α-glucosidase (0.065 U/mL) was added to the mixture and incubated at 37 °C for 15 min. The reaction was stopped by supplementing 1 mL of 200 mM $Na_2CO_3$ in the sample. The release of *p*-nitrophenol from the α-linkage of glucopyranoside was then determined at 400 nm. The percentage of enzyme inhibition (%) was calculated using the following formula: $[(Abs._{control}-Abs._{sample})/Abs._{control}] \times 100\%$. The concentration of the sample required to inhibit 50% of α-glucosidase reaction ($IC_{50}$) was calculated from the plotted graph of the inhibition value of each extract concentration.

*Antibacterial activity*

A standard disk diffusion assay was performed based on a method proposed in a previous study.[19] The process was performed using four targeted bacterial ATCC strains, including *Escherichia coli* ATCC 8739, *Pseudomonas aeruginosa* ATCC 15442, *Bacillus subtilis* ATCC 19659, and *Staphylococcus aureus* ATCC 6538 (IPB University Faculty of Mathematics and Natural Sciences, Department of Biology, Collection of Laboratory of Microbiology). Furthermore, a suspension of bacterial inoculum with a concentration of 1.5% (*v/v*) was applied to Mueller Hinton Agar (MHA) (Himedia) plate medium and allowed to solidify. A total of 20 µL of extract diluted in 99% DMSO was added to sterile filter paper disks with a diameter of approximately 6 mm and placed on the surface of the inoculated agar plate. Antibacterial activity was then evaluated by measuring the diameter of inhibition zones surrounding the disks after incubation for 24 h at 37 °C. Tetracycline (200 µg/mL) and 1% DMSO were used as positive and negative controls, respectively.

*Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)*

The MIC of the *G. pictum* leaf extracts was determined using sterile 96-well plates.[19] The 96 wells of each row was filled with 100 µL of sterilized Mueller Hinton Broth. Furthermore, wells 1-8 of each row were then filled with 100 µL of a mixture of culture medium and plant extract, which were serially diluted to create a concentration sequence from 5000 to 35 µg/mL. Bacterial cultures were prepared in 0.85% NaCl and adjusted to McFarland standard 0.5 (equivalent to $1\times10^8$ colony-forming units/mL), after which 100 µL was added to each well. Tetracycline hydrochloride and 1% DMSO were used as positive and negative controls, respectively. The deep wells were incubated for 24 h at 37 °C, and the turbidity obtained was observed. MIC was determined as the concentration at which no visible cell growth was observed. To evaluate MBC, a portion of liquid (100 µL) from each well with no growth was taken and spread on MHA plate agar, followed by incubation at 37°C for 24 h. The lowest concentration that caused the absence of visible bacterial colonization after sub-culturing was taken as MBC.

*Qualitative phytochemical analysis*

Phytochemical analysis was performed to determine the presence or absence of some classes of compounds, including flavonoids, alkaloids, saponins, tannins, and terpenoids.[20] Furthermore, *G. pictum* extracts were mixed with an appropriate chemical reagent for each analysis. The mixtures obtained were then vortexed and qualitatively observed for the presence of the targeted compound class.

*Determination of total phenolic and flavonoid content*

The analysis of the total phenolic content (TPC) was carried out using the Folin Ciocalteu reagent based on the method used in a previous study.[21] A total of 0.5 mL of the extract (1 mg/mL) was mixed with 0.25 mL Folin Ciocalteu reagent and 3.5 mL distilled water. The solution was then kept at 28 °C for 5-8 min before adding 0.75 mL of 20% sodium carbonate solution. Subsequently, the absorbance was measured at 765 nm after incubation for 2 h at 28 °C. Gallic acid was used as the standard for the calibration curve in this study. The total flavonoid content (TFC) was measured using a colorimetric assay (Priyanto et al.,[15] 2022), and the results were expressed as mg gallic acid equivalents *per* gram of extract (mg GAE/g extract). A total of 500 µL extract (1 mg/mL) and 0.15 mL of 5% sodium nitrite were added to 2.45 mL of distilled water. After 3 min, 0.15 mL of 10% aluminum chloride was added, and the mixture was incubated for 8 min, followed by the addition of 2 mL of 1 M sodium hydroxide. The absorbance was then determined at 510 nm, and quercetin was used as a standard for the calibration curve. The TFC of the extract was expressed as mg quercetin equivalents *per* gram of extract (mg QE/g extract).

*Gas chromatography-mass spectrometry (GC-MS) analysis*

GC-MS analysis was performed using an Agilent 19091S-433: 93.92873 GC-MS. A total of 1 µL extract solution dissolved in *n*-hexane was injected into HP-5MS 5% phenyl methyl silox at 0 °C-325 °C (325 °C) measuring 30 m x 250 µm x 0.25 µm. The initial temperature of the oven was set at 40 °C and increased gradually over 30 min to 300 °C. Furthermore, helium was used as carrier gas at a flow rate of 1 mL/min. MSD Chem-Station Data Analysis software was then used to analyze the mass spectra and chromatograms of the GC-MS results.

*Statistical analysis*

The data obtained from antioxidant, cytotoxicity, antidiabetic, and antibacterial assays are presented as means ± standard deviation from triplicates. One-way analysis of variance was used to compare the mean values with 95% and 99% confidence levels. Further analysis was performed using the Tukey test, and $p$ values $< 0.05$ were considered statistically significant.

## RESULTS

*Antioxidant activity*

*G. pictum* leaf extract showed antioxidant activity with $IC_{50}$ values of 49.00 ± 3.20 µg/mL and 70.18 ± 3.27 µg/mL against ABTS and DPPH, respectively. Furthermore, the extract was significantly ($p < 0.05$) less active than ascorbic acid as a positive control, which had $IC_{50ABTS}$ and $IC_{50DPPH}$ of 10.99 ± 2.66 µg/mL and 3.82 ± 0.59 µg/mL, respectively, as shown in Table 1.

*Cytotoxic property*

A total of 100 µg/mL of *G. pictum*-derived extract inhibited MCF-7 and HepG2 cell growth with inhibition percentages of 74.29 ± 1.53% and 64.90 ± 1.94%, respectively. At this concentration, there was a significant decrease in cellular density, indicating that the treatment affected cancer cell growth. Apoptotic cells of MCF-7 and HepG2 appeared during inverted microscope observation after 48 h of treatment with the extract, as shown in Figure 1. As a positive control, cisplastin (100 µg/mL) was also tested, and it exhibited cytotoxic properties on MCF-7 and HepPG2 cells with growth inhibition percentages of 86.28 ± 0.22% and 64.90 ± 1.94%, respectively.

*Antidiabetic activity*

*G. pictum* leaf extract exhibited antidiabetic activity, as indicated by the inhibition of α-glucosidase activity with an $IC_{50}$ value of 194.59 ± 15.59 µg/mL, as shown in Table 2. The $IC_{50}$ of the extract was higher than that of the positive control quercetin at 3.35 ± 0.01 µg/mL.

*Antibacterial activity*

The methanolic extract of *G. pictum* exhibited various antibacterial activities against *E. coli* ATCC 8739, *P. aeruginosa* strain ATCC 15442, *S. aureus* ATCC 6538, *and B. subtilis* strain ATCC 19659, as indicated by the different inhibition zone diameters, as shown in Table 3. Among the four target bacteria, the extract was most active on *the B. subtilis* ATCC 19659. The inhibition zone was also determined using tetracycline and DMSO as the positive and negative controls, respectively (Figure 2). The extract also had the lowest MIC of 625 µg/mL and an MBC of 1250 µg/mL against the *B. subtilis* strain ATCC 19659, as shown in Table 4.

*Phytochemical profile*

Alkaloids, flavonoids, and terpenoids were found in the *G. pictum* leaf-derived extract, but tannins and saponins were absent. The extract's TPC and TFC were 41.17 ± 2.38 mg GAE/g and 26.52 ± 0.61 mg QE/g, respectively.

*Chemical profile of the G. pictum leaf extract*

GC-MS analysis revealed that the compounds identified in *G. pictum* leaf extract included eicosane, 2,4-Di-*tert*-butylphenol, hentriacontane, tetracosane, octacosane, sulfurous acid, 2-methylhexacosane, docosane, heneicosane, 1-propene-1,2,3-tricarboxylic acid, tributyl ester, and pentacosane, as shown in Table 5.

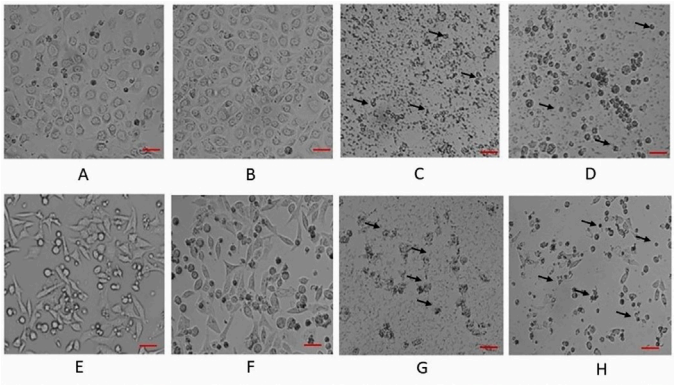


**Figure 1.** Cytotoxicity of *G. pictum* leaf extract on MCF-7 (A-D) and HepG2 cells (E-H); MCF-7 cell line on (A) DMEM medium; treatment with (B) 1% DMSO; (C) Cisplatin 100 µg/mL; (D) Extract 100 µg/mL; (E) HepG2 cell line on DMEM medium; treatment with (F) 1% DMSO; (G) Cisplatin 100 µg/mL; (H) Extract 100 µg/mL. The cell morphology and density were observed under an inverted microscope with magnification 100x. Bars represent 30 µm, and black arrows indicate apoptotic cells.

MCF-7: Michigan Cancer Foundation-7, DMEM: Dulbecco's Modified Eagle Medium

**Table 1. Antioxidant activity of the extract derived from *G. pictum* leaves against DPPH and ABTS**

| Sample | Antioxidant activity ($IC_{50}$ ± SD in µg/mL) | |
|---|---|---|
| | DPPH | ABTS |
| *G. pictum* leaf extract | 70.18 ± 3.27[b] | 49.00±3.20[b] |
| Ascorbic acid | 3.82 ± 0.59[a] | 10.99±2.66[a] |

Values with the same superscript letter in the same column are not significantly different based on one-way ANOVA analysis followed by multiple Duncan test range ($p < 0.05$), DPPH: 2,2 diphenylpicrylhydrazyl, ABTS: 2,2-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid), SD: Standard deviation, IC: Inhibitory concentration

**Table 2. Antidiabetic activity of the *G. pictum* leaf extract**

| Samples | Antidiabetic activity ($IC_{50}$: Average µg/mL ± SD) |
|---|---|
| *G. pictum* leaf extract | 194.59 ± 15.59[b] |
| Quercetin | 3.35 ± 0.01[a] |

Values with the same superscript letter in the same column are not significantly different based on one-way ANOVA analysis followed by multiple Duncan test range ($p < 0.05$), SD: Standard deviation, IC: Inhibitory concentration

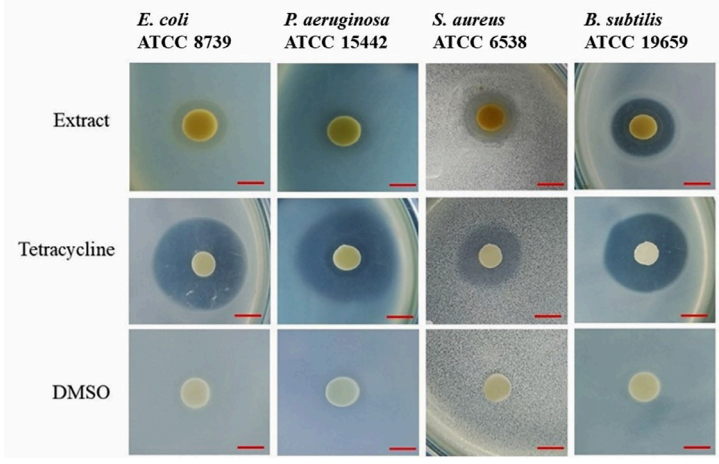


**Figure 2.** Antibacterial activity of *G. pictum* leaf extract (25 mg/mL) against the bacteria tested; 1% DMSO and tetracycline (200 µg/mL) were used as negative and positive controls, respectively. Bars represent 6 mm

**Table 3. Antibacterial activity of *G. pictum* leaf extract by the disk diffusion method**

| Samples | Inhibition zone (mm ± SD) | | | |
|---|---|---|---|---|
| | *E. coli* ATCC 8739 | *P. aeruginosa* ATCC 15442 | *S. aureus* ATCC 6538 | *B. subtilis* ATCC 19659 |
| *G. pictum* leaf extract | 8.5 ±1.4[b] | 7.3 ± 0.4[b] | 10.3 ± 0.2[b] | 13.3 ± 0.4[b] |
| Tetracycline | 22.3 ± 0.9[c] | 22.7 ± 0.9[c] | 13 ± 0.8[c] | 22.3 ± 2.3[c] |
| DMSO | 0 ± 0[a] | 0 ± 0[a] | 0 ± 0[a] | 0 ± 0[a] |

Extract and tetracycline were applied at concentrations of 25 and 200 µg/mL, respectively. Values with the same superscript letter in the same column are not significantly different based on one-way ANOVA analysis followed by multiple Duncan test range ($p < 0.05$), SD: Standard deviation, IC: Inhibitory concentration, ATCC: American Type Culture Collection

**Table 4. MIC and MBC of *G. pictum* leaf extract**

| Samples | MIC/MBC values (µg/mL) | | | |
|---|---|---|---|---|
| | *E. coli* ATCC 8739 | *P. aeruginosa* ATCC 15442 | *S. aureus* ATCC 6538 | *B. subtilis* ATCC 19659 |
| *G. pictum* leaf extract | 2500/ › 2500 | 2500/ › 2500 | 1250/2500 | 625/1250 |
| Tetracycline | 7.81/7.81 | 7.81/7.81 | 3.90/7.81 | 3.90/7.81 |

MIC: Minimum inhibitory concentration, MBC: Minimum bactericidal concentration, ATCC: American Type Culture Collection

**Table 5. Chemical profile of exract from *G. pictum* leaves**

| Number | Proposed compound | Molecular formula | Chemical class | Retention time (min) | Similarity (%) | Bioactivity | References |
|---|---|---|---|---|---|---|---|
| 1 | Eicosane | $C_{20}H_{42}$ | alkane | 18.5011 | 72 | Antifungal; antioxidant and wound healing | [22, 23] |
| 2 | 2,4-Di-*tert*-butylphenol | $C_{14}H_{22}O$ | phenol | 18.9044 | 95 | Antifungal, antioxidant, and cytotoxic on HeLa and MCF-7 cells; antibacterial | [24, 25, 26] |
| 3 | Hentriacontane | $C_{31}H_{64}$ | alkane | 19.0557 | 52 | Anti-inflammatory | [27] |
| 4 | Tetracosane | $C_{24}H_{50}$ | alkane | 20.631 | 60 | Cytotoxic on AGS, MDA-MB-231, HT-29 and NIH 3T3 cells; antioxidant | [28, 29] |
| 5 | Octacosane | $C_{28}H_{57}$ | short chain hydrocarbon | 20.9587 | 86 | Cytotoxic on B16F10-Nex2 cells; antioxidant and wound healing | [30, 23] |
| 6 | Sulfurous acid | $C_{22}H_{46}O_3S$ | mineral acid | 21.299 | 49 | Unknown | |
| 7 | 2-Methylhexacosane | $C_{27}H_{56}$ | fatty acid | 22.4459 | 53 | Unknown | |
| 8 | Docosane | $C_{22}H_{46}$ | alkane | 22.5971 | 58 | Antimicrobial | [31] |
| 9 | Heneicosane | $C_{21}H_{44}$ | alkane | 25.1681 | 90 | Antimicrobial | [32] |
| 10 | 1-Propene-1,2,3-tricarboxylic acid | $C_{18}H_{30}O$ | tricarboxylic acid | 26.2646 | 68 | Unknown | |
| 12 | Pentacosane | $C_{25}H_{52}$ | alkane | 26.9956 | 90 | Volatile attractant | [33] |

## DISCUSSION

This study evaluated the pharmaceutical properties of *G. pictum* leaf extract, including its *in vitro* antioxidant, cytotoxic, antidiabetic, and antibacterial activities. The antioxidant activity of the sample was tested against DPPH and ABTS free radicals. Furthermore, free radicals cause oxidative stress, which facilitates pathological manifestations.[34] Antioxidants have been reported to inhibit these compounds and prevent the occurrence of diseases through scavenging activities or induction of defense mechanisms.[35] Two radicals were used in this study to determine the antioxidant activity of *G. pictum* leaf extract. The DPPH assay was used to assess the electron transfer reaction, whereas ABTS was used to evaluate the hydrogen transfer reaction.[36] The results showed that *G. pictum* leaf extract had stronger effects against ABTS than DPPH, as indicated by the $IC_{50}$ value. Based on previous studies, the smaller the value obtained, the higher the effect. Furthermore, the antioxidant activity of natural extracts can be categorized on the basis of their $IC_{50}$ value, namely solid (‹ 50 µg/mL), strong (50-100 µg/mL), moderate (101-150 µg/mL), and weak (› 150 µg/mL).[37] The methanolic extract of *G. pictum* leaf was shown to have strong effects against DPPH and ABTS free radicals. Scavenging capabilities are essential to avoid the damaging activities of these compounds in different illnesses.

Several studies have shown that antioxidant compounds play a vital role in cancer prevention and treatment.[38,39] In the current study, 100 µg/mL of *G. pictum* leaf extract inhibited the growth of MCF-7 and HepG2 cells, with inhibition percentages of 74.29 ± 1.53% and 64.90 ± 1.94%, respectively. Cell viability was also reduced after the extract was applied for 48 h. The treatment also caused apoptosis and morphological changes in the form of membrane disruption in the cells, as shown in Figure 1. This finding indicated that the extract can induce an apoptotic pathway in MCF-7 and HepG2 cells. A previous study also revealed that it exhibited cytotoxic properties against human colon cancer cell WiDr with an $IC_{50}$ value of 195.61 µg/mL in the *n*-hexane fraction, but was not toxic to Verro cells.[40,41]

The α-glucosidase inhibitory effect of the extract was evaluated to determine its potency as an antidiabetic agent. The α-glucosidase enzyme was responsible for the hydrolysis

of oligosaccharides and disaccharides to glucose.[42] Therefore, blood glucose levels can be controlled by inhibiting its activity. In this study, the methanolic extract of *G. pictum* displayed an inhibitory effect towards α-glucosidase, with an $IC_{50}$ value of 194.59 ± 15.59 µg/mL. These findings are consistent with previous studies, which showed that the *n*-hexane and ethyl acetate extracts derived from the plant showed inhibitory activity.[43]

The methanolic extract of *G. pictum* leaf showed antibacterial effects against four test bacteria, namely *E. coli* ATCC 8739, *P. aeruginosa* ATCC 15442, *B. subtilis* ATCC 19659, and *S. aureus* ATCC 6538. The results also showed that it was more active in the Gram-positive strains, namely *S. aureus* and *B. subtilis*, compared with the Gram-negative bacteria because of differences in cell membrane structure. Gram-negative bacteria are known to have three layers in their external cell structure, including the outer membrane, peptidoglycan layer, and inner membrane, whereas the outer membrane was absent in Gram-positive strains.[44] This absence caused increased sensitivity to antibacterial agents. These results agree with previous studies that showed that the extract had toxic effects on *Aggregatibacter actinomycetemcomittans*, *S. aureus*, *P. aeruginosa*, and *Streptococcus mutans*.[5,45,46,47]

This study also investigated the phytochemical constituents of the methanolic extract of *G. pictum* leaf, and the results showed that it contained alkaloids, flavonoid and terpenoids. Furthermore, these compounds are responsible for several biological activities in natural products such as plants.[48,49] This indicated that they played an essential role in the pharmaceutical properties of the extract, including its antioxidant, cytotoxic, antidiabetic, and antibacterial activities. TPC of the *G. pictum* extract was higher than TFC, namely 41.17 ± 2.38 mg GAE/g and 26.52 ± 0.61 mg QE/g. TFC obtained using methanol as a solvent was higher than that obtained using aqueous, butanol, ethyl acetate, and hexane with values of 2.02, 9.02, 22.45, and 28.21 mg QE/g, respectively. For TPC, higher values were recorded in the ethyl acetate (102.57 mg GAE/g) and butanolic (45.33 mg GAE/g) extracts compared with the methanolic extract with a value of 26.52 ± 0.61 mg QE/g.[12] Based on these results, the solvent used for extraction influenced the TPC and TFC.

The pharmaceutical properties, such as antioxidant, cytotoxic, antidiabetic, and antibacterial activities, of *G. pictum* leaf extract were promoted by the presence of biologically active compounds. GC-MS analysis showed that the extract contained 12 compounds with pharmaceutical activity, as shown in Table 5. Furthermore, it consisted of eicosane, 2,4-Di-*tert*-butylphenol, tetracosane, and octacosane, which were reported to have antioxidant activity and cytotoxic properties on some carcinoma cells.[22-24,29] 2,4-Di-*tert*-butylphenol has also been shown to have toxic effects on microorganisms.[24,26] The other constituent compounds included docosane and heneicosane, which had similar effects against microbes.[31,32] A previous study isolated pentacosane, a volatile attractant, from *G. pictum* leaf extract.[31] Only three compounds, namely sulfurous acid, 2-methylhexacosane, and 1-propene-1,2,3-tricarboxylic acid, have not been reported to have biological activity, but their presence can correlate with pharmaceutical properties.

## CONCLUSION

This study showed the pharmaceutical properties of extract obtained from the leaves of *G. pictum*, including antioxidant, cytotoxic, antidiabetic, and antibacterial activities. Furthermore, the extract contained phytochemicals, such as alkaloids, flavonoid and terpenoids, which are believed to be responsible for its bioactivities. The total phenolic and flavonoid compounds in the sample were also determined. GC-MS analysis showed that it contained eicosane, 2,4-Di-*tert*-butylphenol, hentriacontane, tetracosane, octacosane, sulfurous acid, 2-methylhexacosane, docosane, heneicosane, 1-propene-1,2,3-tricarboxylic acid, tributyl ester, and pentacosane. These compounds contribute to the pharmaceutical activity of the extract. Based on these results, extracts from the leaves of *G. pictum* grown in Cirebon, West Java, Indonesia, are a potential source of therapeutic compounds that can be further studied.

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*Ethics*

**Ethics Committee Approval:** This study does not require any ethical permission.

**Informed Consent:** Not necessary.

*Authorship Contributions*

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