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Bacterial acrylamidase

Assessment of Antioxidant, Antimicrobial and Wound Healing Potential of Golden Chicken Fern (*Cibotium barometz* (L.) J. Sm.) Methanolic Extracts

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ABSTRACT

Cibotium barometz (L.) J. Sm., also known as the golden chicken fern, is valued in traditional Chinese medicine, particularly for its rhizome and golden hairs, which are reputed to have medicinal properties. However, there is limited scientific evidence supporting the wound-healing properties of its golden hairs. This study aims to evaluate the antioxidant, antimicrobial, and wound-healing potential of extracts from these hairs. The golden hairs were extracted with 80% methanol to isolate bioactive compounds. Antioxidant activity was measured using the DPPH radical scavenging assay, and total phenolic content (TPC) and total flavonoid content (TFC) were assessed. TFC was found to be higher than TPC, with values of 331 ± 0.011 mg QE/g and 67.36 ± 0.014 mg GAE/g, respectively. The antimicrobial properties of the extract were tested against *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli*, *and Serratia marcescens* using agar well diffusion; no inhibition zones were observed, indicating a lack of antimicrobial activity. Further assays, including cytotoxicity and wound scratch tests on normal human dermal fibroblast (NHDF) cells, revealed that the extract did not promote wound healing and suppressed NHDF cell growth at a concentration of 10 mg/mL. In conclusion, while *C. barometz* golden hairs are traditionally valued, this study found limited antioxidant activity and no antimicrobial or wound-healing efficacy in the extract.

INTRODUCTION

Medicinal plants are commonly used as raw materials for extracting active ingredients that are utilized in the synthesis of various drugs [1]. Medicine preparation often involves extraction from whole plants or specific plant parts. A plant extract is a substance with desirable properties removed from plant tissue, typically through solvent extraction, for particular applications. Plant extracts are widely used in herbalism and traditional medicine. Medicinal plants have been employed globally to treat numerous diseases and infections due to the presence of secondary metabolites [2]. Key bioactive compounds in plants include alkaloids, flavonoids, tannins, and phenolic compounds. Antibiotic resistance has become a persistent challenge in the healthcare sector globally [3]. The spread of multidrug-resistant bacteria in communities has raised concerns in health services. While synthetic drugs are available to treat infections, they may cause harmful side effects. Natural products, such as plant extracts, offer vast opportunities for new drug discoveries. According to the World Health Organization (WHO), over 80% of the global population relies on traditional medicine for primary

healthcare. In Asia, the use of herbal medicines reflects a longstanding human-environment interaction [4].

Several plants, such as *Centella asiatica* and *Curcuma longa*, exhibit wound-healing properties. Plants are considered potent healers as they naturally support repair mechanisms. Their use could reduce the demand for antibiotics and the associated side effects. The demand for herbal drugs is rising globally due to their safety and tolerability compared to allopathic drugs [5]. Given the potential of plants in wound management, it is crucial to examine all available options to improve wound treatment. Furthermore, scientific validation and safety evaluation are needed before recommending plants for wound healing.

Cibotium barometz (L.) J. Sm., also known as the golden chicken fern as shown in **Fig. 1**, is an important export for both traditional and modern medicine in China, Japan, and France [6]. Various plant parts, including the rhizome, golden hairs, and roots, have medicinal uses. Tap et al. [7] and Wu and Yang [8] demonstrated that *C. barometz* is valued as a medicinal herb, with the golden hairs on its rhizome thought to act as a haemostatic agent when applied to wounds and cuts. Lim [9] had reported that there was a wild distribution of this species in Southeast Asia. They are abundantly present in China, Vietnam, Japan, Thailand, Indonesia and Malaysia. *C. barometz* can grow in the valley, forest edges, along stream-banks and mountainous area from 100 to 1500 m altitude. It is well adapted to warm and humid climatic conditions with optimum temperature between 20ºC and 23°C and the rainfall ranges from 1800 to 2600 mm every year. It prefers red-brown ferralitic and acid soils but will tolerate slightly alkaline soils.

Fig 1. The golden chicken fern (*C. barometz)* plant.

Lai et al. [10] reported *C. barometz* leaf extract to have antioxidative activity that were assessed by 1,1- diphenyl-2-picrylhydrazyl (DPPH) radical scavenging, ferric ion reducing power (FRP), β -carotene bleaching (BCB) and ferrous ion chelating (FIC) and antibacterial activity to *Staphylococcus aureus* and *Bacillus cereus.* Also, Mai et al. [11] reported that methanolic extract of *C. barometz*'s rhizome possess an effective antioxidant activity by various assay including DPPH, ABTS, superoxide anion (O_2^-), and hydroxyl (OH) radical scavenging, Fe^{3+} reducing power and Cu^{2+} reducing power assays which can contribute to its medicinal effects. In Malaysia, the fern grows in Cameron Highlands, where the indigenous community claims it has wound-healing properties. However, scientific evidence to support this claim is limited. Therefore, this study collected the golden hairs of this medicinal plant to assess their antioxidant activity, antimicrobial properties against selected microorganisms, and wound-healing potential.

MATERIALS AND METHODS

Plant material and source of bacteria

The plant *Cibotium barometz* (L.) J. Sm. was commercially obtained from the indigenous community in Cameron Highlands, Pahang, Malaysia. The golden hairs were collected for analysis. Two strains of gram-positive bacteria (*Staphylococcus aureus* and *Bacillus cereus*) and two strains of gram-negative bacteria (*Escherichia coli* and *Serratia marcescens*) were sourced from the culture collection of Department of Microbiology, Faculty of Biotechnology and Biomolecular Sciences, UPM.

Preparation of methanol extraction

The preparation of the methanolic crude extract was carried out according to the method proposed by Chandrasekaran and Venkatesalu [12] with slight modifications. Dried plant material was ground using a blender. A 35 g sample of the ground plant was soaked in 1250 mL of 80% methanol (ensuring the plant material was fully immersed) for a minimum of 48 h at room temperature. The sample was then suction-filtered through Whatman No. 1 filter paper, and the residue was soaked again in another 1250 mL of 80% methanol for an additional 48 h. The filtrate was evaporated to dryness under reduced pressure at 40 °C using a rotary evaporator (Eyela, Japan). The dried sample extracts were weighed using a balance (Setra, USA) and stored at 4 °C until further use. The percentage yield of extraction was calculated using the following equation:

$$Yield\ (\%) = \frac{W1}{W2}\ x\ 100$$

Where W1 is the weight of the extract after drying using the rotary evaporator, and W2 is the initial weight of the plant powder used."

Preparation of test extract solutions for antioxidant assay

The preparation of test solutions for the antioxidant assay was carried out as suggested by Chandrasekaran and Venkatesalu [12]. A series of different concentrations of the methanolic crude extract was prepared by dissolving a known weight of the plant crude extract and standards (ascorbic acid, gallic acid, and quercetin) in 1% (v/v) dimethyl sulfoxide (DMSO), which served as the solvent.

Determination of total phenolic content (TPC)

Twenty-five microliters of gallic acid standard solutions (31.25, 62.5, 125, 250, and 500 µg/mL) and *C. barometz* extract (1000 µg/mL) were mixed with 125 µL of Folin–Ciocalteu reagent (diluted 10-fold with distilled water) in the wells of a 96-well microplate and allowed to react for 10 minutes. Then, 125 µL of sodium carbonate solution (7.5%) was added, and the mixture was allowed to stand for 30 minutes before the absorbance of the reaction mixture was read at 765 nm using a visible–UV microplate kinetic reader (BioTek Instruments, USA). The results were expressed as milligrams of gallic acid equivalent per gram of dry weight (mg GAE/g DW). All standard dilutions and samples were analysed in triplicate (Wu and Yang. [8]).

Determination of total flavonoid content (TFC)

The determination of total flavonoid content (TFC) was carried out according to the method proposed by Wu and Yang [8] with slight modifications. Briefly, 50 µL of quercetin standard solutions (31.25, 62.5, 125, 250, and 500 µg/mL) and *C. barometz* extract (1000 µg/mL) were mixed with 20 µL of

$NaNO_2$ (0.5 mol L^{-1}) and 20 µL of $AlCl_3$ (0.3 mol L−1) reagent in a 96-well microplate and allowed to incubate at room temperature for 10 minutes. Next, 200 µL of NaOH solution (0.5 mol L^{-1}) was added and allowed to stand for 30 minutes before the absorbance of the reaction mixture was read at 510 nm using a visible–UV microplate kinetic reader (BioTek Instruments, USA). The results were expressed as milligrams of quercetin equivalent per gram of dry weight (mg QE/g DW). All samples were analysed in triplicate.

DPPH radical scavenging activity

The antioxidant properties were evaluated using the DPPH radical scavenging activity method described by Ayele et al. [13] with some modifications. Briefly, a DPPH solution (0.2 mM) was prepared by dissolving 3.94 mg of DPPH in 50 mL of pure methanol. Plant extracts with varying concentrations (250, 200, 100, 50, and 25 µg/mL) and standards (ascorbic acid) at concentrations of 200, 100, 50, 25, 12.5, 6.25, and 3.125 µg/mL were prepared. Then, 10 µL of each concentration was incubated in the dark with 200 µL of the 0.2 mM DPPH solution at room temperature for 30 minutes in a 96-well plate. The absorbance of the reaction mixture was read at 517 nm using a visible–UV microplate kinetic reader (BioTek Instruments, USA). All determinations were performed in triplicate.

The radical scavenging activity was estimated using the following formula:

$$\% \, inhibition = \left\{\frac{(AB - AA)}{AB}\right\} X \, 100$$

where AB is the absorbance of the control and AA is the absorbance of the tested sample at 517 nm."

Preparation of test extract solution for antimicrobial assay

A series of different concentrations of methanolic crude extract (10, 5, and 2.5 mg/mL) were prepared by dissolving a known weight of the plant crude extract and a standard (chloramphenicol) at a concentration of 100 µg/mL in 1% (v/v) dimethyl sulfoxide (DMSO), which served as the solvent.

Agar well diffusion assay

For the agar well diffusion assay, 10 mL of LB agar was prepared. Forty microliters of *S. aureus*, *B. cereus*, *E. coli*, and *S. marcescens*, which had previously been grown in LB broth, were mixed well with the hot LB agar. The mixture was then poured into LB agar plates and left to solidify. Wells with a diameter of 6 mm were created using a sterile yellow tip. After that, 100 µL of the sample was added to each well and left at 4 °C for 2 h. All plates containing *S. aureus*, *B. cereus*, and *E. coli* were incubated at 37 °C, while the plate with *S. marcescens* was incubated at room temperature for 24 h. Finally, the diameter of the clear zone surrounding each well was measured as the inhibition zone to the nearest millimetre. An agar well (6 mm) showing no zone of inhibition was considered to exhibit no antimicrobial activity. Chloramphenicol (100 µg/mL) was used as the positive control, while 1% DMSO was used as the negative control. All tests were conducted in triplicate for each type of microorganism (modified from Shami et al. [14]).

NHDF cell culture

Normal human dermal fibroblast (NHDF) cells were obtained and maintained according to the ATCC protocol. The cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 5% fetal bovine serum (FBS) and incubated at 37 °C in a 5% CO2 atmosphere. Cells at 80% to 90% confluence were used for seeding and treatment throughout the experiment (Muhammad et al. [15]).

Crystal violet assay

NHDF cells were seeded in 24-well plates at a concentration of 1.5×10^5 cells/well. The media were pipetted out and discarded, and the cells were rinsed with phosphate-buffered saline (PBS). DMEM with serial dilutions of different concentrations ofmethanolic plant extract (200 µL) at 1, 10, 100, 1000, and 10,000 µg/mL was added, and the plates were incubated at 37 °C in a 5% CO2 atmosphere for 24 h. The cells were then rinsed with PBS to remove any dead cells, and formaldehyde was added for 10 to 15 minutes to fix the living cells. The formaldehyde was then removed, and the cells were washed with PBS. Next, 500 µL of crystal violet solution (0.5%) was added to the wells, allowing the dye to be taken up by viable cells for 10 to 15 minutes. The plate was washed with tap water and allowed to dry at room temperature. Images were captured and analysed (modified from Al-Fatimi et al. [16]).

Wound scratch assay

NHDF cells were seeded in 6-well plates at a concentration of 5×10^5 cells/well and cultured in fibroblast media containing 5% FBS. A small area was then scratched using a 200 µL pipette tip. The media were pipetted out and discarded, and the cells were rinsed with PBS to remove any loosened debris. DMEM with serial dilutions of different concentrations of methanolic plant extract at 10, 100, 1000, and 10,000 µg/mL was added, and the plates were incubated at 37 °C in a 5% CO2 atmosphere. The distance between the two layers of cells that were scratched by the pipette tip was inspected microscopically at 0 and 24 h. As the NHDF cells migrated to fill the scratched area, images were captured using a digital camera attached to a microscope (Cole-Parmer, USA) and a computer system with ToupTek ToupView software. The experiments were performed in triplicate [13].

Statistical analysis

All experiments have been conducted in triplicate and its value expressed as mean ± standard deviation (SD) using Microsoft Excel.

RESULTS AND DISCUSSION

Yield of extraction

Biological assays often begin with the crucial step of extracting bioactive compounds from plants. The ultimate goal of extraction is to maximize the yield of high-quality substances that contain a high concentration of desirable compounds [17]. Various extraction methods have been employed to isolate compounds from plant materials, including Soxhlet extraction and maceration. The biological activities of plant extracts can vary significantly depending on the extraction method used, highlighting the importance of selecting the appropriate technique [18]. In this study, maceration was chosen as the methodology because the targeted constituents are thermolabile and the process can be conducted at room temperature.

Additionally, the extraction solvent is a critical factor that affects the efficiency of bioactive compound extraction from plant materials and their associated health benefits. In the present study, the dried hair of the plant was macerated using methanol. Methanol was selected because Yao et al. [19] reported it as a suitable solvent for extracting polyphenols from fresh plants due to its ability to inhibit the action of polyphenol oxidases, which could otherwise affect antioxidative activity, as well as its ease of evaporation. The extraction yield was measured to determine the efficiency of the solvent in extracting bioactive compounds from the original material and is presented as a percentage (%). Murugan and Parimelazhagan [20] defined extraction yield as the

amount of recovered extract compared to the initial amount of plant material used. Based on this equation, the percentage extraction yield of the methanolic plant extract obtained was 8.3%.

DPPH free radical scavenging activity of *C. barometz* extract

The antioxidant potential of *C. barometz* extracts was determined by performing the DPPH free radical scavenging assay, using ascorbic acid as a positive control. **Fig. 2** shows the percentage inhibition of both extracts and ascorbic acid at concentrations ranging from 25 to 250 µg/mL and 3.125 to 200 µg/mL, respectively. The DPPH stable free radical method is a sensitive technique for assessing the antioxidant activity of plant extracts [21]. The results indicated that ascorbic acid exhibited higher free radical scavenging activity at a concentration of 200 µg/mL, with an inhibition of 82.89% ± 0.012, compared to the plant extract, which showed an inhibition of 14.83% ± 0.01. The significant difference in percentage inhibition between ascorbic acid, a known potent antioxidant, and the sample extract suggests that the methanolic *C. barometz* hair extract possesses low antioxidant activity.

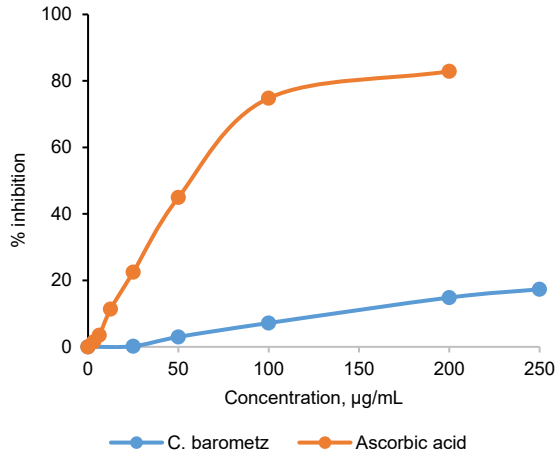

Fig. 2. DPPH free radical activity of *C. barometz* extracts at the concentration ranging from 25 - 250 µg/mL with a comparison to ascorbic acid. The data were analysed as the mean of triplicate measurements, with error bars representing the standard errors.

TPC and TFC of *C. barometz* extract

Phenolics are the most widespread secondary metabolites in the plant kingdom and have garnered significant attention as potential natural antioxidants due to their abilities to act as both efficient radical scavengers and metal chelators [22]. In this study, the Folin-Ciocalteu method was employed to determine total phenolic content. The principle of the assay is as follows: the Folin-Ciocalteu reagent can oxidize total phenolic compounds, which reduces the reagent to a blue compound, with the colour intensity being positively related to the total phenolic content. In this assay, gallic acid was used as the standard, and the results demonstrated a strong linear relationship, as described by the linear regression equation: y=0.0056x+0.2861y = 0.0056x + 0.2861y=0.0056x+0.2861 (R^2 = 0.9952, where x is the sample concentration and y is the absorbance). The total phenolic content of the methanolic extract of *C. barometz* was found to be 67.36 ± 0.014 mg GAE/g. Due to the structural complexity of bioactive compounds in plants, it is essential to explore various solvents with different polarities to identify the most effective options for antioxidant extraction. In this context, Zarib et al. [23] reported that the ethyl acetate extract of *C. barometz* exhibited the highest total phenolic content, exceeding that of methanol, cyclohexane, and dichloromethane extracts.

In addition to phenolics, flavonoids also play an important role in scavenging free radicals in the body and act as antioxidants. In this study, the aluminium chloride colorimetric assay was used to determine the total flavonoid content. Quercetin was used as the standard, and the results showed a good linear relationship, as indicated by the linear regression equation: y=0.0005x+0.1275y = 0.0005x + 0.1275y=0.0005x+0.1275 (R^2 = 0.9651, where x is the sample concentration and y is the absorbance). The total flavonoid content of the methanolic extract of *C. barometz* was 331 ± 0.011 mg QE/g. The comparison of TFC and TPC of the sample at a concentration of 1 mg/mL is shown in **Fig. 3**, where the amount of flavonoid extracted is higher than the amount of phenolics present.

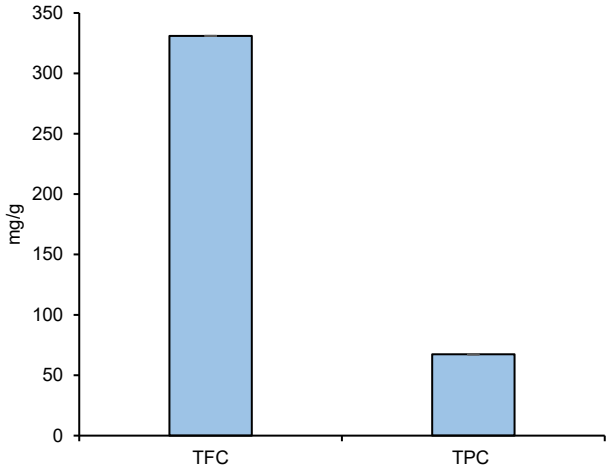

Fig. 3. Total flavonoid content (TFC) and total phenolic content (TPC) of *C. barometz* extract at concentration 1 mg/mL. The data were analysed as the mean of triplicate measurements, with error bars representing the standard errors.

A previous study by Al-Wajeeh et al. [24] reported that *C. barometz* possesses high free radical scavenging activity and contains many phenolic compounds that are potent antioxidants. However, the amount of phenolics obtained in this study was low, likely due to environmental conditions that are less suitable for this plant species. According to Lai et al. [22], phenolic compounds are generated by plants in response to environmental stress. Therefore, plants in high-altitude areas exposed to stressors such as low temperatures and decreased pressure tend to have a higher concentration of polyphenols. In this study, the habitat of the plant was altered. *C. barometz* typically thrives in mountainous regions at high altitudes, which allows for a higher total phenolic content (TPC). However, when transferred to a low-altitude environment, the accumulation of phenolic compounds is less stimulated, resulting in a lower TPC value upon analysis.

Moreover, the concentration of phenolic compounds in plant material is directly related to its antioxidant activity, suggesting that a significant portion of the antioxidant activity in plant extracts may be attributed to phenolic compounds [11,25] . As the hair extract of *C. barometz* possesses low total phenolic content, its antioxidant activity is also low, as demonstrated by the DPPH radical scavenging activity. This result indicates that phenolic compounds contribute significantly to the antioxidant capacity of the investigated plant species, consistent with

findings from numerous research groups that reported a positive correlation between total phenolic content and antioxidant activity [26-28].

Fig. 3 shows that more flavonoids were extracted from *C. barometz*, as indicated by the higher total flavonoid content (TFC) compared to the total phenolic content (TPC). However, this high flavonoid content did not influence the antioxidant activity, as the radical scavenging effect observed in this study was low. This suggests that there is a stronger correlation between phenolic compounds and the inhibition of free radicals compared to flavonoids. This result aligns with the findings of Anokwuru et al. [29], who reported that the plant extract with the highest flavonoid content exhibited the lowest antioxidant activity. They suggested that the flavonoid content in the extract may be glycosylated, which could render the flavonoids less available for antioxidant activity. This is further supported by Chavan et al. [30], who stated that flavonoids contribute minimally to antioxidant activities, indicating a relatively low correlation between these two factors.

Antimicrobial activity of *C. barometz* extract

In this study, *C. barometz* hair that underwent the extraction process was tested for its antimicrobial properties using the agar well diffusion assay. Four types of bacteria were used in this experiment*: B. cereus* and *S. aureus* (gram-positive bacteria) and *E. coli* and *S. marcescens* (gram-negative bacteria). **Fig. 4** shows the inhibition zones observed after 24 h. The study found no inhibition zones for both gram-negative and gram-positive bacteria when the plant extract was added at concentrations of 2.5, 5.0, and 10 mg/mL, which was similar to the negative control of 1% DMSO. The inhibition zone was only present around chloramphenicol, the positive control at a concentration of 100 µg/mL. Chloramphenicol exhibited inhibition zones for all four microorganisms: 11 ± 1.41 mm for *S. aureus*, 7.5 ± 0.71 mm for *B. cereus*, 9.5 ± 0.71 mm for *E. coli*, and 13.5 ± 2.12 mm for *S. marcescens*.

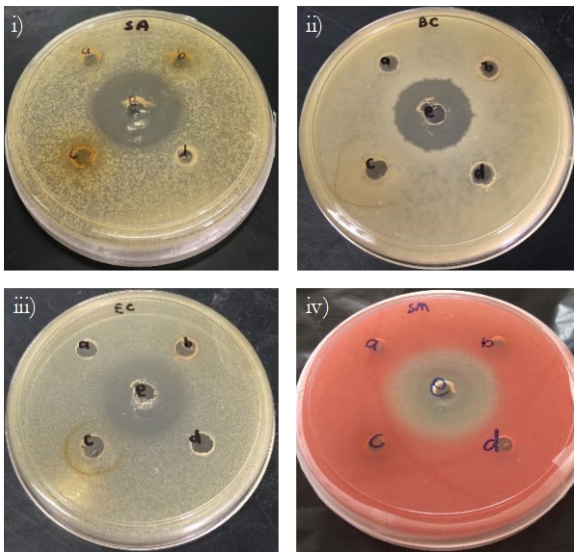

Fig. 4. Inhibition zone from agar well diffusion assay for gram-positive and gram-negative bacteria at different concentration: The alphabets indicate different concentrations [a) 2.5 mg/mL b) 5.0 mg/mL c) 10 mg/mL d) 1% DMSO e) 100 µg/mL chloramphenicol] and the roman numbers indicate different species [i) *S. aureus* ii) *B. cereus* iii) *E. coli* iv) *S. marcescens*].

Previous studies have reported similar findings for gram-negative bacteria, which are more resistant to fern extracts, including *C. barometz*. According to Lai et al. [10], some fern extracts such as *C. barometz*, *D. linearis*, and *B. orientale* exhibit no antimicrobial activity against *E. coli*, *P. aeruginosa*, *E. aerogenes*, and *K. pneumoniae*, all of which are gram-negative bacteria. The insensitivity of the fern extract against gram-negative bacteria is likely due to the impermeable nature of their outer membrane.

The outer membrane of gram-negative bacteria is enriched with lipopolysaccharides (LPS), which confer hydrophobic properties and significantly reduce membrane permeability to various substances, including plant-derived extracts. The limited efficacy of *C. barometz* extract against gram-negative bacteria may be attributed to their chemical composition, potentially consisting of larger or less lipophilic molecules that are unable to effectively traverse this barrier. Furthermore, the presence of efflux pumps and selective porins in gram-negative bacteria exacerbates the restriction of intracellular uptake of these compounds. Consequently, despite the presence of bioactive compounds within the extract, their inability to bypass these structural and functional defences renders them ineffective against gram-negative bacteria.

In addition, the outer membrane of gram-negative bacteria surrounds the cell wall, providing an additional layer of protection that antibiotics must overcome to kill the bacteria. The outer membrane consists of macromolecules formed by a combination of lipid bilayer and protein compositions, which significantly impact the sensitivity of bacteria to various antibiotics [31]. Previous reports indicate that gram-negative bacteria are resistant to most plant extracts [32]. Hence, this study demonstrates that the gram-negative bacteria, *E. coli* and *S. marcescens*, are resistant to the methanolic hair extract of *C. barometz*.

In contrast, the cell wall of gram-positive bacteria is composed of peptidoglycan layers that help stabilize the cell membrane, which consists of a phospholipid bilayer. Unlike gram-negative bacteria, gram-positive bacteria do not have an outer membrane to protect them from antibiotics. The cell wall in gram-positive bacteria consists of a single layer, while the gram-negative cell wall is multi-layered. However, this study also showed that the extract exhibited no antimicrobial activity against gram-positive bacteria. This result differs from findings reported by Lai et al. [10], where the methanolic extract of *C. barometz* possessed antimicrobial activity against gram-positive bacteria, *S. aureus* and *B. cereus*. Additionally, previous studies have reported that most plant extracts show activity against gram-positive strains [33].

This discrepancy may be related to the antioxidant activity of the extract. As phenolic compounds possess antibacterial, antiviral, and antifungal actions in plants, their content may influence the antimicrobial properties of the plant. Abram et al. [34] reported that the antimicrobial activity of a plant is correlated with its total phenolic content, which was consistent across the samples. Thus, since the TPC value is low, the antimicrobial activity of the extract is absent.

Cytotoxicity of *C. barometz* extract

Before conducting the scratch assay to evaluate the wound healing potential, it is essential to assess the cytotoxicity of the extract to ensure that it does not adversely affect NHDF cells.

Therefore, a crystal violet assay was performed, as it is a rapid and reliable method for this purpose. This assay relies on the ability of viable cells to incorporate and bind the dye. **Fig. 5** illustrates the crystal violet staining of NHDF cells treated with various concentrations of plant extract after 24 h of incubation.
In general, cells detach from the tissue culture plate upon death, a characteristic that can be utilized to identify cell viability following the addition of the extract. The attachment of cells was assessed by staining the attached cells with crystal violet dye, which binds to cellular proteins and DNA. Dead cells become detached, resulting in a decrease in crystal violet staining within the culture [35]. As observed in **Fig. 5**, the crystal violet staining for cells treated with different concentrations of extract, ranging from 0.001 mg/mL to 10 mg/mL, was similar to that of the control (0 mg/mL). This finding indicates that the extract was nontoxic to NHDF cells after 24 h of incubation. However, microscopic observations during the wound scratch assay (**Fig. 6**) revealed that NHDF cells shrank at the concentration of 10 mg/mL, suggesting slight toxicity of the extract at this higher concentration.

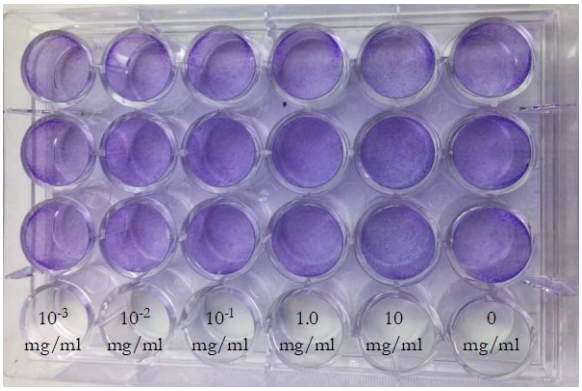

Fig. 5. Uptake of crystal violet dye by viable cells observed after 24 h.

Wound scratch assessment of *C. barometz* extract

To identify the wound healing activity of the plant extract, a wound scratch assay was conducted, which is a simple and widely used method for studying cell migration. This method involves creating an artificial gap, referred to as a "scratch," in a confluent cell monolayer. Over time, the cells at the edge of the gap migrate toward the cell-free area to close the scratch and re-establish cell contacts [36,37]. In this study, human normal skin cell lines were utilized to evaluate both the cytotoxicity of the extract and its wound healing activity. Human dermal fibroblast cells, located in the dermis layer of the skin, are responsible for generating connective tissue. The most common approach to simulate wounding is to create a gap by scratching a confluent monolayer of cells using a pipette tip, needle, or other sharp instruments. In this study, a pipette tip was used to create the scratch. Since the scratch is created manually, achieving consistent wounds can be challenging. Therefore, it is crucial to maintain consistent pressure and angle of the pipette tip throughout the assay to ensure uniformity in the wound formation. Additionally, rinsing the injured cells with PBS or another suitable buffer before replacing the cell culture medium is essential. This rinsing step removes debris from damaged or dead cells, especially after the scratching process. Furthermore, scratching may release growth factors from the damaged cells; thus, replacing the growth medium with fresh medium after scratching helps regulate the factors available for cell migration [38].

The results are presented in **Fig. 6**, showing images captured at 0 h and 24 h post-wounding. After 24 h, the migration of fibroblasts treated with the methanolic crude extract at 0.01 mg/mL was comparable to the control group (0 mg/mL). At 0.1 mg/mL, cell migration was slower than that of the control, and at 1 mg/mL, there was little difference in cell lines between 0 h and 24 h. At 10 mg/mL, no migration was observed, and it was evident that this concentration caused shrinkage of fibroblast cells, indicating toxicity at higher concentrations. The lack of significant difference between the control and treated cells suggests that the methanolic plant extract does not accelerate the wound healing process of the NHDF cells. However, in diabetic rats, wounds treated with *C. barometz* exhibited a smaller wound area and a faster rate of wound closure, independent of dosage and healing duration. The observed differences in wound healing between NHDF cells and diabetic rats maybe be attributed to several factors, including the cellular environment, metabolic conditions, and the nature of the extracts used [39].

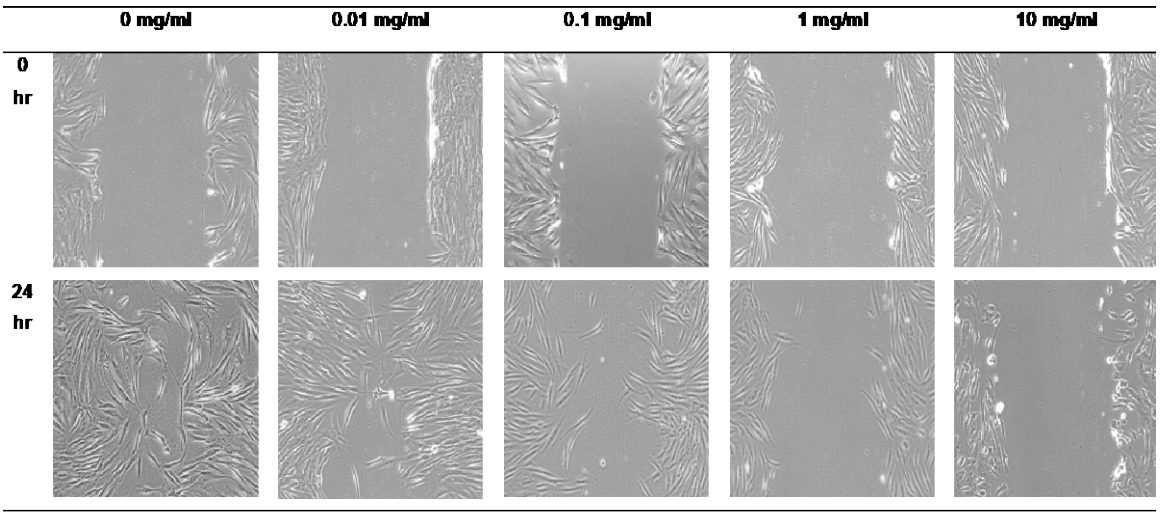

Fig. 6. Digital images showing the effect of different concentrations of the sample on normal human dermal fibroblasts in a wound scratch assay at 0 and 24 h. The yellow line indicates the wound created by scratching the NHDF cells.

Generally, the wound healing process can be categorized into three stages: the inflammatory phase, proliferative phase, and remodeling phase, all of which determine the appearance of the healed tissue. The inflammatory phase involves preparing the wound area for healing and restricting movement around the wound. During this phase, haemostatic mechanisms are initiated to halt blood loss at the wound site, involving platelet aggregation to promote blood clotting and inflammation [5]. According to Wang and Ming [40], extracts of C. barometz can be combined with gelatin paste to form an effective haemostatic agent, helping to stop or minimize bleeding. Their blood coagulation studies observed significant clot formation with 10% extract. Therefore, the plant extract appears to play a role primarily in haemostatic mechanisms rather than in the wound healing process.

CONCLUSION

The hair extract of *C. barometz* was obtained through maceration using 80% methanol. To assess its antioxidant activity, a DPPH radical scavenging assay was conducted, and the total phenolic content (TPC) was determined using the Folin-Ciocalteu method, while the total flavonoid content (TFC) was measured using the aluminum trichloride ($AlCl_3$) method. The results indicated that the DPPH scavenging activity of the extract was low, corresponding with the low TPC value compared to the TFC. The agar well diffusion assay was performed to evaluate the antimicrobial activity against *S. aureus*, *B. cereus*, *E.coli*, and *S. marcescens*. However, the plant extract exhibited no antimicrobial activity, as no inhibition zones were observed against both gram-positive and gram-negative bacteria. Additionally, the cytotoxicity and wound healing activity of the extract were assessed using the crystal violet assay and wound scratch assay with normal human dermal fibroblast (NHDF) cells. The results showed that the extract was slightly toxic to the cells at high concentrations and exhibited no wound healing activity. Further studies on blood coagulation and haemostatic activity should be conducted to analyse the properties of the hair extract. Moreover, exploring alternative extraction methods or solvents may improve the extraction efficiency of bioactive compounds.

CONFLICT OF INTEREST

The authors have declared that no conflict of interest exists.

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None.

ETHICS STATEMENT

Not applicable

REFERENCES

1. Singh R. Medicinal plants: A review. J Plant Sci. 2015;3(1-1):50-5.
2. León J, Rojo E, Sánchez-Serrano JJ. Wound signalling in plants. J Exp Bot. 2001;52(354):1-9.
3. Valle DL, Andrade JI, Puzon JJM, Cabrera EC, Rivera WL. Antibacterial activities of ethanol extracts of Philippine medicinal plants against multidrug-resistant bacteria. Asian Pac J Trop Biomed. 2015;5(7):532-40.
4. Duraipandiyan V, Ayyanar M, Ignacimuthu S. Antimicrobial activity of some ethnomedicinal plants used by Paliyar tribe from Tamil Nadu, India. BMC Complement Altern Med. 2006;6(1):35.
5. Rawat S, Singh R, Thakur P, Kaur S, Semwal A. Wound healing agents from medicinal plants: a review. Asian Pac J Trop Biomed. 2012;2(3).
6. Praptosuwiryo TN, Pribadi DO, Rugayah. Growth, development and morphology of gametophytes of golden chicken fern (*Cibotium barometz* (L.) J. Sm.) in natural media. Biodiversitas. 2015;16(2):303-10.
7. Tap N, Son LT, Phuong ND, Nga NQ, Huyen PT, Tien N. Non-detriment finding for *Cibotium barometz* in Viet Nam. 2006;1:1-10.
8. Wu Q, Yang XW. The constituents of *Cibotium barometz* and their permeability in the human Caco-2 monolayer cell model. J Ethnopharmacol. 2009;125(3):417-22.
9. Lim TK. Edible medicinal and non-medicinal plants: Volume 12, modified stems, roots, bulbs. Edible Med Non-Med Plants. 2016;12:1-690.
10. Lai HY, Lim YY, Tan SP. Antioxidative, tyrosinase inhibiting and antibacterial activities of leaf extracts from medicinal ferns. Biosci Biotechnol Biochem. 2009;73(6):1362-6.
11. Mai W, Chen D. Antioxidant activity of *Rhizoma cibotii in vitro*. Adv Pharm Bull. 2012;2(1):107-14.
12. Chandrasekaran M, Venkatesalu V. Antibacterial and antifungal activity of *Syzygium jambolanum* seeds. J Ethnopharmacol. 2004;91(1):105-8.
13. Ayele Y, Kim JA, Park E, Kim YJ, Retta N, Dessie G, Kim H. A methanol extract of *Adansonia digitata* L. leaves inhibits pro-inflammatory iNOS possibly via the inhibition of NF-κB activation. Biomol Ther (Seoul). 2013;21(2):146.
14. Shami AMM, Philip K, Muniandy S. Synergy of antibacterial and antioxidant activities from crude extracts and peptides of selected plant mixture. BMC Complement Altern Med. 2013;13(1):360.
15. Muhammad AA, Pauzi NAS, Arulselvan P, Abas F, Fakurazi S. *In vitro* wound healing potential and identification of bioactive compounds from *Moringa oleifera* Lam. Biomed Res Int. 2013;2013:1-9.
16. Al-Fatimi M, Wurster M, Schröder G, Lindequist U. Antioxidant, antimicrobial and cytotoxic activities of selected medicinal plants from Yemen. J Ethnopharmacol. 2007;111(3):657-66.
17. Spigno G, De Faveri DM. Antioxidants from grape stalks and marc: Influence of extraction procedure on yield, purity and antioxidant power of the extracts. J Food Eng. 2007;78(3):793-801.
18. Hayouni EA, Abedrabba M, Bouix M, Hamdi M. The effects of solvents and extraction method on the phenolic contents and biological activities *in vitro* of Tunisian *Quercus coccifera* L. and *Juniperus phoenicea* L. fruit extracts. Food Chem. 2007;105(3):1126-34.
19. Yao L, Jiang Y, Datta N, Singanusong R, Liu X, Duan J, et al. HPLC analyses of flavanols and phenolic acids in the fresh young shoots of tea (*Camellia sinensis*) grown in Australia. Food Chem. 2004;84(2):253-63.
20. Murugan R, Parimelazhagan T. Comparative evaluation of different extraction methods for antioxidant and anti-inflammatory properties from *Osbeckia parvifolia* Arn.–An *in vitro* approach. J King Saud Univ Sci. 2014;26(4):267-75.
21. Sylvie DD, Anatole PC, Cabral BP, Veronique PB. Comparison of *in vitro* antioxidant properties of extracts from three plants used for medical purpose in Cameroon: *Acalypha racemosa*, *Garcinia lucida* and *Hymenocardia lyrata*. Asian Pac J Trop Biomed. 2014;4.
22. Lai HY, Lim YY, Tan SP. Antioxidative, tyrosinase inhibiting and antibacterial activities of leaf extracts from medicinal ferns. Biosci Biotechnol Biochem. 2009;73(6):1362-6.
23. Zarib ANM, Zollappi ANH, Abidin MZ, Rahim MBHA, Gani SA, Hamid M, Khayat ME. Total phenolic, total flavonoid and antioxidant activity of extract from rhizome of *Cibotium barometz* prepared by various solvents. Malays J Biochem Mol Biol 2018;21(2):17-22.
24. Al-Wajeeh NS, Hajerezaie M, Noor SM, Halabi MF, Al-Henhena N, Azizan AH, et al. The gastro protective effects of *Cibotium barometz* hair on ethanol-induced gastric ulcer in Sprague-Dawley rats. BMC Vet Res. 2016;13(1):27.
25. Chauke AM, Shai LJ, Mphahlele PM, Mogale MA. Radical scavenging activity of selected medicinal plants from Limpopo province of South Africa. Afr J Tradit Complement Altern Med. 2012;9(3):426-30.
26. Cai Y, Luo Q, Sun M, Corke H. Antioxidant activity and phenolic compounds of 112 traditional Chinese medicinal plants associated with anticancer. Life Sci. 2004;74(17):2157-84.

27. Tawaha K, Alali FQ, Gharaibeh M, Mohammad M, El-Elimat T. Antioxidant activity and total phenolic content of selected Jordanian plant species. Food Chem. 2007;104(4):1372-8.
28. Zheng W, Wang SY. Antioxidant activity and phenolic compounds in selected herbs. J Agric Food Chem. 2001;49(11):5165-70.
29. Anokwuru CP, Esiaba I, Ajibaye O, Adesuyi AO. Polyphenolic content and antioxidant activity of *Hibiscus sabdariffa* calyx. Res J Med Plant. 2011;5(5):557-66.
30. Chavan JJ, Gaikwad NB, Kshirsagar PR, Dixit GB. Total phenolics, flavonoids and antioxidant properties of three Ceropegia species from Western Ghats of India. S Afr J Bot. 2013;88:273-7.
31. Delcour AH. Outer membrane permeability and antibiotic resistance. Biochim Biophys Acta. 2009;1794(5):808-16.
32. Cos P, Vlietinck AJ, Berghe DV, Maes L. Anti-infective potential of natural products: how to develop a stronger *in vitro* 'proof-of-concept'. J Ethnopharmacol. 2006;106:290–302.
33. Ali NA, Jülich WD, Kusnick C, Lindequist U. Screening of Yemeni medicinal plants for antibacterial and cytotoxic activities. J Ethnopharmacol. 2001;74(2):173-9.
34. Abram V, Čeh B, Vidmar M, Hercezi M, Lazić N, Bucik V, et al. A comparison of antioxidant and antimicrobial activity between hop leaves and hop cones. Ind Crops Prod. 2015;64:124-34.
35. Feoktistova M, Geserick P, Leverkus M. Crystal violet assay for determining viability of cultured cells. Cold Spring Harb Protoc. 2016;2016(4):343-6.
36. Liang CC, Park AY, Guan JL. *In vitro* scratch assay: a convenient and inexpensive method for analysis of cell migration *in vitro*. Nat Protoc. 2007;2(2):329.
37. Balekar N, Katkam NG, Nakpheng T, Jehtae K, Srichana T. Evaluation of the wound healing potential of *Wedelia trilobata* (L.) leaves. J Ethnopharmacol. 2012;141(3):817-24.
38. Jonkman JE, Cathcart JA, Xu F, Bartolini ME, Amon JE, Stevens KM, et al. An introduction to the wound healing assay using live-cell microscopy. Cell Adhes Migr. 2014;8(5):440-51.
39. Al-Wajeeh, NS. Evaluation of gastro protective effect and wound healing potential of cibotium barometz and vitex pubecsens in an animal model [Thesis (PhD)]. Kuala Lumpur: University of Malaya; 2017.
40. Wang, Y. L. & Ming. X. Haemostatic preparation containing an extract of golden moss. World Intellectual Property Organization. 2012; PCT/US20 1l/065326.