Research article

UHPLC-MS/MS-GNPS based phytochemical investigation of *Dryopteris ramosa* (Hope) C. Chr. and evaluation of cytotoxicity against liver and prostate cancer cell lines

Zia-ur-Rehman a, Hafiz Majid Rasheed a, Kashif Bashir a, Aleksandra Gurgul b, Fazli Wahid c, Chun-Tao Che b, Irum Shahzadi d, Taous Khan a, *

a Department of Pharmacy, COMSATS University Islamabad, Abbottabad Campus, 22060, Pakistan
b Department of Pharmaceutical Sciences, College of Pharmacy, University of Illinois at Chicago, Chicago, IL 60612, USA
c Department of Biomedical Sciences, Pak-Austria Fachhochschule: Institute of Applied Sciences and Technology, Mang, Khanpur Road, Haripur, Pakistan
d Department of Biotechnology, COMSATS University Islamabad, Abbottabad Campus, 22060, Pakistan

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**ABSTRACT**

*Dryopteris ramosa* (family: Dryopteridaceae) has been reported for its medicinal importance in cancer, gastrointestinal disorders, and infections. The present study aimed to investigate the detailed phytochemical profile of *D. ramosa* and its cytotoxic potential using various cancer cell lines. The phytochemical profile of *D. ramosa* methanolic extract and its fractions were established by employing UHPLC-MS/MS and Global Natural Product Social (GNPS) molecular networking. Moreover, the cytotoxic activity of extract and fractions was evaluated against human liver (HepG-2) and prostate cancer (PC-3) cells using MTT assay. Overall, 18 compounds including flavonoids, flavonoid O-glycosides, isoflavone di-C-glycoside, flavanol, flavanone, rotenoid, phloroglucinol derivative, coumarin derivative, benzofuranone, abietic acid, and phenolic acid were observed as the major phytochemical bioactive constituents in the extract and fractions of *D. ramosa*. In MTT assay, chloroform fraction showed highest anti-proliferative activity against liver cancer cells (IC$_{50}$ = 53.49 μg/mL) followed by *n*-hexane fraction (IC$_{50}$ = 55.36 μg/mL), *D. ramosa* extract (IC$_{50}$ = 85.67 μg/mL) and ethyl acetate (IC$_{50}$ = 125.00 μg/mL) fraction. However, *n*-hexane and chloroform fractions presented maximum cytotoxic effect against prostate cancer cells with respective IC$_{50}$ values of 214.53 and 281.47 μg/mL. Moreover, all the tested samples showed negligible toxicity against non-cancer (BHK-21) cells. The results indicated that *D. ramosa* is rich in flavonoids, phloroglucinol derivative, and phenolic acids and showed positive results in cytotoxic studies, especially against liver cancer. Therefore, it can be considered safe for the development of anticancer drugs, especially against liver cancer.

1. Introduction

Cancer is one of the leading causes of death in the globe. Cancer causes death as a result of excessive cell growth and proliferation [1]. Conventional anticancer therapeutics including chemotherapy and radiotherapy are known to induce senescence in cancer cells [2]. Chemotherapeutic drugs, on the other hand, have been discovered to assault both healthy and malignant cells, resulting in side effects such as bone marrow suppression, hair loss, and cardio-vascular, pulmonary, ophthalmic, and central nervous system toxicity [3]. Plants have been utilised to treat cancer since ancient times [4], and many bioactive secondary metabolites, particularly in the anti-tumour and anti-infection fields, have been discovered. Natural products from plant sources such as vinblastine, vincristine, paclitaxel, and podophyllotoxin analogues (e.g. etoposide) are being employed in the treatment of various cancers [5, 6]. Plant-based anticancer medicines have a variety of molecular pathways and have provided a variety of targets for future anticancer therapies [7].

* D. ramosa is locally known as “Ateer or Pakha or Kunji”. It is distributed in Pakistan, Nepal, Bhutan, Afghanistan, and India. In Pakistan, it is found in shady and moist places of the lesser Himalaya (Galiyat region). It has ascending rhizome, which is covered with fronds. Its ramenta are large, broad, and pale brown in colour [8]. In recent

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* Corresponding author.
E-mail address: taouskhan@cuiatd.edu.pk (T. Khan).

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2405-8440/© 2022 Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).
years, the market value of D. ramosa has been increased 6 folds and the current market value is 7.1 US dollars per kg. D. ramosa is considered as one of the most valuable plant species for the local people [9]. Many researchers have reported the folkloric applications of D. ramosa. Traditionally, it has been used in gastrointestinal disorders like gastric pain, ulcer, and constipation [10]. It is also used as a tonic, antimicrobial, cytotoxic [9], and aphrodisiac [11] in folk medicines. D. ramosa has been reported to contain secondary metabolites such as flavonoids, saponins, glycosides, triterpenoids, and tannins. Recent pharmacological research proves that the plants of Dryopteris genus also have antiviral, antitumor, antimicrobial, anti-inflammatory, and antioxidative activities [12]. Despite its ethnomedicinal importance, D. ramosa has never been subjected to determine its phytochemical profile and anticancer activities, although preliminary phytochemical analysis and brine shrimp lethality assay were performed by Alam et al. [13]. Therefore, the present work aimed to explore the phytochemical profile of D. ramosa and to evaluate its cytotoxic potential against liver and prostate cancer cells.

2. Materials and methods

2.1. Plant material

The whole plant of Dryopteris ramosa was collected from Kaghan (altitude of 2500 m; Latitude is 34.5417°N, 73.3500°E), Khyber Pakhtunkhwa, Pakistan and authenticated by a plant taxonomist, Dr. Abdul Nazir in the Department of Environmental Sciences, COMSATS University Islamabad, Abbottabad Campus. The voucher specimen (Number; CUHA-213/201) was deposited in the same institute.
### Table 1. Tandem mass-based metabolomics analysis for the chemical composition of *D. ramosa* crude extract and fractions.

| Compound No. | Rt (min) | Precursor ion | Fragment ions (MS<sup>2</sup>) | Molecular formula | Exact mass | Dereplication result | Cosine score |
|--------------|----------|---------------|--------------------------------|------------------|-----------|----------------------|--------------|
| 1            | 4.67     | 449.110       | 330.0, 413.088, 165.019         | C<sub>23</sub>H<sub>29</sub>O<sub>11</sub> | 448.101   | Luteolin 6-C-glucoside | 0.89         |
| 2            | 4.84     | 433.114       | 313.072, 337.072, 283.062       | C<sub>12</sub>H<sub>20</sub>O<sub>10</sub> | 432.106   | Apigenin 8-C-glucoside | 0.90         |
| 3            | 4.90     | 289.236       | 153.01, 89.04, 153.048          | C<sub>12</sub>H<sub>16</sub>O<sub>3</sub> | 288.063   | Eriodictyol           | 0.83         |
| 4            | 5.30     | 197.118       | 91.054, 105.071, 133.102        | C<sub>11</sub>H<sub>16</sub>O<sub>3</sub> | 196.110   | Loliolid              | 0.80         |
| 5            | 7.93     | 419.163       | 211.097, 205.086, 149.06        | C<sub>22</sub>H<sub>28</sub>O<sub>8</sub> | 418.163   | Flavaspidic acid AB   | 0.86         |

**n-Hexane fraction of *D. ramosa* crude extract**

| Compound No. | Rt (min) | Precursor ion | Fragment ions (MS<sup>2</sup>) | Molecular formula | Exact mass | Dereplication result | Cosine score |
|--------------|----------|---------------|--------------------------------|------------------|-----------|----------------------|--------------|
| 1**          | 6.76     | 315.195       | 187.113, 171.081, 199.113       | C<sub>20</sub>H<sub>20</sub>O<sub>3</sub> | 314.188   | 7-Dehydroabietic acid | 0.77         |
| 5**          | 7.93     | 419.163       | 211.097, 205.086, 149.06        | C<sub>22</sub>H<sub>28</sub>O<sub>8</sub> | 418.163   | Flavaspidic acid AB   | 0.86         |
| 7            | 8.09     | 359.717       | 176.072, 190.087                | C<sub>12</sub>H<sub>20</sub>O<sub>7</sub> | 358.105   | Excavatin L           | 0.72         |

**Chloroform fraction of *D. ramosa* crude extract**

| Compound No. | Rt (min) | Precursor ion | Fragment ions (MS<sup>2</sup>) | Molecular formula | Exact mass | Dereplication result | Cosine score |
|--------------|----------|---------------|--------------------------------|------------------|-----------|----------------------|--------------|
| 8            | 5.06     | 547.217 [M + Na]<sup>+</sup> | 350.134, 159.044, 251.126      | C<sub>20</sub>H<sub>20</sub>O<sub>11</sub> | 524.226   | Dihydrodehydrodiconiferyl alcohol-9-O-α-rhamnosypyranoiside | 0.72         |
| 5**          | 7.93     | 419.163       | 211.097, 205.086, 149.06        | C<sub>22</sub>H<sub>28</sub>O<sub>8</sub> | 418.163   | Flavaspidic acid AB   | 0.86         |
| 7            | 8.09     | 359.717       | 176.072, 190.087                | C<sub>12</sub>H<sub>20</sub>O<sub>7</sub> | 358.105   | Excavatin L           | 0.72         |

**Ethyl acetate fraction of *D. ramosa* crude extract**

| Compound No. | Rt (min) | Precursor ion | Fragment ions (MS<sup>2</sup>) | Molecular formula | Exact mass | Dereplication result | Cosine score |
|--------------|----------|---------------|--------------------------------|------------------|-----------|----------------------|--------------|
| 8            | 5.06     | 547.217 [M + Na]<sup>+</sup> | 350.134, 159.044, 251.126      | C<sub>20</sub>H<sub>20</sub>O<sub>11</sub> | 524.226   | Dihydrodehydrodiconiferyl alcohol-9-O-α-rhamnosypyranoiside | 0.72         |
| 11           | 5.13     | 273.077       | 153.02, 91.055, 119.049         | C<sub>12</sub>H<sub>16</sub>O<sub>3</sub> | 272.068   | Naringenin           | 0.88         |
| 4**          | 5.30     | 197.118       | 91.054, 105.071, 133.102        | C<sub>11</sub>H<sub>16</sub>O<sub>3</sub> | 196.110   | Loliolid             | 0.80         |
| 12           | 5.35     | 303.051       | 153.019, 137.033, 229.05        | C<sub>12</sub>H<sub>20</sub>O<sub>7</sub> | 302.043   | Quercetin            | 0.86         |
| 13           | 5.38     | 287.056       | 153.019, 121.029, 165.019       | C<sub>11</sub>H<sub>16</sub>O<sub>3</sub> | 286.048   | Kaempferol           | 0.87         |
| 5**          | 7.93     | 419.163       | 211.097, 205.086, 149.06        | C<sub>22</sub>H<sub>28</sub>O<sub>8</sub> | 418.163   | Flavaspidic acid AB   | 0.86         |

**n-Butanol fraction of *D. ramosa* crude extract**

| Compound No. | Rt (min) | Precursor ion | Fragment ions (MS<sup>2</sup>) | Molecular formula | Exact mass | Dereplication result | Cosine score |
|--------------|----------|---------------|--------------------------------|------------------|-----------|----------------------|--------------|
| 10**         | 6.76     | 315.195       | 187.113, 171.081, 199.113       | C<sub>20</sub>H<sub>20</sub>O<sub>3</sub> | 314.188   | 7-Dehydroabietic acid | 0.77         |
| 14           | 4.31     | 453.141       | 163.04, 123.045, 181.05         | C<sub>21</sub>H<sub>24</sub>O<sub>11</sub> | 452.132   | Epicatechin-8-C-beta-d-glucopyranoside | 0.76         |
| 15           | 4.51     | 595.168       | 325.072, 337.072, 379.082       | C<sub>22</sub>H<sub>30</sub>O<sub>15</sub> | 594.158   | Paniculatin           | 0.91         |
| 1**          | 6.76     | 449.110       | 330.071, 413.088, 165.019       | C<sub>21</sub>H<sub>29</sub>O<sub>11</sub> | 448.101   | Luteolin 6-C-glucoside | 0.89         |

(continued on next page)
2.2. Extraction and fractionation

The collected plant was garbled, washed with tap water, and shade dried, followed by pulverization to get a fine powder. The powdered plant material (12 kg) was macerated with methanol (50 L) at room temperature. The extracted material was filtered using a muslin cloth and then with Whatman 42 filter paper. The filtrate was concentrated under reduced pressure using a rotary evaporator (Büchi Rotavapor R-300 (BÜCHI Labortechnik AG, Flawil, Switzerland)) to afford a crude extract (650 g). The polarity-based fractions of D. ramosa extract were prepared by solvent-solvent extraction technique. For this purpose, the crude extract (625 g) was suspended in distilled water and was extracted successively with organic solvents including n-hexane, chloroform, ethyl acetate, and n-butanol to obtain n-hexane-soluble (257 g), chloroform-soluble (33 g), ethyl acetate-soluble (14 g), n-butanol-soluble (118 g) and water-soluble (160 g) fractions.

2.3. Phytochemical analysis

2.3.1. Sample preparation for UHPLC-MS/MS analysis

The methanolic crude extract and fractions of D. ramosa were dissolved in LC-MS grade methanol (1 mg/mL) and filtered through a 0.2 μm membrane filter in HPLC autosampler glass vials.

2.3.2. UHPLC-MS/MS analysis

Metabolomics profiling of the extract and fractions of D. ramosa was carried out using a Shimadzu (Kyoto, Japan) Nexera UHPLC system coupled to an Impact II quadrupole/time-of-flight (Q/TOF) mass spectrometer (Bruker, Billerica, MA, USA). The electrospray ionization (ESI) technique was used in positive ion mode to fragments the ions. For chromatographic separation, a CORTECS UHPLC column packed with C-18 silica gel of 1.6 μm particle size with an internal diameter of 2.1 mm and 100 mm column length (Waters, Milford, MA, USA), guardned by a pre-column (2.1 mm × 5 mm) was used. The gradient program was set using 0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile (B). The gradient elution profile was as follows: 5% B (2.5 min), 5-100% B (6 min), 100% B (2.40 min), and 5% B (3 min) and the total run time was 14 min. The oven temperature was set at 40 °C. The sample injection volume was 5 μL and the flow rate was kept at 0.4 mL/min. Spectra were recorded in full scan mode with m/z range of 80–1000.

2.3.3. Molecular networking

The LC-MS/MS data of the D. ramosa crude extract and fractions were subjected to GNPS molecular networking analysis available online at (http://gnps.ucsd.edu/ProteoSAFe/static/gnps-splash.jsp). The data were converted to GNPS compatible (.mzML format) files via the MSConvert package (Version 3.0.19330, Proteowizard Software Foundation, USA). The converted .mzML files were uploaded on the GNPS platform using WinSCP version 5.17.6. The spectral networks were visualized with the help of Cytoscape 3.7.2 [14].

2.4. Cytotoxic activity

The cytotoxicity of extract and fractions of D. ramosa was evaluated against normal (BHK-21) cells as well as human liver (HepG-2) and prostate cancer (PC-3) cells [15, 16]. The cells were cultured in Dulbecco’s modified Eagle’s medium (DMEM) containing 10% FBS, 100 U/mL penicillin, and streptomycin (all from Gibco/Invitrogen Life Technologies, US) at 37 °C in an incubator having 5% CO2. For MTT assay, cells at a concentration of 1 × 104 cells/well were seeded into 96-well flat-bottom culture plates (Costar® , Corning Inc., Corning, NY, USA). Each well was supplied with 0.1 mL DMEM media. After 24 h culture (at about 80% confluence), cells were treated with indicated concentrations (31.25–125 μg/mL) of crude extract and fractions for 24 h. Similarly, DMSO, a control (0.03–0.5%) was mixed with media and added to the control wells. After that, media was replaced in each well with serum-free media containing MTT reagent (BioShop, Canada) (1:10). The plates were further incubated at 37 °C (under 5% CO2) for an additional 4 h in a humidified environment. After media aspiration, formazan crystals were dissolved with DMSO (100 μL) and the absorbance of the formazan dye, generated by the reaction between dehydorgenase and MTT in metabolically active cells, was measured using a microplate reader (Chem Plate reader, China) at 492 nm. Experiments were performed at least thrice. The % cell viability was calculated using the following formula;

Table 1 (continued)

| Compound No. | Rt (min) | Precursor ion | Fragment ions (MS²) | Molecular formula | Exact mass | Dereplication result | Cosine score |
|--------------|----------|---------------|---------------------|-------------------|-----------|----------------------|-------------|
| 16           | 4.80     | 579.172       | 313.073, 337.073,   | C₂₅H₃₀O₁₄        | 578.164   | Isovitexin 2-O-rhamnoside | 0.81        |
| 2***         | 4.84     | 433.114       | 313.072, 337.072,   | C₂₂H₂₂O₁₀        | 432.106   | Apigenin 8-C-glucoside | 0.90        |
| 3**          | 4.90     | 289.236       | 153.019, 89.04,     | C₁₅H₁₀O₆         | 288.063   | Eriodictyol           | 0.83        |
| 17           | 5.13     | 463.089       | 287.057, 153.02,    | C₂₂H₁₈O₁₂        | 462.080   | Kaempferol 3-O-glucuronide | 0.91        |
| 12*          | 5.35     | 303.051       | 153.019, 137.033,   | C₁₅H₁₀O₅         | 302.043   | Quercetin             | 0.86        |
| 13*          | 5.38     | 287.056       | 153.019, 121.029,   | C₁₅H₁₂O₆        | 286.048   | Kaempferol            | 0.87        |

Aqueous fraction of D. ramosa crude extract

| Compound No. | Rt (min) | Precursor ion | Fragment ions (MS²) | Molecular formula | Exact mass | Dereplication result | Cosine score |
|--------------|----------|---------------|---------------------|-------------------|-----------|----------------------|-------------|
| 10**         | 4.23     | 162.957 [M+ H] | 84.96, 89.037,     | C₆H₆O₄          | 180.042   | Caffeic acid         | 0.78        |
| 15*          | 4.51     | 595.168       | 325.072, 337.072,   | C₂₂H₂₂O₁₇        | 594.158   | Paniculatin          | 0.91        |
| 18           | 4.91     | 465.105 [M+ K] | 289.072, 153.019,   | C₂₂H₁₈O₉        | 426.131   | Dalbinol             | 0.86        |

* Precursor ion is a protonated molecule [M + H] unless stated otherwise.
** Most intense fragments are in bold text.
***, **, *** represents compounds identified in two or three or four subsequent fractions, respectively.
2.5. Statistical analysis

Results were statistically analysed via two-tailed Student’s t-test by applying GraphPad prism software (v 5.0). Experiments were carried out in triplicate and data were presented as mean ± standard deviation (SD) with the confidence interval (CI) of 95%.

3. Results and discussion

3.1. Phytochemical analysis

The UHPLC-ESI-MS/MS analysis, GNPS molecular networking, and MolnetEnhancer techniques were applied to establish the phytochemical composition of the *D. ramosa* extract and fractions. In total, 552 nodes and 738 edges were identified through Cytoscape as shown in Figure 1. The results indicated the presence of simple flavonoids, flavanones,
flavonoid O-glycosides, isoflavone di-C-glycosides, flavanol glycoside, rotenoid, phloroglucinol derivative, coumarin derivative, benzofuranone, abietic acid, and phenolic acids (Figure 1). For tentative identification of compounds, a cosine score greater than 0.7 was set in the molecular networking analysis. Eighteen compounds were noted in the crude extract and fractions of D. ramosa. The bioactive n-butanol and ethyl acetate fractions were rich in flavonoids and their derivatives. The list of tentatively identified compounds with their spectrometric characteristics is given in Table 1. Flavonoids (flavonols) include eriodictyol (3), quercetin (12) and kaempferol (13). Naringenin (11) and were annotated as flavanone type flavonoids. Kaempferol 3-O-glucuronide (17), a flavonoid O-glycoside was annotated by MS² fragmentation pattern that showed a loss of 176 Da indicating the presence of glucuronide moiety. Flavonoid C-glycosides included luteolin 6-C-glucoside (1), apigenin 8-C-glucoside (2), and epicatechin 8-C-beta-o-glucopyranoside (14) showing fragmentation patterns similar to those of C-glycosides [17]. Paniculatin (15) and isovitexin 2′-O-rhamnoside (16) were annotated as isoflavone di-C-glycosides based on the MS² fragmentation pattern. These compounds displayed a characteristic fragmentation pattern of flavonoid C-glycosides with major fragments loss of 60, 90, and 120 Da [18]. Protocatechuic acid (9) and caffeic acid (10) were annotated as phenolic acids. Loliolide (4) was identified as a benzofuranone and dalbinol (18) as rotenoid. Phloroglucinol derivative flavaspidic acid AB (5), abietic acid derivative 7-dehydroabietic acid (6), and coumarins derivative excavatin L (7) were also present. The structures of these identified compounds are presented in Figure 2. All these compounds except caffeic acid and quercetin reported by Baloach et al. [19] are being reported for the first time from D. ramosa.

Ferns are well-known for their use in food, crafts, fibers, as well as use as ornamental plants and building materials [20]. In South Asia, some of the fern species are also used as traditional medicines [1]. Ferns have been reported to possess phytochemicals such as isoprenoids, phenols, and flavonoids [10]. Many scientists have reported different pharmacological effects of these phytoconstituents from fern species on human health [10, 12]. Similarly, different researchers have reported the ethnomedicinal values of ferns from Pakistan like D. ramosa (plant family: Dryopteridaceae) [21]. D. ramosa has never been explored for its detailed phytochemical profile and anticancer activities. Therefore, this study aimed to investigate the comprehensive phytochemical profile of D. ramosa and to evaluate its cytotoxic potential against liver and prostate cancer cells.

Tandem mass spectrometry coupled with liquid chromatography (LC-MS²) is widely employed for the separation and identification of complex mixtures from botanical sources [22]. On the other hand, GNPS molecular networking utilizes tandem mass spectrometric data and has been adopted for the determination of phytochemical composition and identification of chemical constituents [23]. Such approaches were therefore applied to establish a comprehensive and detailed phytochemical composition of D. ramosa. MolnetEnhancer technique was also applied to identify major classes of phytochemical constituents present in the extract and fractions. Eighteen compounds were noted in the crude extract and respective fractions of D. ramosa including simple flavonoids, flavonoid O-glycosides, isoflavone di-C-glucoside, flavanols, flavanones, rotenoid, phloroglucinol derivative, coumarin derivative, benzofuranone, abietic acid, polyunsaturated essential fatty acid, and phenolic acids. The bioactive n-butanol and ethyl acetate fractions were rich in flavonoids and their derivatives. The chemical constituents identified by LC-MS/MS analysis in D. ramosa were reported for in the current study and include luteolin 6-C-glucoside (1), apigenin 8-C-glucoside (2), eriodictyol (3), loliolide (4), flavaspidic acid AB (5), 7-dehydroabietic acid (6), excavatin L (7), dihydrodebr-drodimconiferyl alcohol-9-O-a-rhamnopyranoside (8), protocatechuic acid (9), caffeic acid (10), naringenin (11), quercetin (12), kaempferol (13),

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Table 2. The IC₅₀ values of D. ramosa extract and its fractions against liver (HepG-2) and prostate (PC-3) cancer cells at indicated concentrations.

| Extract/Fraction      | IC₅₀ (µg/mL) HepG-2 cells | IC₅₀ (µg/mL) PC-3 cells |
|----------------------|---------------------------|------------------------|
| D. ramosa extract    | 85.67                     | NA                     |
| n-Hexane fraction    | 55.36                     | 214.53                 |
| Chloroform fraction  | 53.49                     | 281.47                 |
| Ethyl acetate fraction | 125.00                   | >500                   |
| n-Butanol fraction   | NA                        | NA                     |
| Aqueous fraction     | NA                        | >500                   |

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Figure 3. Presentation of effects of D. ramosa extract and its fractions on HepG-2 cells proliferation. The results are expressed as the percentage of the negative control. Data are means ± SD of three independent experiments. *, ** and *** represent p ≤ 0.05, 0.01 and 0.001. ns presents non-significance.
epicatechin 8-C-beta-D-glucopyranoside (14), paniculatin (15), isovitexin 2’-O-rhamnoside (16), kaempferol 3-O-glucuronide (17), dalbinol (18). This study has extended the spectrum of the phytochemical profile of D. ramosa.

3.2. Cytotoxic activity

Treatment of cells by the D. ramosa extract and fractions for 24 h resulted in a reduction of HepG-2 and PC-3 cells viability in comparison to the control. In general, the n-butanol fraction presented the least cytotoxic effect (% cell death) (35.03 ± 2.84) on HepG-2 cells, while the aqueous fraction showed mild effect (46.49 ± 5.97). However, a stronger effect was observed for chloroform fraction (IC$_{50}$ = 53.49 μg/mL), followed by n-hexane fraction (IC$_{50}$ = 55.36 μg/mL), D. ramosa extract (IC$_{50}$ = 85.67 μg/mL) and ethyl acetate (IC$_{50}$ = 125.00 μg/mL) fraction (Figure 3, Table 2). In the case of PC-3 cells, crude extract showed mild toxicity (20.02 ± 0.10% cell inhibition) while, ethyl acetate, aqueous and n-butanol fractions displayed moderate toxicity (% cell inhibition) (63.24 ± 4.03, 50.32 ± 1.87 and 40.34 ± 1.54, respectively). However, highest activity was revealed by n-hexane (IC$_{50}$ = 214.53 μg/mL) and chloroform fraction (IC$_{50}$ = 281.47 μg/mL) (Figure 4, Table 2). In addition, the crude extract and all fractions of D. ramosa were found to be safe in BHK-21 (non-cancer) cells (Figure 5). The ethyl acetate fraction showed mild toxicity against non-cancer cells but it is negligible and this fraction can be considered as safe.

In the current study, the cytotoxic activity of the crude extract and fractions of D. ramosa was evaluated using the MTT assay. Treatment of cells with the extract or fractions of D. ramosa for 24 h resulted in the reduction of liver cancer cells viability in comparison to DMSO (control) treated cells (no cell death). In the case of prostate cancer cells, crude extract of D. ramosa presented mild cytotoxicity. However, all the tested fractions were found active against prostate cancer cells at high concentrations (250 and 500 μg/mL). The major concern about conventional medications is that some of these drugs are toxic to normal cells. Therefore, safety is very crucial for the development of new and novel anticancer medicines [24]. In the current study, it was observed that crude extract and fractions induced negligible toxicity to BHK-21 (non-cancer) cells at maximum tested dose (125 μg/mL). These findings mirrored those reported by various scientists [16, 25, 26], who demonstrated the cytotoxic potential of different medicinal plant extracts on the same type of non-cancer cell lines following comparable treatments. In this study, mostly flavonoids have been reported in extracts and fractions of D. ramosa. It was important to mention that flavonoids like apigenin, baicalein, and trihydroxy flavones do not possess any major cytotoxicity against non-cancer cells [27]. The safety of extract and fractions of D. ramosa may be due to the presence of flavonoids. Therefore, these results indicated that fractions of D. ramosa extract may be proceeded for the isolation of anticancer constituents.
Natural compounds and/or their structural analogs have historically made major contributions to pharmacotherapy, especially for infectious diseases and cancer [28]. In this study, flavonoids and phenolic acid were identified as major constituents in the plant extract and fractions. Among the identified compounds, luteolin 6-C-glucoside (1), apigenin 8-C-glucoside (2), eriodictyol (3), flavaspic acid AB (5), excavatin L (7), protocatechuic acid (9), naringenin (11), quercetin (12), kaempferol (13), epicatechin-8-C-beta-gluco-pyranoside (14), isovitexin 2’-O-rhamnoside (16), kaempferol 3-O-glucuronide (17) and dalbinol (18) have been reported to possess antiproliferative properties against cancers cells including liver, melanoma, colorectal, ovarian and breast cancer [29, 30, 31, 32, 33, 34]. Flavones increase lipophilicity due to their specific structure and have high potential cellular activities [35]. Apigenin possesses cytotoxic action against various cancer cell lines including hepatoma, breast cancer, neuroblastoma, colon cancer, esophageal carcinoma, prostate cancer, and lung cancer cells [36]. Studies have also shown that the novel flavone derivatives with substitutions of chloride, methoxy, nitro, and isopropyl groups on flavone structures are effective anticancer agents against hepato-carcinoma (HepG-2) cells [37]. Kawai et al. (1999) [38] confirmed that in flavonoids, the double bond between C-2 and C-3, and C-3 hydroxyl group are crucial for antiproliferative activity. In general, hydroxyl derivatives of plant polyphenols (chalcones) have more potent antiproliferation effects on HepG-2 cancer cells as compared to other flavonoids and chalcone derivatives [39]. For example, the addition of OH substitution at 2’ position led to an increase (about 2.5 times) in HepG-2 cells growth inhibition.

Luteolin, a flavone, possesses the anticancer property, which is possibly related to the inhibition of cell proliferation and induction of apoptosis. Besides, it sensitizes cancer cells to drug-induced cytotoxicity along with suppressing the cell survival pathways like PI3K/Akt, nuclear factor kappa B, and X-linked inhibitors of apoptotic protein. It has been reported that the antiproliferative potential of quercetin against cancer cells especially liver cancer cells is due to the presence of hydroxy group at the C-3 position of ring B, and C-5 of ring A of the quercetin [40]. Kaempferol (no hydroxylation at 3’) has a lower antiproliferative effect on HepG-2 cancer cells [41]. On the basis of the above discussion, it can be concluded that these constituents alone or in combination with other identified phytoconstituents might have contributed to the cytotoxic activity of the tested samples. This study suggested the isolation and purification of individual bioactive molecules and exploration of their anticancer mechanism.

4. Conclusions

The present work has established the phytochemical profile of D. ramosa using tandem high-resolution mass spectrometry that indicated 18 compounds in the crude extract and its fractions comprising mostly of flavonoids and phenolic acids. Moreover, D. ramosa extract and fractions presented promising cytotoxic effects against liver cancer and mild effect on prostate cancer cell lines. Moreover, all the tested samples were found safe for non-cancer cells. This study suggested that D. ramosa has anti-tumor potential and may be used for the isolation and development of safer anticancer drugs.

Declarations

Author contribution statement

Taous Khan, Chun Tao Chee: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data.
Zia ur Rehman, Hafiz Majid Rasheed: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.
Alessandra Gurgal: Performed the experiments.
Kashif Bashir: Analyzed and interpreted the data; Wrote the paper.

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Data availability statement

Data will be made available on request.

Declaration of interest’s statement

The authors declare no conflict of interest.

Additional information

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