

Research Article

Pilot Scale Extraction and Standardization of *Begonia medicinalis* Herbs as Raw Material for Anticancer Herbal Medicine

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Begonia medicinalis is an endemic plant in Central Sulawesi that is traditionally used as an herbal medicine to treat cancer. This study is aimed at performing pilot-scale extraction of *B. medicinalis* herbs followed by standardization of the obtained extract, including determination of phytochemical components and anticancer activity. The plant sample was extracted in a 100 L macerator using 70% ethanol and evaporated using an evaporator machine for 20 h to obtain a concentrated extract. The determination of macroscopic and microscopic tests and standardization parameters included organoleptic, moisture content, total ash content, water and ethanol soluble content, heavy metal content, microbial contamination, oxalic acid level, total phenolics, total flavonoids, total saponins, and quercetin as marker compound. The anticancer activity in HeLa cancer cells was evaluated using the microculture tetrazolium (MTT) method. The results of the pilot-scale standardization of *B. medicinalis* extract met the quality standards for extract raw materials. The extract also showed high levels of total phenolics, total flavonoids, and total saponins (1.68 mg GAE/g, 64.49 mg QE/g, and 17.19 mg QBE/g), respectively. The marker compound of quercetin was highly found with the concentration of 554.07 ± 0.52 mg/kg. The extract showed potential anticancer activity with an IC_{50} value of 66.00 ± 8.20 μ g/mL. This study demonstrates the potential of the standardized pilot-scale extract of *B. medicinalis* grown in Central Sulawesi to be developed as an anticancer herbal medicine.

Keywords: anticancer; *Begonia medicinalis*; extraction; pilot scale; standardization

1. Introduction

The genus *Begonia* (Begoniaceae) is one of the most diverse angiosperm groups, comprising more than 2000 species distributed worldwide. Many species are used in traditional medicine and have been shown to produce structurally diverse secondary metabolites with pharmacological relevance, including antioxidant, antimicrobial, immunomodulatory, and anticancer activities [1]. Among them, *Begonia medicinalis* was recently described as a new endemic species from Central Sulawesi, Indonesia, providing unique opportunities for phytochemical exploration and drug-lead discovery.

Ethnomedicinal knowledge in Central Sulawesi highlights the use of *Begonia* species for managing inflammation, infection, and other ailments. Scientific evidence is now accumulating to support these claims. Extracts of *B. medicinalis* have been shown to contain abundant phenolics, flavonoids, and saponins and exhibit anticancer, antioxidant, antiviral, and immunomodulatory activities, strengthening its potential as a candidate for therapeutic development [2, 3]. Previous studies have identified 70% ethanol as a solvent for extracting bioactive compounds with immunomodulatory properties from *B. medicinalis* at the laboratory scale. In addition, 70% ethanol is used as the optimal solvent for

dissolving polar and semipolar metabolites from several medicinal plants [3, 4]. Therefore, our extraction conditions were designed to replicate this efficacy while adapting parameters for pilot-scale processing, ensuring technical feasibility and maintaining bioactivity.

Several phytochemical classes commonly detected in *B. medicinalis* are strongly associated with anticancer activity. Flavonoids and phenolic compounds act as antioxidants and modulators of redox-sensitive signaling pathways that may induce apoptosis, inhibit proliferation, and interfere with cancer-promoting kinases and transcription factors. Quercetin, a flavonol frequently detected in plant extracts, has been reported to induce apoptosis and cell-cycle arrest through modulation of p53, NF- κ B, PI3K/AKT, and MAPK pathways [5, 6]. The presence and determination of the concentration of this compound represent high-value chemical markers in evaluating *B. medicinalis* for anticancer activity.

Cervical cancer remains a major global health burden, and the HeLa cell line (human cervical adenocarcinoma) is widely employed for preliminary cytotoxicity testing of botanical extracts. Prior studies on *B. medicinalis* have demonstrated cytotoxic effects against HeLa and other cancer cell lines, indicating the presence of bioactive constituents with therapeutic potential [7, 8]. However, species-specific differences and variability in extraction procedures highlight the need for standardized and scalable extraction protocols.

Pilot-scale extraction plays a critical role in bridging laboratory discovery and translational applications. It ensures reproducibility of extract yield, scalability of production, and suitability for downstream pharmacological testing. Standardization is also essential to meet quality requirements outlined in WHO and national guidelines for herbal medicines, including botanical identification, physicochemical characterization, chromatographic fingerprinting, and quantification of marker compounds such as total phenolics, flavonoids, saponins, and quercetin [9].

Based on this context, the present study aimed to perform pilot-scale extraction of *B. medicinalis*, standardize the extract and further determine the total phenolic, flavonoid, and saponin levels, as well as quantify quercetin levels and evaluate its anticancer activity against HeLa cells. By linking standardized phytochemical markers with cytotoxic activity, this work expands on our earlier research and provides new gate for herbal product development of *B. medicinalis*.

2. Materials and Methods

2.1. Plant Collection. The aerial herb parts including leaves and stems of *Begonia medicinalis* herb were collected from local farmers in Toddopoli village, Soyojaya district, North Morowali Regency, Indonesia. A voucher specimen was identified at UPT. Sumber Daya Hayati Sulawesi, Tadulako University with the number: 253/UN.28.UPT-SDHS/LK/2024.

2.2. Sample Authentication. Macroscopic and microscopic characteristics were initially determined on herbs simplisia samples to authenticate the raw plant material.

2.2.1. Macroscopic Test. Macroscopic characteristics, including morphology and color of *B. medicinalis* leaf simplicia, were examined visually with or without a magnifying glass [10].

2.2.2. Microscopic Test. Microscopic analysis of *B. medicinalis* leaf simplicia powder was conducted under a light microscope at appropriate magnifications. Observations included diagnostic fragments such as vascular bundles, mesophyll tissue, and epidermal cells [11].

2.3. Pilot-Scale Extraction. *B. medicinalis* extraction on pilot scale was performed under a fixed condition of 70% ethanol, room temperature, and maceration time (72 h) based on laboratory-scale extraction on our previous study [3]. Approximately 10 kg of *B. medicinalis* powder was extracted using a macerator with a 100 L capacity at room temperature. The extraction process employed 70% ethanol with a material-to-solvent ratio of 1:10. The resulting extract was concentrated by evaporating the solvent at 55°C for 20 h using an evaporator machine until a liquid extract was obtained.

2.4. Extract Standardization

2.4.1. Organoleptic Evaluation. Organoleptic properties such as color, odor, and taste were assessed after exposing the extract to air for 15 min [11].

2.4.2. Determination of Water-Soluble Extract. Approximately 10 g of sample was mixed with 50 mL of water, stirred for 1 h, and allowed to stand for 18 h before filtration. A 25 mL portion of the filtrate was evaporated to dryness in a shallow, preweighed dish at 80°C, then further dried at 105°C until a constant weight was reached. The water-soluble extract content was calculated as a percentage of the air-dried material [11].

2.4.3. Determination of Ethanol-Soluble Extract. About 10 g of sample was mixed with 50 mL of 95% ethanol, stirred for 1 h, and allowed to stand for 18 h before filtration. A 25 mL aliquot of the filtrate was evaporated at 80°C and heated further at 105°C until a constant weight was obtained. Ethanol-soluble extract content was expressed as a percentage of the air-dried material [11].

2.4.4. Determination of Moisture Content. Moisture content was determined gravimetrically following the Indonesian National Standard (SNI 01-2891-92). Approximately 1 g of extract was dried in an oven at 105°C for 5 h. Drying and weighing continued hourly until the weight difference between two consecutive measurements was less than 0.25% [12].

2.4.5. Determination of Total Ash Content. One gram of extract (W1) was placed in a preweighed silicate crucible (W0), previously incinerated and stabilized. The sample was incinerated at 600°C $\pm$ 25°C until complete ash formation, cooled in a desiccator, and weighed (W2). Total ash

content was calculated as percentage w/w relative to the initial sample weight with the formula as follows [12]:

$$\text{Total Ash Content} = W2 - W0/W1$$

2.4.6. Determination of Heavy Metal Content. Arsenic (As), lead (Pb), mercury (Hg), and cadmium (Cd) contents were determined using the wet digestion method. One gram of extract was dissolved in 10 mL of HNO_3 and heated until the volume was reduced by half. After cooling, 5 mL of HClO_4 was added and reheated until white fumes ceased. The digest was cooled, diluted with distilled water, filtered, and adjusted to 50 mL in a volumetric flask. Heavy metal concentrations were analyzed using inductively coupled plasma-mass spectrometry (ICP-MS, Agilent 7900, Agilent Technologies, United States) [13].

2.4.7. Microbial Contamination Test. Total plate count (TPC) and yeast-mold counts were performed. One gram of sample was suspended in 9 mL of buffer peptone water (BPW), homogenized (10^{-1} dilution), and serially diluted up to 10^{-3} . Aliquots (1 mL) were inoculated onto Petri dishes, followed by pouring sterile PCA medium (for TPC) or PDA medium (for yeast-mold count) at $\sim 45^\circ\text{C}$. Plates were incubated at $35^\circ\text{C} \pm 1^\circ\text{C}$ for 24–48 h (TPC) and at 20°C – 25°C for 3–5 days (yeast-mold count). Colonies were counted as CFU/g according to SNI 19-2897-92 [12].

2.4.8. Detection of *Escherichia coli* and *Staphylococcus aureus*. A 1 mL aliquot of diluted sample was inoculated onto CompactDry PA, EC, and XSA plates. After incubation at 35°C – 37°C for 24 ± 2 h, colonies were identified: *S. aureus* appeared as blue colonies on CompactDry XSA, whereas *E. coli* appeared as purple-blue colonies on CompactDry EC [14].

2.4.9. Detection of *Salmonella* sp. A 25 g sample was inoculated into 225 mL BPW and incubated at $36^\circ\text{C} \pm 1^\circ\text{C}$ for 22 ± 2 h. Subsequently, 100 μL of culture was inoculated onto CompactDry SL and overlaid with 1 mL sterile distilled water. Plates were incubated at $42^\circ\text{C} \pm 1^\circ\text{C}$ for 22 ± 2 h. Positive *Salmonella* colonies appeared as yellow or green with black centers. Confirmation was performed by subculturing onto EMB agar, incubated for 18–24 h at 37°C , where *Salmonella* colonies appeared vigorous and colorless [14].

2.4.10. Total Phenolic Content. Total phenolic content was determined using the Folin-Ciocalteu method with gallic acid as standard. Briefly, 0.5 mL of extract solution in ethanol was sonicated (50% intensity, 10 min), mixed with 0.5 mL Folin-Ciocalteu reagent and 5 mL distilled water, incubated for 10 min, then reacted with 1.5 mL of 20% sodium carbonate. The solution was adjusted to 10 mL, diluted as necessary, and absorbance measured at 760 nm using spectrophotometer UV-Vis (UV-1800, Shimadzu, Tokyo, Japan) [11].

2.4.11. Total Flavonoid Content. Flavonoid content was quantified by the aluminum chloride colorimetric method using quercetin as standard. A 0.5 mL sample was hydro-

lyzed with 2 mL of 4N HCl in an autoclave at 110°C for 2 h, followed by ether extraction. The dried ether fraction was treated sequentially with 0.3 mL 5% sodium nitrite, 0.6 mL 10% aluminum chloride, and 2 mL 1 M sodium hydroxide, then diluted to 5 mL with distilled water. Absorbance was measured at 510 nm using a spectrophotometer UV-Vis (UV-1800, Shimadzu, Tokyo, Japan) [11].

2.4.12. Total Saponin Content. Total saponins were determined using Quillaja bark saponin as reference. About 100 mg of extract was hydrolyzed with 2 mL of 25% H_2SO_4 in an autoclave at 110°C for 120 min, followed by ether extraction. The ether fraction was dried, mixed with 1 mL water, and reacted with 50 μL anisaldehyde. After 10 min, 2 mL of 50% H_2SO_4 was added and heated at 60°C for 10 min. The mixture was diluted to 10 mL, further diluted tenfold, and absorbance measured at 435 nm using spectrophotometer UV-Vis (UV-1800, Shimadzu, Tokyo, Japan) [15].

2.4.13. Determination of Quercetin and Oxalic Acid. Determination of quercetin and oxalic acid was analyzed by high-performance liquid chromatography (HPLC, Agilent 1260 Infinity II, Agilent Technologies, USA) with variable wavelength detector (VWD). For quercetin, about 200 mg of extract was dissolved in 70% ethanol, vortexed, and sonicated for 60 min. After centrifugation, the supernatant was filtered (0.45 μm) and injected (20 μL) into an HPLC system operating under isocratic conditions (mobile phase: 0.5% phosphoric acid:methanol=45:55; C18 column; flow rate: 1 mL/min; detection: 370 nm). The analysis was performed for 20 min. Quercetin standard solutions were prepared at concentrations of 0.2, 0.5, 1, 2, 4, 6, 8, and 10 mg/L for calibration. Method validation was performed assessing linearity, precision, accuracy, limit of detection and limit of quantification. [16]. For oxalic acid, about 1 mL of liquid extract was dissolved by ethanol until 25 mL. Then injected (20 μL) into an HPLC system operating under isocratic conditions (mobile phase: 0.005 M sulphuric acid; Agilent Hi-Plex H 300 $\times$ 7,7 column; flow rate: 0.5 mL/min; detection: 210 nm). Oxalic acid standard solutions were prepared at concentrations of 2, 5, 10, 20, 50, 100 and 200 mg/L for calibration [17].

2.4.14. Cytotoxicity Assay. Cytotoxic activity was evaluated using the MTT assay against HeLa cervical cancer cells obtained from the Laboratory of Parasitology, Faculty of Medicine, Universitas Gadjah Mada, Indonesia. Cells (1×10^4 cells/well) were seeded into 96-well plates with RPMI-1640 medium supplemented with 10% fetal bovine serum and incubated at 37°C with 5% CO_2 for 24 h. Extracts were added at concentrations ranging from 31.25 to 1000 $\mu\text{g}/\text{mL}$ and incubated for an additional 24 h. Subsequently, 10 μL of MTT solution (5 mg/mL) was added per well and incubated for 4 h. The reaction was terminated by adding 100 μL of 10% sodium dodecyl sulfate in 0.01 M HCl. Plates were shaken for 10 min and incubated overnight in the dark. Absorbance was measured at 550 nm using an ELISA reader (Biorad, United States). Experiments were conducted in

triplicate, and cytotoxicity was expressed as percentage cell viability relative to untreated controls [18].

$$\% \text{Cell viability} = (\text{Absorbance of treated cells} / \text{Absorbance of control cells}) \times 100\%$$

3. Results and Discussions

3.1. Macroscopic and Microscopic Characterization. Macroscopic analysis confirmed the morphological identity of *B. medicinalis* leaves, characterized by 7–9 cm length, 6 cm width, brownish-green coloration, serrated edges, and distinctive aroma (Figure 1). Microscopic observations revealed mesophyll and vascular bundle structures (Figure 2). This is critical for ensuring traceability, preventing adulteration, and maintaining reproducibility across batches, all of which are essential steps in the standardization of herbal medicines [11]. Beyond mesophyll and vascular bundles, microscopic examination revealed characteristic fragments such as glandular trichomes and crystal idioblasts unique to *Begonia* species. These features align with observations from fresh sectional specimens reported by [19], facilitating accurate microscopic identification.

3.2. Extraction Yield and Organoleptic Characteristics. The pilot-scale extraction of *B. medicinalis* produced 4.431 kg of thick extract from 10 kg of dried leaf powder, resulting in an extraction yield of 44.31% (Table 1). This yield is considered relatively high compared with similar herbal extractions, where yields commonly range between 10%–30% depending on solvent polarity and plant matrix composition [20]. The high pilot-scale extraction yield corroborates earlier laboratory findings, underscoring the efficacy of 70% ethanol in dissolving active metabolites due to its polarity, which favors the extraction of both polar and semipolar compounds [3, 4]. This confirms the scalability of laboratory conditions to pilot scale.

Organoleptically, the extract appeared as a thick dark brown mass, with a bitter taste and nearly odorless characteristics, consistent with polyphenolic-rich extracts reported in other medicinal plants [11].

3.3. Standardization Parameters. Standardization results, that can be seen in Table 2, revealed a moisture content of $24.22\% \pm 0.00\%$. The moisture content in the range of 5%–30% is in accordance with the moisture content requirements for viscous extracts according to the Indonesian Herbal Pharmacopoeia [11]. This value indicates a low possibility of microorganism growth during storage if packaged and stored properly. Moisture content is also directly related to the stability and shelf life of herbal products [21].

The total ash content was obtained of $1.21\% \pm 0.00\%$ (w/w). Ash content provides information on the amount of inorganic residue remaining after combustion, whereas acid insoluble ash indicates the presence of insoluble materials such as sand and silicates [11]. The values obtained indicate that the extract is relatively clean of inorganic contaminants.

The value of water-soluble content was $18.95\% \pm 0.49\%$, whereas the ethanol soluble content was $9.27\% \pm 0.26\%$. These results indicate that the active compounds in *B. med-*



FIGURE 1: Macroscopic observations of *B. medicinalis* leaf simplisia. Length 7–9 cm, width 6 cm, brownish green color, sharp and serrated tip shape and distinctive aroma.

icalis extracts are more soluble in polar solvents such as water than ethanol. This suggests the predominance of polar to semipolar compounds such as flavonoids, saponins, and steroid glycosides [22], which aligns with earlier phytochemical reports on *Begonia* species [18, 23]. “In addition to flavonoids, saponins, and steroid glycosides, literature reports the presence of alkaloids, tannins, and phenolic acids in 70% ethanol extracts of *B. medicinalis* [24], which may contribute to the biological activity observed.”

The percentage of soluble juice content is an indicator of the effectiveness of the solvent in extracting bioactive compounds and is used as a parameter in evaluating the quality of the extract [25].

Heavy metal analysis confirmed the absence of toxic metals such as As, Hg, Pb, and Cd, ensuring extract safety for further pharmacological application. The microbial contamination test including total plate count (ALT) and yeast mold test showed that the sample had colony counts of 3.7×10^5 colonies/g and 3.2×10^4 colonies/g, respectively. This result is far below the maximum allowable limit for herbal extracts, which are $\text{ALT} \leq 5 \times 10^7$ colonies/g and yeast mold test $\leq 5 \times 10^5$ [26]. These results indicate that the production and storage processes adhered to hygienic standards and met microbiological safety standards.

The extract contained significant levels of phenolic compounds (64.49 mg GAE/g), then followed by saponins (17.19 mg/g) and flavonoids (1.68 mg QE/g). Quercetin, identified as a key bioactive marker, was detected in the extract with a retention time of 4.69 min, which was almost identical to that of the quercetin reference standard (R_t 4.687 min) (Figure 3). Quantitative analysis using the calibration curve of the standard indicated that the extract contained 554.07 ± 0.52 mg/kg of quercetin (Table 3). This amount exceeded the levels previously reported in another

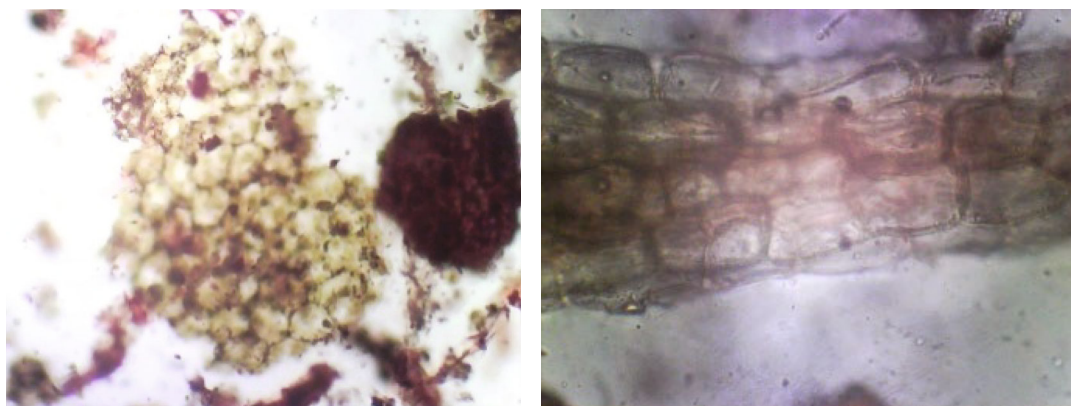


FIGURE 2: Microscopic observations on the herb of *B. medicinalis* that showed the mesophyll and vascular bundle.

TABLE 1: *B. medicinalis* pilot-scale extract.

Parameters	Results
Name of extract	<i>B. medicinalis</i>
Powder weight (kg)	10
Extract weight (kg)	4.431
Yield (%)	44.31
Odor	Almost odorless
Color	Dark brown
Taste	Bitter
Form	Thick

Begonia species, *B. nelumbiifolia* [27], thereby establishing quercetin as one of the predominant flavonoids in *B. medicinalis*. Quercetin is known to exert cytotoxicity against cervical carcinoma cells by downregulating the PI3K/AKT and MAPK signaling pathways, thereby promoting apoptosis and inhibiting metastasis [28].

Previous ethnobotanical surveys and phytochemical studies have reported that many *Begonia* species contain high levels of oxalic acid, particularly in their leaves and stems [27]. Excessive oxalate intake is associated with reduced mineral bioavailability and an increased risk of kidney stone formation. As shown in Table 2, oxalate levels in *Begonia* species (1.95% b/v) are considerably higher than those found in spinach, moringa, or cassava leaves [29]. According to the European Food Safety Authority [30], safe daily oxalate intake should not exceed 250 mg/day. Based on the quantified oxalate content of 1.95% in the standardized *B. medicinalis* extract, the administration of 100 mg extract in SNEDDS formulation [31], will deliver approximately 1.95 mg oxalic acid per dose. This represents only 0.78% of the EFSA-recommended safe daily intake (250 mg/day), confirming that oxalate exposure from the formulation remains within a negligible and safe margin.

3.4. Cytotoxic Activity Against HeLa Cells. The anticancer activity was selected based on documented pharmacological potential of *B. medicinalis* extracts as anticancer, antioxidant, and immunomodulatory agents, aligning with thera-

TABLE 2: Quality standardization parameters of *B. medicinalis* pilot-scale extract.

Parameters	Values
<i>Physicochemical properties</i>	
Moisture content (%)	24.22 ± 0.014
Total ash content (%)	1.21 ± 0.007
Water soluble content (%)	18.95 ± 0.494
Ethanol soluble content (%)	9.27 ± 0.262
<i>Heavy metals</i>	
Arsenic (As)	Not detected
Mercury (Hg)	Not detected
Lead (Pb)	Not detected
Cadmium (Cd)	Not detected
<i>Microbial contamination</i>	
<i>Staphylococcus aureus</i>	Negative
<i>Escherichia coli</i>	Negative
<i>Salmonella</i> sp.	Negative
Total plate count (colony/g)	3.7 × 10 ⁵
Yeast mold test (colony/g)	3.2 × 10 ⁴
<i>Phytochemical profile</i>	
Total phenolic (mg GAE/g)	64.49 ± 0.298
Total flavonoid (mg QE/g)	1.68 ± 0.052
Total saponin from Quillaja bark (mg/g)	17.19 ± 0.140
Oxalic acid (%)	1.95 ± 0.007

peutic relevance and enabling biological validation of extract standardization. The cytotoxic potential of the *B. medicinalis* extract was evaluated against HeLa cervical cancer cells, showing a clear dose-response relationship (Figure 4a). Compared with doxorubicin as a positive control (Figure 4b), the extract demonstrated potential cytotoxicity with an IC₅₀ value of 66.00 ± 8.20 µg/mL within the pharmacologically active range. Previous reports on *Begonia* extracts highlight their ability to induce apoptosis through mitochondrial dysfunction and caspase activation [18]. The presence of quercetin in the extract may have contributed

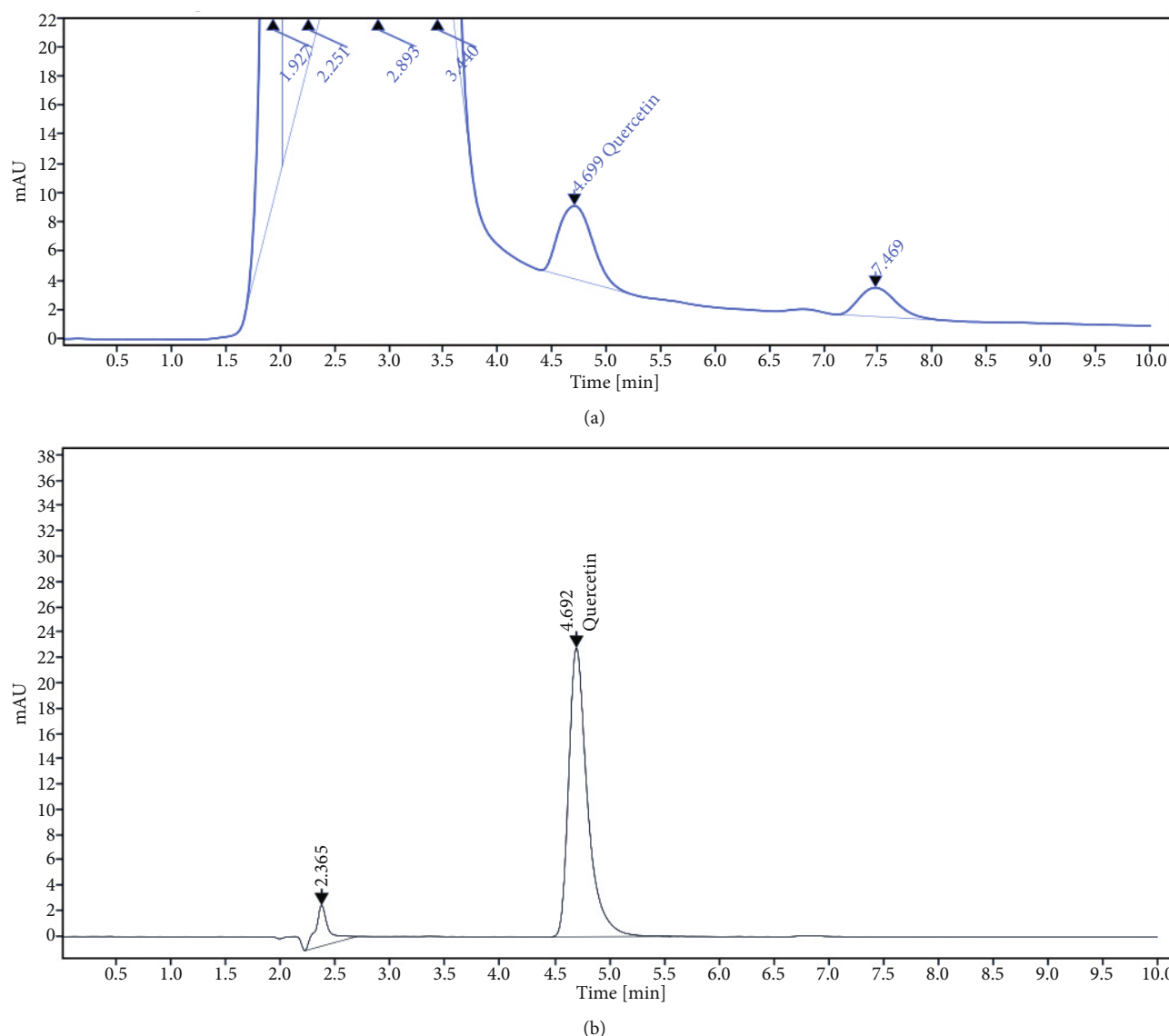


FIGURE 3: HPLC chromatogram of quercetin in *B. medicinalis* herbs pilot-scale extract (a) and quercetin standard (b).

TABLE 3: Analytical parameter for quercetin quantification using HPLC.

Standard compound	tR (min)	Concentration (mg/kg)	Calibration curve	R2	LOD (mg/L)	LOQ (mg/L)
Quercetin	11.557	554.07 ± 0.52	$y = 45.4747x - 0.0379$	0.999	0.736	2.23

Abbreviations: LOD, limit of detection; LOQ, limit of quantification; R, correlation coefficients; tR, retention times.

significantly, as quercetin is well-documented to downregulate proliferative signaling pathways such as PI3K/AKT and MAPK in cervical carcinoma cells [32].

Pearson correlation matrix demonstrated variable associations between phytochemical constituents and the inhibitory concentration (IC_{50}) of the standardized extract (Figure 5). A strong negative correlation was observed between quercetin and IC_{50} ($r \approx -1.00$), indicating that higher quercetin levels corresponded with lower IC_{50} values. This result suggests that quercetin plays a predominant role in enhancing the biological activity of the extract. These

findings are consistent with previous reports that quercetin exhibits potent anticancer and antioxidant mechanisms through apoptosis induction and ROS modulation [33, 34]. Saponins also showed a consistently strong negative association with IC_{50} ($r \approx -1.00$), implying their significant contribution to bioactivity. Saponins have been well documented for their cytotoxic and antitumor potential, including membrane-mediated apoptosis and immune stimulation [35]. Their combined interaction with quercetin may contribute synergistically to the inhibitory potency of the extract.

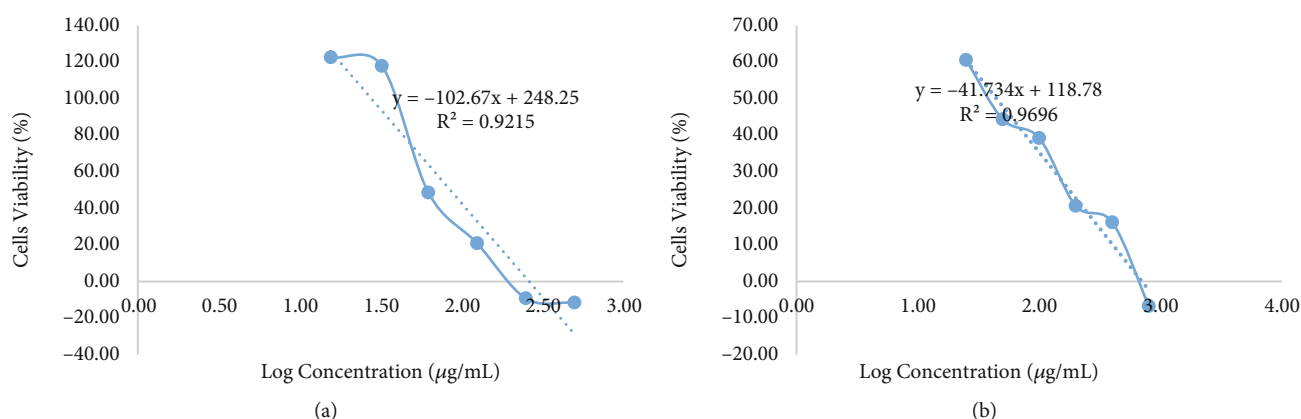


FIGURE 4: Dose-response of *B. medicinalis* pilot-scale extract (a) and doxorubicin (b) on HeLa cells.

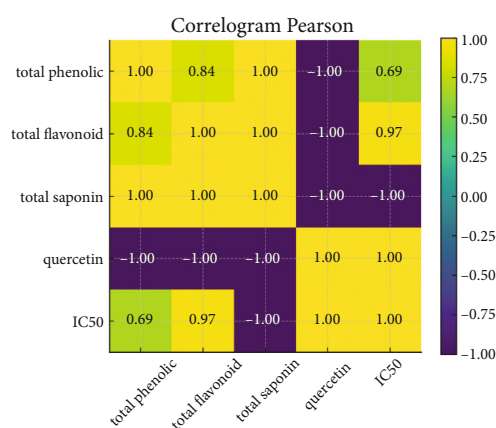


FIGURE 5: Correlation plot (correlogram) that shows Pearson's correlation coefficients between phytochemical constituents and anticancer activities of the extracts. Blue color represents negative correlation (values from 0 to -1), which indicates an inverse proportional relationship between the compared variables, whereas the red color represents positive correlation (values from 0 to +1), which indicates a direct proportional relationship between the compared variables.

On the contrary, total flavonoid content exhibited a strong positive correlation with IC_{50} ($r \approx 0.97$), suggesting that higher flavonoid content did not enhance and may even reduce extract potency. This result supports the notion that not all flavonoid classes possess cytotoxic benefits, and some may antagonize the bioactivity of principal compounds [36]. Additionally, total phenolic content showed only a moderate positive correlation with IC_{50} ($r \approx 0.69$), indicating that phenolics were not the primary determinant of biological activity within this extract.

Collectively, the correlation analysis confirms that the inhibitory potency of the extract is predominantly driven by quercetin and saponin constituents rather than the total flavonoid or phenolic fractions. These observations highlight the importance of targeted standardization and fractionation strategies focused on quercetin- and saponin-rich fractions for subsequent formulation development.

Overall, this study provides the first pilot-scale evidence of *B. medicinalis* as a feasible and pharmacologically active

extract with anticancer potential. In-depth mechanistic studies investigating apoptotic markers (e.g., Bax, Bcl-2, and caspase-3) and signaling pathways (e.g., PI3K/AKT, MAPK, and NF- κ B) will provide insights into the precise molecular mechanisms underlying its activity. Additionally, pharmacokinetic and toxicity profiling are necessary to evaluate bioavailability and safety in animal models.

4. Conclusion

The pilot-scale extraction of *Begonia medicinalis* yielded a high extract percentage (44.31%) enriched with phenolics, flavonoids, saponin, and particularly quercetin. Standardization confirmed compliance with pharmacopeial limits for ash and moisture content, water and ethanol soluble content, heavy metals, oxalic acid level, and microbial contamination. Importantly, the extract demonstrated cytotoxic activity against HeLa cells, highlighting its potential anticancer properties. Collectively, these findings provide foundational evidence to support further formulation development and preclinical evaluation of *B. medicinalis* as a promising phytopharmaceutical product candidate.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Disclosure

The authors took full responsibility for the content of the publication.

Conflicts of Interest

All authors declare no conflicts of interest.

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