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

Short Title: Pharmaceutical Properties of *Graphtophylum pictum* leaves

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ABSTRACT
Graptophyllum pictum (L.) Griff is a medicinal shrub belonging to the Acanthaceae family and is traditionally used to treat various diseases. Therefore, this study aims to evaluate the pharmaceutical properties and phytochemical profiles of the methanolic extract of *G*. pictum. The results showed that the extract had potent antioxidant activity against 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) 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 the 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 analysis 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* strain ATCC 19659, with MIC and MBC values of 625 µg/mL and 1250 µg/mL, respectively. Phytochemical tests indicated the presence of alkaloids, flavonoids, and terpenoids in the extract, with total phenolic content (TPC) and total flavonoid content (TFC) of 41.17±2.38 mg GAE/g and 26.52±0.61 mg QE/g, respectively. Based on the Gas Chromatography-Mass Spectrometry (GC-MS) analysis, 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.
Keywords: Antioxidant; cytotoxic; flavonoids; phytochemical; phenols; α-glucosidase inhibitor.

INTRODUCTION

Several plants have gained recognition for their potential in becoming primary sources of medicine in drug discovery. These natural sources of herbal medicine offer an alternative to synthetic and modern drugs due to their lower potential to cause 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, acting as analgesics, anticancer, 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 several regions.[3] *Graptophyllum pictum* (L.) Griff, locally known as daun ungu, daun wungu, and handeuleum, is a herbal shrub from the Acanthaceae family. 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, analgetic, 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. properties[8,5,9] *In vivo* studies also revealed that it can decrease

blood glucose levels, as well as act as an anti-hemorrhoid, antioxidant, and anti-inflammatory agent.[10,11,12] These biological activities have been linked to its phytochemicals content, namely phenols, flavonoids, tannins, alkaloids, saponins, terpenoids, and steroids.[8]

Although several studies have reported the biological properties and metabolites 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 leaves obtained 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 the 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 calculation of the percentage inhibition (%). For the ABTS assay, radicals were produced by reacting 7 mM ABTS solution and 2.45 mM potassium persulfate (1:1) with incubation for 12-14 hours at room temperature in dark conditions. Furthermore, 170 µL of the radicals was mixed with 30 µL extract and incubated for 30 minutes, with the determination of absorbance at 734 nm. The inhibition of both assays was calculated using the formula: % = [(A1-A2)/A1] × 100%. A_1 represents the absorbance of DPPH/ABTS blank (without samples), and A_2 = the absorbance of samples. The concentration of sample required to scavenge 50% free radical (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 the positive controls.

Cytotoxicity assay

This study used human breast adenocarcinoma MCF-7 and liver carcinoma HepG2 cell lines (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. The cells were cultured in DMEM 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 carried out by seeding MCF-7 and HepG2 cells on a 96-well microplate at a concentration of 1×10^4 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 MTT (Roche) stock solution (0.5 mg/ml) was added and incubated for 3 hours 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 Thermo Scientific Varioskan Flash (Thermo Fischer). The inhibition percentage was then calculated using the formula: [1- (Abs. sample - Abs. DMSO control)] × 100%. DMSO at a final concentration of 0.05% and 100 µg/mL Cisplatin (Sigma) were used as the negative and positive controls, respectively.[17]

Antidiabetic assay

The antidiabetic activity was carried out 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 as the positive control ranged 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 with the supplementation of 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 formula: [(Abs. control - Abs. sample)/Abs. control] × 100%. The concentration of 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 disc diffusion assay was carried out based on a method proposed by a previous study.[19] The process was performed using four targeted bacterial ATCC strains, including *E. coli* ATCC 8739, *P. aeruginosa* ATCC 15442, *B. subtilis* ATCC 19659, and *S. aureus* ATCC 6538 (Collection of Laboratory of Microbiology, Department of Biology, Faculty of Mathematics and Natural Sciences, IPB University). 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 discs with a diameter of approximately 6 mm and placed on the surface of the inoculated agar plate. The antibacterial activity was then evaluated by measuring the diameter of inhibition zones surrounding the discs after incubation for 24 h at 37°C. Tetracycline and 1% DMSO were used as the positive and negative controls, respectively.

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

The minimum inhibitory concentration of the plant extracts was determined using sterile 96-well plates.[19], and each row was filled with 100 µL of sterilized Mueller Hinton Broth (MHB). 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. The bacterial cultures were prepared in 0.85% NaCl and adjusted to McFarland standard 0.5 (equivalent to 1×10^8 CFU/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 turbidity obtained was observed. The MIC was determined as the concentration at which no visible cell growth was observed. To evaluate the MBC, a portion of liquid (100 µL) from each well with no growth, was taken and spread on Mueller Hinton Agar (MHA) plate agar, followed by incubation at 37°C for 24 hr. The lowest concentration that caused the absence of visible bacterial colonization after sub-culturing was taken as MBC.

Qualitative phytochemical analysis

Phytochemical analysis was carried out 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 observed qualitatively for the presence of the targeted compound class.

Determination of total phenolic and flavonoid

The analysis of 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 was carried out for 2 h incubation at 28°C. Gallic acid was used as a standard for the calibration curve in this study. The total flavonoid content was measured using a colorimetric assay (Priyanto *et al.,* 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 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 total flavonoid content of the extract was expressed as mg quercetin equivalents per gram of extract (mg QE/g extract).

GC-MS analysis

GC-MS analysis was carried out using an Agilent 19091S-433: 93.92873 GC-MS. A total of 1 µL extract solution dissolved in n-hexane was injected into the HP-5MS 5% Phenyl Methyl Silox 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 it increased gradually over 30 minutes to 300 °C. Furthermore, helium gas was used as a carrier agent with 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 assay, were presented as means ± standard deviation from triplicates. One-way analysis of variance (ANOVA) was used to compare the mean values with 95% and 99% confidence levels. Further analysis was calculated 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 compared to 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 bacterial 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 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 compared to the positive control, quercetin at 3.35±0.01 µg/mL.

Antibacterial activity

Methanolic extract of *G. pictum* exhibited various antibacterial activities against *E. coli* strain ATCC 8739, *P. aeruginosa* strain ATCC 15442, *S. aureus* strain ATCC 6538, *B. subtilis* strain ATCC 19659, as indicated by the different inhibition zone diameter, as shown in Table 3. Among the four target bacteria, the extract was most active on *B. subtilis* strain ATCC 19659. The inhibition zone was also performed 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 *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 total phenolic content (TPC) and total flavonoid content (TFC) were 41.17±2.38 mg GAE/g and 26.52±0.61 mg QE/g, respectively.

Chemical profile of 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).

DISCUSSION

This study evaluated the pharmaceutical properties of *G. pictum* leaf extract, such as 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 are known to cause oxidative stress, which facilitated pathological manifestations.[34] Antioxidants have been reported to have the ability to inhibit these compounds and prevent the occurrence of diseases through scavenging activities or induction of defense mechanisms.[35] Two radicals were utilized in this study to determine the antioxidant activity of *G. pictum* leaf extract. The DPPH assay was used to assess the electron transfer reaction, while ABTS evaluated the hydrogen transfer reaction.[36] The results showed that *G. pictum* leaf extract had stronger effects against ABTS compared to DPPH, as indicated by the IC_{50} value. Based on previous studies, the smaller value obtained, the higher the effect. Furthermore, the antioxidant activity of natural extracts can be categorized based on their IC_{50} value, namely very strong (< 50 µg/mL), strong (50-100 µg/mL), moderate (101-150 µg/mL), 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 were essential to avoid the damaging activities of these compounds in different illnesses.

Several studies have shown that antioxidant compounds played 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. The cell viability was also reduced after the extract was applied for 48 hours. 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 of 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 through the inhibition of 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 result with previous studies, that the n-hexane and ethyl acetate extract showed inhibitory activity.[43]

The methanolic extract of G. pictum leaf showed antibacterial effects against four test bacteria, namely *E. coli* strain ATCC 8739, *P. aeruginosa* strain ATCC 15442, *B. subtilis* strain ATCC 19659, and *S. aureus* strain ATCC 6538. The results also showed that it was more active in the Gram-positive strains, namely *S. aureus* and *B. subtilis* compared to the Gram-negative bacteria due to differences in cell membrane structure. Gram-negative bacteria were known to have three layers in their external cell structure, including the outer membrane, peptidoglycan layer, and inner membrane, while the outer membrane was absent in Gram-positive strains.[44] This absence caused increased sensitivity to antibacterial agents. These results are in line with previous studies 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, flavonoids, and terpenoids. Furthermore, these compounds have been reported to be responsible for several biological activities in natural products, such as plants.[48,49] This indicated that they played an essential role in the pharmaceutical property of the extract, including its antioxidant, cytotoxic, antidiabetic, and antibacterial activities. The total phenolic content () of the *G. pictum* extract was higher compared to the total flavonoid content, namely 41.17±2.38 mg GAE/g and 26.52±0.61 mg QE/g, respectively. The TFC obtained through the use of methanol as a solvent was higher compared to 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 to the methanolic extract with a value of 26.52±0.61 mg QE/g.[12] Based on these results, the solvent used for the extraction influenced the TPC and TFC.

The pharmaceutical properties, such as antioxidant, cytotoxic, antidiabetic, and antibacterial activities, from *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 have also been shown to have toxic effects on microorganisms.[24,26] Other constituent compounds included docosane and heneicosane, which had similar effects against microbes.[31,32] A previous study had isolated pentacosane, a volatile attractant, from *G. pictum* leaf extract.[31] Based on previous reports, 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 property of extract obtained from the leaves of *G. pictum*, including antioxidant, cytotoxic, antidiabetic, and antibacterial activities. Furthermore, the extract contained phytochemicals, such as alkaloids, flavonoids, and terpenoids, which were believed to be responsible for its bioactivities. The total phenolic and flavonoid compounds in the sample were also determined in this study. The 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 have been reported to contribute to the pharmaceutical activity of the extract. Based on the results, extract from the leaves of *G. pictum* grown in Cirebon, West Java, Indonesia, is a potential source of therapeutic compounds, which can be further studied.

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Table and Figure Legend

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

Sample	**Antioxidant activity (IC_{50}±Standard deviation 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]

Note: Value with the same superscript letter at the same column is not significantly different based on one-way ANOVA analysis followed by multiple Duncan test range ($p < 0.05$).

Table 2. Antidiabetic activity of *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]

Note: Value with the same superscript letter at the same column is not significantly different based on one-way ANOVA analysis followed by multiple Duncan test range ($p < 0.05$).

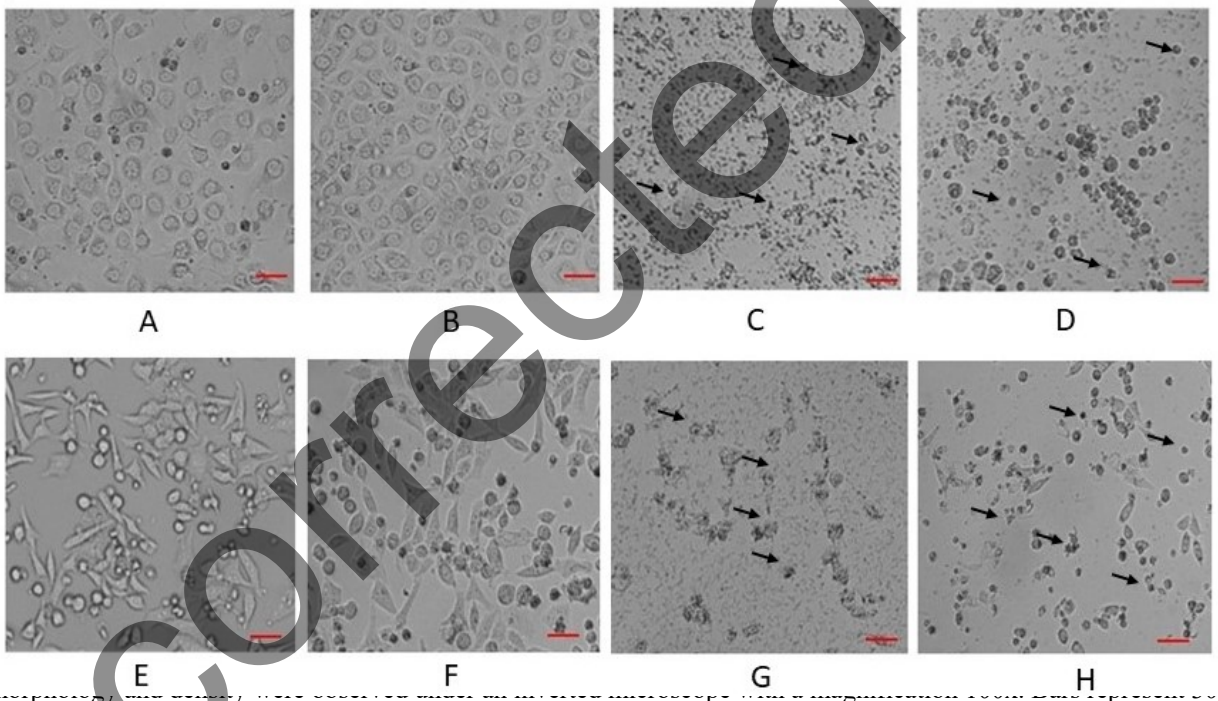
Table 3. Antibacterial activity of *G. pictum* leaf extract by disc diffusion method

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]

Note: Extract and tetracycline were applied at the concentration of 25 mg/mL and 200 µg/mL, respectively. Value with the same superscript letter at the same column is not significantly different based on one-way ANOVA analysis followed by multiple Duncan test range ($p < 0.05$).

Table 4. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (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



morphology and density were observed under an inverted microscope with a magnification 400×. Bars represent 50 µm, and black arrows indicate apoptotic cells.

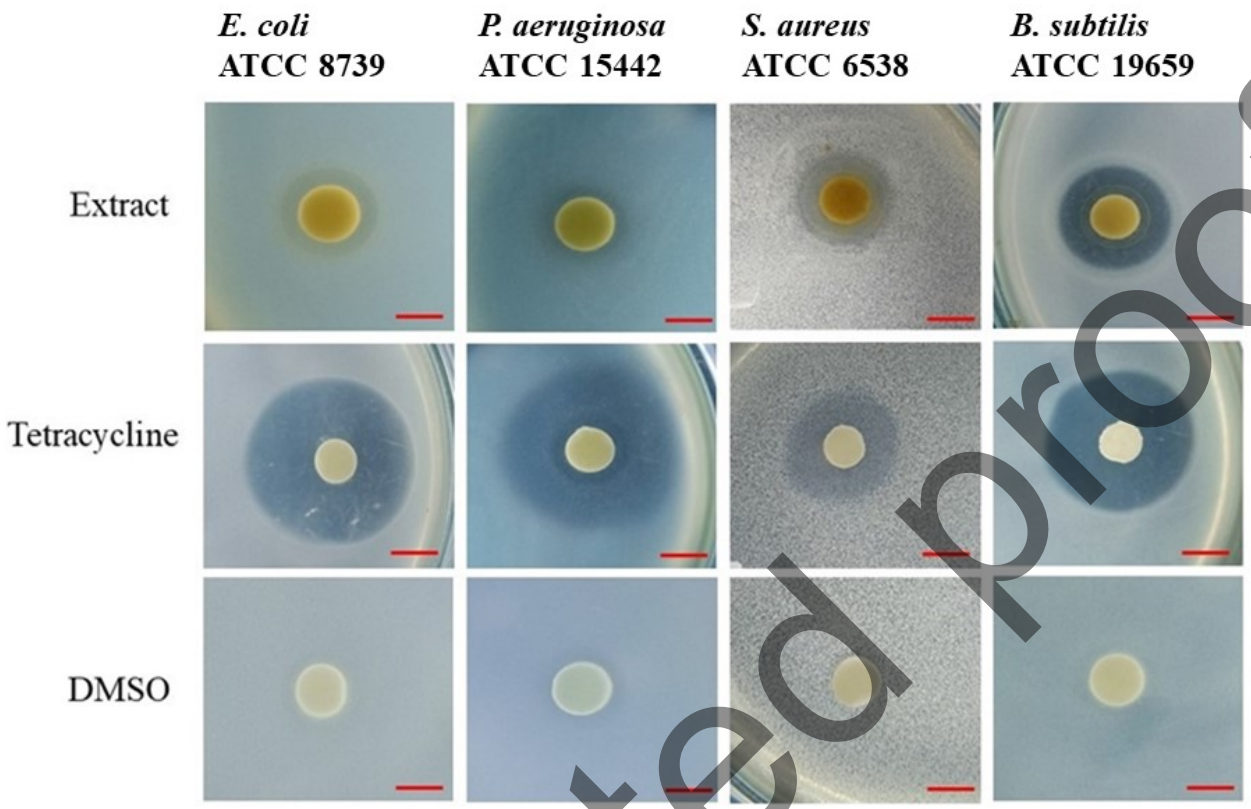


Figure 2. Antibacterial activity of *G. pictum* leaf extract (25 mg/mL) against bacterial tested; 1% DMSO and tetracycline (200 µg/mL) were used for negative and positive control, respectively. Bars represent 6 mm.

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

No.	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	unkown	
12.	Pentacosane	$C_{25}H_{52}$	alkane	26.9956	90	volatile attractant	33