Research Article

In Vitro Anticancer Activity of Imperata cylindrica Root’s Extract toward Human Cervical Cancer and Identification of Potential Bioactive Compounds

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Imperata cylindrica is traditionally used to cure several diseases including cancer, wounds, and hypertension. The present study was designed to investigate the anticancer activity of the methanolic root extract of I. cylindrica (IC-MeOH). The water-soluble tetrazolium-1 and colony formation assays were used to check the proliferation ability of the cells. Cell apoptosis and cell cycle were measured by flow cytometry-based fluorescence-activated cell sorting. The ultrahigh-performance liquid chromatography-high-resolution mass spectrometry (UHPLC-HRMS) analysis was used for the metabolites profiling of IC-MeOH. Based on high-mass accuracy, spectral data, and previous reports, tentative compound identifications were assigned. Our findings revealed that IC-MeOH inhibited the proliferation of HeLa and CaSki cells. The plant extract was also found to induce a concentration- and time-dependent apoptosis and cell cycle arrest in the G0/G1 phase (IC50 value) in CaSki cell line. Analysis of IC-MeOH permitted the identification of 10 compounds already reported for their anticancer activity, epicatechin, curcumin, (-)-yatein, caffeic acid, myricetin, jatrorrhizine, harmaline, cinnamaldehyde, dobutamine, and syringin. In conclusion, IC-MeOH is a rich source of cytotoxic metabolites that inhibits human cervical cancer proliferation via apoptosis and cell cycle arrest.

1. Introduction

Cervical cancer is the fourth most common cancer in women. In 2018, an estimate of 570000 women were diagnosed with cervical cancer worldwide and about 311000 died of the disease. Almost all cervical cancer cases (99%) are caused by high-risk human papillomaviruses (HPV), an extremely common virus transmitted through sexual contact. Although most HPV infections are cured spontaneously with no symptoms, persistent infection can cause cervical cancer in women. Cervical cancer is one of the most successfully curable forms of cancer when diagnosed [1]. The chemodrugs used to treat cervical cancer include cisplatin, carboplatin, oxaliplatin, paclitaxel, and topotecan. Nonetheless, cervical cancer cells may develop resistance to cisplatin, main chemotherapy drug used for patients suffering from the cervical cancer. This substantially compromises the efficacy of cisplatin in the treatment of advanced or recurrent cervical cancer [2]. Given the fact that cisplatin can damage the kidney (nephrotoxicity) and display other common side effects such as anaphylaxis, leukopenia, neutropenia, thrombocytopenia, anaemia, hepatotoxicity, and cardiotoxicity [3], natural bioactive compounds may offer a better solution. Drug development using natural products has been extensively explored by researchers [4], and the use of plant-derived molecules is frequent in cancer research. The plant kingdom is made up of around 250000 species [5], which are regularly exposed to stressful conditions due to biotic...
and abiotic factors in their living environment [6]. To sur-

vive under such difficult and stressful conditions, plants

undergo some important modifications leading to the syn-

thetic stimulation of secondary metabolites, which are

known for their various pharmacological activities [7]. The

in vitro investigations of Kue et al. [8, 9], and Nayim

et al. [10] have shown the cytotoxic effects of the methanolic

root extract of I. cylindrica against a panel of cancer cell lines

including leukemia cells (CCRF-CEM and HL-60), breast

cancer cells (MDA-MB-231-bcrp Clone 23), human wild-
type HCT116 (P53+/+) colon cancer cells, and pancreatic
cancer cells Mia PaCa-2. The in vitro study conducted by
Keshava et al. [11] had revealed the weak cytotoxic effect of
the methanol leaf extract of I. cylindrica against the
human oral squamous carcinoma cell line SCC-9, and from

investigations of Kwok et al. [12], the ethyl acetate extract of
I. cylindrica leaf showed an antiproliferative effect against
colorectal cancer cells HT-29. Plants’ biological activities rely
on their phytochemical composition, and chromatography
coupled to mass spectrometry is the most widely applied
technology used for the analysis of samples in very complex
matrices such as plant extracts [13]. To the best of our
knowledge, no research work has highlighted the anticancer
mode of action of the methanolic extract of Imperata cylin-

drica root against cervical cancer. Thus, this study aimed at
evaluating the metabolic profile of IC-MeOH using an
ultrahigh-performance liquid chromatography-high-
resolution mass spectrometry, and its anticancer mode of
action toward human cervical cancer cells.

2. Materials and Methods

2.1. Collection of Plant Material. Roots of I. cylindrica (Gram-

ineae) were collected in June 2019, from the “Menoua”
Division in the West Region. The collected plant was iden-
tified by Mr. Nana Victor of the National Herbarium of
Cameroon (Yaounde) under the voucher number
30139/SRF-Cam.

2.2. Extraction of Plant Material. The dried roots of I. cylin-

drica (100 g) were ground and macerated in methanol
(500 mL) at room temperature for two (02) days. During
extraction, the sample was shaken repeatedly. The obtained
solution was filtered using Whatman No. 1 paper. Subse-

quently, the solvent was recovered in a rotary evaporator
(BUCHI R- Rotavapor model R-2000) and the resulting
product was dried and lyophilized (Labconco Freeze
Dryer—105°C, ThermoFisher, USA). The extraction yield
was 7%.

2.3. Ultrahigh-Performance Liquid Chromatography-High-

Resolution Mass Spectrometry. The ultrahigh-performance
liquid chromatography-high-resolution mass spectrometry
(UHPLC-HRMS) analysis was used to assess the chemical
profile of IC-MeOH [14]. Seventy-five (75) mg of IC-

MeOH was weighed, and 1 mL of 100% methanol was added
and vortexed well. The sample was sonicated and centri-
fuged at 14800 rpm at 4°C for 10 min. The obtained superna-
tants were spiked with reserpine (positive ion mode) and

Taurocholate-D8 (negative ion mode) for reverse phase
and hydrophilic interaction liquid chromatography (HILIC)
analyses. Afterward, the samples were vortexed and centri-
fuged at 14500 rpm at 4°C for 10 min. Ten (10) μL of the
supernatant was injected into the UHPLC-HRMS system.
The mass spectrometer employed for UHPLC-HRMS analysis
was a Q-Exactive Orbitrap (ThermoFisher Scientific, San
Jose, CA, USA) equipped with a heated electrospray ionisa-
tion (HESI) source. It also houses a HCD (higher-energy
collision dissociation) cell for carrying out MSn experi-
ments. The Q-Exactive Orbitrap was coupled to a Dionex Ulti-
Mate 3000 UHPLC system (ThermoFisher Scientific, San Jose,
CA, USA). This system was provided with a column oven
(set at 40°C), an autosampler, and a thermocontroller (set
at 4°C). Separation of the IC-MeOH was done using a C18
column (150 mm × 4.6.1 mm, 5 μm) (Phenomenex Luna,
India Pvt. Ltd.) and HILIC column (150 mm × 4.6 mm,
5 μm) (Phenomenex Luna, India Pvt. Ltd.) at 40°C. Experi-
ments were performed with sample analysis in reverse-
phase chromatography (positive and negative) and HILIC
(positive and negative) modes. The MS operating conditions
for all three experimental replicate analyses were as follows:
- spray voltage, +2500 V (-2500 V for negative mode); capil-

lary temperature, 280°C; vaporizer temperature, 320°C;
- sheath gas, 30 arbitrary units (40 for negative mode); and
- auxiliary gas, 10 arbitrary units. Injector settings were as fol-

lows: 0-2 mins: waste, 2-45 mins: load, and 45-55 mins:
- waste. The UHPLC-HRMS instrumentation method was
used for the HILIC phase, and the UHPLC-HRMS instru-
mentation method for the reverse phase is compiled in
Tables S1 and S2. After mass analysis, the tentative
identification of compounds was achieved based on the
matching of accurate m/z with local library of authentic
standards from PlantCyc (10 ppm) database, METLIN
Mass Spectral Database (accurate mass and/or MS/MS
library), and the literature data.

2.4. Human Cervical Cell Line Culture. The human cervical
cancer cell lines HeLa and CaSki purchased from American
Type Culture Collection (ATCC, Rockville, MD, USA) were
maintained in Dulbecco’s Modified Eagle Medium (Gibco,
ThermoFisher, USA) supplemented with 10% Foetal Bovine
Serum (Gibco, ThermoFisher, USA) and 1% penicillin and
streptomycin (10 mL/L) (Gibco, ThermoFisher, USA).
The nontumor human cervical cell line HCK1T (donated by
the National Cancer Center Research Institute (NCCRI),
Japan) was maintained in 3:1 (v/v) Nutrient Mixture-
Dulbecco’s Modified Eagle Medium supplemented with
5% FBS, 0.4 μg/mL hydrocortisone, 5 μg/mL insulin,
8.4 ng/mL toxin cholera, 10 ng/mL epidermal growth factor,
24 ng/mL adenine, and 5 μmol/mL Y-27632. All cells were
incubated in a humidified atmosphere of 5% CO₂ at 37°C.
All experiments were performed with cells in the logarith-
mic growth phase.

2.5. Cell Viability Assay. The cell viability assay of IC-MeOH
against HeLa, CaSki, and HCK1T cell lines was performed
using the cell proliferation reagent WST-1 (Sigma-Aldrich,
Roche Diagnostics, Germany) [15]. The stable tetrazolium
salt WST-1 was cleaved to a soluble formazan by a complex cellular mechanism that occurs primarily at the cell surface. This bioreduction is a largely dependent dye formed directly on the glycolytic production of NAD(P)H in viable cells. Therefore, the amount of formazan dye formed directly correlates to the number of metabolically active cells in the culture. Briefly, cells were detached by treatment with 0.5% trypsin/EDTA (Gibco, Canada) and seeded at a density of 5.10^3 cells/mL and 6.10^3 cells/well of a 96-well cell culture plate (Greiner, Bio-One, CELLSTAR, Germany), respectively, for HeLa and CaSki in a total volume of 100 μL; cells were allowed to attach overnight. Afterward, they were immediately treated with different concentrations of crude extract ranging from 3 to 384μg/mL dissolved in 0.1% dimethyl-sulfoxide (Sigma-Aldrich) and incubated for 24, 48, and 72 h; cisplatin (Celon Laboratories, India) was used as a positive control and tested from 0.1 to 100 μg/mL. After the treatment periods, 10 μL of WST-1 reagent was added to each well and incubated for 1 h. Absorbance was measured at 450 nm wavelength (Spectra-Max M5 Multimode reader). Each assay was performed at least three times, and the cell viability was evaluated with respect to untreated cells. IC50 values (concentration of the tested compounds required to reduce cell density to 50%) were calculated by concentration-response curve fitting using GraphPad Prism version 8.1.0.

2.6. Clonogenic Assay. Colony formation assay was performed to assess the effect of IC-MeOH on CaSki cell line’s clonogenic ability. The following protocol has been modified from a published version [16]. Briefly, cells were harvested by trypsinization from 70 to 80% confluent monolayer cell culture, washed with PBS, and resuspended in DMEM containing 10% FBS. Afterward, cells were seeded in 6-well plates (Greiner, Bio-One, CELLSTAR, Germany) at a density of 1000 cells/well and incubated at 37°C in a humidified incubator. After 24 h, the medium was replaced with fresh medium and cells were treated with different concentrations of the plant extract (10, 15, 20, 25, and 30 μg/mL) and 0.1% DMSO vehicle for 24 h. The medium was then replaced with fresh DMEM containing 10% FBS. The cells were allowed to grow for an additional 11 days. After this period, the media were removed and cells were washed with PBS, fixed with acetic acid-methanol (1:1 v/v), and incubated for 5 to 10 min at room temperature (RT). Afterward, colonies of cells were stained with 0.5% crystal violet (Sigma-Aldrich) and incubated for 2 h at room temperature. Crystal violet was discarded, cells were washed in tap water and dried overnight, and plates were imaged. The colonies containing at least 50 cells were counted under a Nikon inverted microscope Eclipse TE2000-S. The data were collected from three independent experiments performed in triplicate.

2.7. Apoptosis Analysis. A quantitative assessment of apoptosis was performed using phycoerythrin (PE) Annexin V Apoptosis Detection Kit I (BD Biosciences, Pharmagen, USA) [17]. CaSki cells were seeded in 6-well plates at a density of 4 × 10^5 cells/well for 24 h, then treated with 0.1% DMSO or 0.3% saline, either with IC-MeOH (1/2 IC_{50}, IC_{50}, and 2 IC_{50}) or with cisplatin used as a positive control (1/2 IC_{50}, IC_{50}, and 2 IC_{50}) for 24 and 48 h. After the different treatment days, cells were taken out, washed twice with PBS, trypsinized, centrifuged for pellet collection, and resuspended in cold PBS and later in a 1× binding buffer (1 × 10^6 cells/mL). Afterward, 100 μL of cell resuspension solution was transferred in 1.5 mL Eppendorf; 5 μL of phycoerythrin-conjugated annexin V (annexin V-PE) and 5 μL of 7-Amino Actinomycin D (7-AAD) were added and followed by 15 min incubation in the dark at room temperature. The stained cells were then diluted with 1× binding buffer and immediately analyzed using a flow cytometer (Becton Dickinson FACSVerse). Data from 10,000 events were collected per data file. In four zones of the drawn quadrant, we had viable cells (Q1), cells bound to annexin V-PE only (early apoptotic cells, Q2), and cells bound both to annexin-PE and 7-AAD (late apoptotic cells, Q3).

2.8. Cell Cycle Analysis. The effect of the methanolic root extract of *I. cylindrica* on CaSki cell cycle was determined by flow cytometry-based fluorescence-activated cell sorting (FACS) analysis of propidium iodide- (PI-) stained cells [18]. Cells were seeded in 6-well plates at a density of 1.5 × 10^5 cells/well and incubated overnight. After serum starvation for 24 h, they were treated with either 0.1% DMSO (negative controls) or IC-MeOH (1/2 IC_{50}, IC_{50}, and 2 IC_{50}) for 24 and 48 h. After the different treatment days, cells were trypsinized, washed with cold phosphate-buffered saline (PBS), fixed in ice-cold 70% ethanol overnight, and redissolved in 1 mL of PBS solution supplemented with RNase and stained with PI. After 15 min of incubation at 37°C, analysis was done using flow cytometry (Becton Dickinson FACSVerse). FlowJo software was used to process the data.

2.9. Statistical Analysis. Each experiment was performed three times, on independent cell passages. Statistical analysis was performed using GraphPad Prism version 8.1.0. The data are plotted as the mean ± SD. Differences between the means of treated and untreated samples were evaluated using one-way analysis of variance (one-way ANOVA) followed by *post hoc* Dunnett’s multiple comparison test. *p* values < 0.05 were considered to be statistically significant, and significance was marked as *p* values < 0.05, **p** values < 0.01, ***p*** values < 0.001, and ****p*** values < 0.0001.

3. Results

3.1. The Methanol Extract of Imperata cylindrica Root Cytotoxicity toward Cervical Cancer Cells. The antiproliferative activity of IC-MeOH on the human cervical cancer cell lines HeLa and CaSki was evaluated using the water-soluble tetrazolium-1 reagent (WST-1). Multiple concentrations of IC-MeOH and cisplatin were used, and IC_{50} values were determined from the dose-response curve. The cytotoxicity results of both IC-MeOH and the positive control against the abovementioned cell lines are shown in Figure 1 and Table 1. IC-MeOH showed a concentration- and time-dependent growth inhibition, with IC_{50} values
The dose-response curves of IC-MeOH and cisplatin. IC-MeOH: *Imperata cylindrica* root’s methanol extract.

### Table 1: IC$_{50}$ concentrations (µg/mL) of *cylindrica* root methanol extract and cisplatin.

| Cell line | Treatment time | IC$_{50}$ (µg/mL) |
|-----------|----------------|-------------------|
|           | 24 h           | 48 h              | 72 h              |
| HeLa      | IC-MeOH        | 84.17 ± 4.00      | 75.05 ± 3.42      | 68.00 ± 2.39      |
|           | Cisplatin      | 17.63 ± 1.20      | 1.6375 ± 0.09     | 0.9347 ± 0.08     |
| CaSki     | IC-MeOH        | 65.14 ± 3.35      | 55.52 ± 0.82      | 50.71 ± 1.53      |
|           | Cisplatin      | 26.29 ± 1.70      | 12.07 ± 1.00      | 6.40 ± 0.37       |
| HCK1T     | IC-MeOH        | 55.54 ± 2.10      | 45.19 ± 1.70      | 40.91 ± 2.00      |
|           | Cisplatin      | 28.23 ± 1.41      | 18.15 ± 1.01      | 9.83 ± 0.70       |

IC$_{50}$: inhibitory concentration 50; IC-MeOH: *Imperata cylindrica* root’s methanol extract.

($\mu$g/mL) of 84.17 ± 4.00, 75.05 ± 3.42, and 68.00 ± 2.39 for HeLa and 65.14 ± 3.35, 55.52 ± 0.81, and 50.51 ± 1.53 for CaSki, respectively, after 24, 48, and 72 h of treatment periods. Cisplatin also impaired HeLa and CaSki cell growth in a concentration- and time-dependent manner. However, IC-MeOH showed the best IC$_{50}$ values with CaSki compared to HeLa. On the nontumor cervical cell line HCK1T, both IC-MeOH and cisplatin displayed a concentration- and time-dependent cytotoxicity, as their IC$_{50}$ values on this cell line were in a decreasing order with increasing incubation periods.

#### 3.2. Effect of *I. cylindrica* Root Extract on CaSki Cell Line’s Clonogenic Ability

Clonogenic assay is the method of choice to determine cell reproductive death after treatment with ionising radiation but can also be used to determine the effectiveness of other cytotoxic agents. This method was used in our study to test whether IC-MeOH can reduce the clonogenic survival of CaSki cells after 24 h of treatment with different concentrations. From the obtained results displayed in Figure 2, it was notable that IC-MeOH significantly inhibited the ability of CaSki cells to form colonies at concentrations of 10, 15, 20, 25, and 30 $\mu$g/mL compared to the control (untreated cells). Moreover, the anticlonogenic effect of IC-MeOH was concentration-dependent as shown by the diagram in Figure 2.

#### 3.3. *I. cylindrica* Root Extract Induces Apoptosis in CaSki Cells

7-AAD-annexin-V double staining has been used to differentiate healthy CaSki cells from early and late apoptotic CaSki cells (Figure 3(a)). Fluorescence-activated cell sorting analysis of the untreated (control) and treated cells revealed that IC-MeOH (IC$_{50}$ and 2 IC$_{50}$) as well as cisplatin (1/2 IC$_{50}$, IC$_{50}$, and 2 IC$_{50}$) significantly induced apoptosis in CaSki cells after 24 h and 48 h of treatment. The percentages of apoptotic cells induced by IC-MeOH and cisplatin are shown in Figure 3(b).

#### 3.4. *I. cylindrica* Root Extract Arrests CaSki’s Cell Cycle at the $G_0/G_1$ Phase

CaSki cell cycle distribution was studied in the...
absence and in the presence of IC-MeOH at different concentrations equivalent to 1/2 IC_{50}, IC_{50}, and 2 IC_{50}. Compared to untreated cells, at all treatment periods, the percentage of cells was increased at the G0/G1 phase and decreased at the S and G2/M phases in the group of cells treated at 1/2 IC_{50} and IC_{50}. However, as shown in Figures 4(a) and 4(b), the aforementioned changes in cell’s population percentage were significant at IC_{50} but not at 1/2 IC_{50}. The same observation was made for the group cells treated at 2 IC_{50} except a significant decrease of cells at the G0/G1 phase and a large number of dead cells in the sub-G1 phase.

3.5. UHPLC-HRMS Analysis of I. cylindrica Root Methanol Extract. A study was conducted on the IC-MeOH based on UHPLC-HRMS in the negative and positive ion mode to identify the potential bioactive chemicals that may be responsible for its recorded anticancer activity. The chromatogram of the UHPLC-HRMS analysis of IC-MeOH is shown in Figure 5. The analysis led to the identification of 46 compounds with high-resolution mass spectrometry and MS/MS data (Table 2).

A total of nine compounds already reported for their anticancer activity have been tentatively identified in IC-MeOH, including 05 flavonoids (epicatechin (1), curcumin (2), (-)-yatein (3), caffeic acid (4) and myricetin (5)), 02 alkaloids (jatroharrhizine (6) and harmaline (7)), 01 phenylpropanoid (cinnamaldehyde (8)), 01 synthetic catecholamine (dobutamine (9)), and 01 monosaccharide derivative that is trans-sinapyl alcohol attached to a β-D-glucopyranosyl residue at position 1 via a glycosidic linkage (syringin (10)). The chemical structures of the compounds are shown in Figure 6.
or cisplatin (1/2 IC50, IC50, and 2IC50) against CaSki cells. All data presented are the mean ± SD and are representative of three independent experiments. p values < 0.05 were considered to be statistically significant, and significance was marked as * p values < 0.05, **p values < 0.01, and ***p values < 0.001. IC-MeOH: *Imperata cylindrica* root’s methanol extract; IC50: inhibitory concentration 50 of IC-MeOH; 1/2 IC50: half of the inhibitory concentration 50 of IC-MeOH; 2 IC50: two times the inhibitory concentration 50 of IC-MeOH; CaSki_24 h: CaSki cells treated during 24 h; CaSki_48 h: CaSki cells treated during 48 h.

Figure 3: (a) Apoptotic effect of IC-MeOH in CaSki cells. Untreated CaSki cells (control) and treated CaSki cell population with IC-MeOH or cisplatin (1/2 IC50, IC50, and 2IC50) at different stages of apoptosis after 24 h and 48 h. Data from 10,000 cells had been collected per data file; in four zones of the drawn quadrant, we had viable cells (Q1), cells bounded to annexin V-PE only (Q2: early apoptotic cells), and cells bounded to both annexin V-PE and 7-AAD (Q3: late apoptotic cells). IC-MeOH: *Imperata cylindrica* root’s methanol extract; IC50: inhibitory concentration 50 of IC-MeOH; 1/2 IC50: half of the inhibitory concentration 50 of IC-MeOH; 2 IC50: two times the inhibitory concentration 50 of IC-MeOH; 24 h, 48 h, and 72 h: treatments’ times. (b) Percentage of apoptotic cells (early and late) in the control (untreated CaSki cells) and treated CaSki cells. All data presented are the mean ± SD and are representative of three independent experiments. p values < 0.05 were considered to be statistically significant, and significance was marked as * p values < 0.05, **p values < 0.01, and ***p values < 0.001. IC-MeOH: *Imperata cylindrica* root’s methanol extract; Cntrl: control; IC50: inhibitory concentration 50 of IC-MeOH; 1/2 IC50: half of the inhibitory concentration 50 of IC-MeOH; 2 IC50: two times the inhibitory concentration 50 of IC-MeOH; CaSki_24 h: CaSki cells treated during 24 h; CaSki_48 h: CaSki cells treated during 48 h.
4. Discussion

The present study was designed to identify the anticancer chemicals of IC-MeOH and to assess the inhibitory potential of the latter against human cervical cancer cell lines and the mode (s) of action (s). The UHPLC-HRMS analysis used for the metabolite profiling of IC-MeOH indicated the presence of several compounds already reported for their anticancer effects, belonging in majority to alkaloids and phenolic compounds groups. Naturally derived phenolic compounds and alkaloids are known to exhibit potent anticancer activities as well as combat various diseases through specific modes of actions. Among the identified active ingredients of IC-MeOH, epicatechin, curcumin, and myricetin are cytotoxic agents causing cancer cell death through induced apoptosis and cell cycle arrest [19–24]. Yatein is known to induce cell cycle arrest and microtubule destabilisation in human lung adenocarcinoma cells [25], and caffeic acid initiates cancer cell death by increasing intracellular ROS, altering mitochondrial membrane potential, lipid peroxidation, and apoptosis in HeLa and ME-180 cervical carcinoma cell lines [26]. Jatrorrhizine inhibits growth and induces C8161 metastatic melanoma cell cycle arrest at G0/G1 transition [27]. Harmaline induces apoptosis and prevents the proliferation and migration of human breast cancer cell lines [28]. Dobutamine displays antitumor activity against human osteosarcoma cells, via cell apoptosis and cell cycle arrest in the G2/M phase [29]. Cinnamaldehyde promotes apoptosis by

![Figure 4: Effect of IC-MeOH on CaSki cell cycle after 24 h (a) and 48 h of treatment (b). IC-MeOH: Imperata cylindrica root’s methanol extract; DMSO Cntrl: control (untreated cells); IC<sub>90</sub>: inhibitory concentration 50 of IC-MeOH; 1/2 IC<sub>90</sub>: half of the inhibitory concentration 50 of IC-MeOH; 2 IC<sub>90</sub>: two times the inhibitory concentration 50 of IC-MeOH; CaSki_24 h: CaSki cells treated during 24 h; CaSki_48 h: CaSki cells treated during 48 h.](image-url)
Figure 5: (a) UHPLC-HRMS chromatogram of IC-MeOH in the reverse phase, negative and positive ion mode. IC-MeOH: *Imperata cylindrica* root’s methanol extract. (b) UHPLC-HRMS chromatogram of IC-MeOH in the HILIC phase, negative and positive ion mode. IC-MeOH: *Imperata cylindrica* root’s methanol extract.
| No. | MW (m/z)   | RT (min) | MF               | Tentatively identified compounds                           | References  |
|-----|------------|----------|------------------|-------------------------------------------------------------|-------------|
| 1   | 125.014    | 4.077    | C₂H₄NO₃S        | Taurine                                                      |             |
| 2   | 273.966    | 4.09     | C₂H₁₁AsO₈        | Ribose-1-arsenate                                           |             |
| 3   | 155.034    | 4.885    | C₂H₆NO₃P        | N-Methylethanolamine phosphate                               |             |
| 4   | 256.079    | 4.931    | C₁₁H₁₃NO₆        | β-D-Ribosylinocitinate                                      |             |
| 5   | 198.052    | 6.192    | C₆H₁₂O₃          | Vanillylmandelic acid (100)                                 |             |
| 6   | 173.069    | 7.833    | C₆H₁₁NO₄        | N-Acetyl-L-glutamate 5-semialdehyde                         |             |
| 7   | 314.155    | 10.193   | C₂₀H₂₄N₄        | A bacteriochlorin                                           |             |
| 8   | 153.042    | 14.784   | C₂H₂NO₃          | 3-Hydroxyxanthanilate                                       |             |
| 9   | 225.100    | 15.732   | C₁₂H₁₃N₅        | Benzyladenine                                               |             |
| 10  | 180.041    | 20.723   | C₆H₄O₃          | Caffeic acid [41, 42]                                        |             |
| 11  | 339.204    | 22.805   | C₁₈H₂₈NO₅       | (-)-7-Epi-12-hydroxyjasmonoyl-L-isoleucine                 |             |
| 12  | 241.110    | 25.209   | C₂₂H₂₇N₅O₩      | O-Methylxandrocymbine                                       |             |
| 13  | 297.193    | 23.445   | C₁₆H₃₂N₄O₅      | N-(3-Oxododecanoyl) homoserine lactone                     |             |
| 14  | 164.057    | 23.652   | C₁₃H₁₄N₅O₆      | β-D-Ribosylinocitinate                                      |             |
| 15  | 289.167    | 24.843   | C₁₇H₂₃NO₃       | Atropine                                                    |             |
| 16  | 346.118    | 24.921   | C₁₈H₁₈N₄        | Miranxanthin-V                                              |             |
| 17  | 385.188    | 25.290   | C₂₂H₃₇NO₅       | 1-naphthol                                                  |             |
| 18  | 400.150    | 26.31    | C₂₂H₂₇N₅Oₓ      | (-)-yateine                                                 |             |
| 19  | 354.165    | 27.242   | C₁₈H₃₂N₄O₂      | N-(3-Oxododecanoyl) homoserine lactone                     |             |
| 20  | 306.144    | 27.409   | C₁₉H₁₈N₅O₥      | EGTA                                                        |             |
| 21  | 380.145    | 27.561   | C₁₂H₂₄O₂        | Coniferyl acetate                                           |             |
| 22  | 222.088    | 27.797   | C₂₀H₂₄N₅O₆      | 1,17-Dihydro-16α, 17-dihydroxy giberellin A9                |             |
| 23  | 222.088    | 28.052   | C₂₀H₂₄N₅O₆      | 3,6,7,3'-4'-Pentamethylquercetagenin                        | [19]        |
| 24  | 346.118    | 28.052   | C₂₀H₂₄N₅O₆      | 1,17-Dihydro-16α, 17-dihydroxy giberellin A9                |             |
| 25  | 339.204    | 28.264   | C₁₃H₂₆N₅O₇      | 3,6,7,3'-4'-Pentamethylquercetagenin                        |             |
| 26  | 142.063    | 28.713   | C₁₂H₁₆O₂        | Homofuranone                                                |             |
| 27  | 250.172    | 29.409   | C₁₈H₃₂N₅Oₐ₆     | Sphingosine 1-phosphate                                     |             |
| 28  | 284.125    | 29.643   | C₁₉H₂₇NO₆        | 2-Phenylethyl β-D glucopyranoside                           |             |
| 29  | 379.246    | 29.806   | C₁₉H₃₂N₆O₅      | Taxusin                                                     |             |
| 30  | 504.273    | 30.052   | C₂₀H₂₄O₄        | 1,17-Dihydro-16α, 17-dihydroxy giberellin A9                |             |
| 31  | 169.082    | 30.173   | C₆H₁₉CHO        | Benzaldehyde                                                |             |
| 32  | 388.316    | 30.208   | C₂₀H₂₉O₈        | 3,6,7,3'-4'-Pentamethylquercetagenin                        |             |
| 33  | 372.142    | 30.297   | C₁₂H₂₆O₂        | Syringin                                                    |             |
| 34  | 132.057    | 30.398   | C₁₀H₈O₂         | Cinnamaldehyde                                              |             |
| 35  | 328.125    | 30.898   | C₁₂H₂₄O₄        | L-Dihyrophenylanine                                         |             |
| 36  | 252.172    | 31.638   | C₁₃H₂₆O₃        | 3-Hydroxyxubimin                                            |             |
| 37  | 273.193    | 31.616   | C₁₉H₂₆N        | 1,17-Dihydro-16α, 17-dihydroxy giberellin A9                |             |
| 38  | 338.138    | 31.837   | C₂₀H₂₉NO₄⁺₁      | Jatrorhizine                                                |             |
| 39  | 440.204    | 32.834   | C₂₀H₂₉O₈        | 10-Deacetyl-2-debenzoylebacatin III                        |             |
| 40  | 518.253    | 35.686   | C₂₂H₄₄O₂P₂       | Geranylfernsyl diphosphate                                  |             |
| 41  | 186.067    | 35.887   | C₁₂H₁₈O₂        | 3,5-Dihydroxybiphenyl                                      |             |
| 42  | 504.253    | 36.186   | C₂₈H₂₇FO₇       | β-Methasone dipropionate                                   |             |
| 43  | 368.125    | 37.257   | C₂₁H₂₀O₆        | Curcumin                                                    |             |
| 44  | 287.151    | 40.314   | C₁₇H₂₃N₆        | Galanthamine                                                |             |
| 45  | 431.303    | 41.630   | C₂₈H₁₈NO₄        | Malyngamide H                                               |             |

MW: molecular weight; RT: retention times; MF: molecular formula. Compounds were tentatively identified based on accurate m/z, standards from PlantCyc (10 ppm) database, METLIN Mass Spectral Database, and literature data.
inhibiting NF-κB and AP-1 activity in cancer cells [30, 31], and syringin exhibited anticancer effects in HeLa human cervical cancer cells by inducing apoptosis, cell cycle arrest, and inhibition of cell migration [32]. The active ingredients identified in IC-MeOH are cytotoxic through apoptosis induction and cell cycle arrest. These reported findings correlate our investigations on the anticancer properties of IC-MeOH. The cytotoxicity and apoptosis assays of IC-MeOH revealed that IC-MeOH displayed a concentration- and time-dependent cytotoxicity against all the tested cell lines. Moreover, IC-MeOH inhibited the ability of CaSki cells to form colonies. The potential activity of a test substance such as a plant extract or secondary metabolite against cancer is not only associated with the cytotoxic or antiproliferative effect but is also related to the ability to inhibit mechanism concerning cancer’s hallmarks [33]. The primary goal of anticancer chemotherapeutic drugs is to destroy cancer cells by inducing apoptosis in affected cells [34]. Apoptosis is a highly systematic and programmed cell death, wherein the cell debris is phagocytosed by the adjacent cells; the plant bioactive chemicals have molecular target for inducing apoptosis in different cancer cells [35–37]. In our study, IC-MeOH was found to significantly induced apoptosis in CaSki cells after 24 h and 48 h of treatment at IC_{50} and 2IC_{50} values. IC-MeOH was also found to induce G0/G1 cell cycle arrest in CaSki after 24 h and 48 h of treatment periods. These outcomes correlate with previous investigations carried out on this plant species, which revealed its in vitro cytotoxicity, apoptosis-induced and G0/G1 cell cycle arrest against other types of cancer, including breast cancer, blood cancer, and human liver hepatocellular carcinoma [9]. Cell cycle checkpoints can be activated by DNA damage. In this case, the growth arrest caused by checkpoints allows the cell to repair the damage. If the damage is severe and cannot be repaired, mitochondrial mechanisms kick in to convert the cell cycle arrest signal into apoptotic signal, where p53 directly and indirectly through Bax targets mitochondrial membrane potential [38]. Furthermore, cell cycle arrest in response to DNA damage activates p53 and causes a G1 arrest by inducing expression of p21 and the consequent inhibition of cyclin D/Cdk [39]. Hence, the anticancer chemicals identified in IC-MeOH may be responsible for its cytotoxicity via induced apoptosis and G0/G1 cell cycle arrest toward cervical cancer cells. Results displayed by Figure 1 and Table 1 show higher cytotoxicity of IC-MeOH toward nontumor cells than cancer cells that could represent adverse effects. Nonetheless, our previous investigations on IC-MeOH regarding its toxicity in vivo revealed nontoxic effects for acute and repeated administration [40]. To the best of our knowledge, the present study is highlighting for the first time the antiproliferative mode of action of IC-MeOH toward human cervical cancer cells.

The use of herbal medicinal products for treating cancer is gaining acceptance, and many formulations have been patented and tested at the clinical trial stage.

5. Conclusion

The use of herbal medicinal products for treating cancer is gaining acceptance, and many formulations have been patented and tested at the clinical trial stage. The overall results provide promising baseline information to deeply investigate
IC-MeOH’s secondary metabolites for their anticancer activities. The UHPLC-HRMS analysis of IC-MeOH revealed the presence of anticancer chemicals belonging to various classes, which may be responsible for the cytotoxicity via apoptosis induction and G0/G1 cell cycle arrest shown by IC-MeOH toward cervical cancer cells.

Data Availability
The datasets used and/or analyzed during the current study are available from the corresponding authors on reasonable request.

Conflicts of Interest
The authors declare no conflict of interest.

Authors’ Contributions
V.K., S.K., S.M., and M.T.A. designed the study. N.P. performed the experimental work and wrote the manuscript. S.M. revised the manuscript. All authors have reviewed the manuscript.

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Supplementary Materials
Table S1: UHPLC-HRMS instrumentation method for the HILIC phase. Table S2: UHPLC-HRMS instrumentation method for the reverse phase. (Supplementary Materials)

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