Cell Cycle Arrest in Different Cancer Cell Lines (Liver, Breast, and Colon) Induces Apoptosis under the Influence of the Chemical Content of Aeluropus lagopoides Leaf Extracts

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Abstract: Natural products, especially secondary metabolites produced by plants under stressed conditions, are shown to have different pharmacological impacts from one to another. Aeluropus lagopoides is one of the common halophyte plants that survive under stressed conditions, and has been used for healing wounds and as a painkiller. The bioactivity and the chemical composition of this plant have been poorly investigated. Consequently, the chemical components of A. lagopoides leaves were extracted using hexane (nonpolar), ethyl acetate (semi-polar), and n-butanol (polar) to extract the most extensive variety of metabolites. The cytotoxicity and anticancer impact of extracted secondary metabolites were evaluated against breast (MCF-7), colon (HCT-116), and liver (HepG2) cancer cell lines using a SulphoRhodamine-B (SRB) test. Their mechanisms of action were verified by observing the appearance of apoptotic bodies using the fluorescent microscope, while their antiproliferative impacts were evaluated using a flow cytometer. Results revealed that secondary metabolites extracted using hexane and ethyl acetate had the highest cytotoxicity and thus the greatest anticancer activity effect on HepG2 with IC\textsubscript{50} (24.29 ± 0.85 and 11.22 ± 0.679 µg/mL, respectively). On the other hand, flow cytometer results showed that secondary metabolites could inhibit the cell cycle in the G0/G1 phase. To ascertain the chemical composition–function relationship, the extracts were analyzed using LC-MS/MS. Accordingly, A. lagopoides hexane and ethyl acetate extracts may contain agents with anticancer potential.

Keywords: Aeluropus lagopoides; HepG2; HCT-116; MCF-7; apoptosis; LC-MS/MS

1. Introduction
Cancer, a multistage process consisting of different molecular and morphological aspects, has become the second leading cause of death around the world [1]. In Saudi Arabia over 15,800 people were diagnosed with cancer in 2015 [2]. The production of anticancer drugs is challenging, because cancer cells create complications and resist drugs’ effects. Consequently, monitoring pathways inside the cancer cell to observe the enzymes and their catalysis is the best way to keep cancer cells under...
control. Secondary metabolites that different organisms produce to adapt to their environment may have roles as toxins that initiate or inhibit different pathways inside targeted cells. Meanwhile, apoptosis is one of the most critical pathways inside cancer and normal cells. Like other pathways, apoptosis controlled by enzymes such as caspase or cyclin, which can be activated by many catalysts. In any case, natural products are considered to be the best possible anticancer drugs because of their relatively few side effects. Consequently, natural products became the primary source of almost 80 percent of drugs approved by the Food and Drug administration (FDA) [3]. Therefore, the global focus has been on the isolation of bioactive compounds, and identifying and measuring their therapeutic potential in cancer prevention and treatment [4]. Plants, especially medicinal plants, have been used as a source of medicine since the beginning of civilization [5]. Plants have contributed to famous drugs that used as anticancer or chemoprevention agents, such as taxol, vinca alkaloids, camptothecin, and topoisomerase inhibitors [6–8]. Halophyte plants are cultivated under stressed abiotic conditions [9]. Consequently, they are the plants most likely to develop different molecular, physiological, and morphological mechanisms for their survival [10]. Halophyte plants have been investigated as sources of nutrition [11], fuel [12], and bioremediation [13]. Medical halophytes are rich in bioactive secondary metabolites such as antioxidants, polyphenols, and flavonoid compounds. These components show antimicrobial, antiviral, anticancer, and anti-inflammatory biological activity, and are often nontoxic to normal cell lines [14–16]. Aeluropus lagopoides, a halophyte plant from the family Poaceae, is a perennial grass that tolerates salinity and grows in coastal salt marshes [17]. A. lagopoides is a productive plant used as a source of nutrition for livestock [18,19] and, in Arabian countries, for healing wounds and killing pain [20]. To our knowledge, this is the first report on LC-MS/MS profiling and anticancer activity of crude A. lagopoides extracts within cancer cell lines. The current study aims to investigate the ability of crude non-polar, semi-polar, and polar extracts of A. lagopoides leaves to activate different necessary mechanisms that can prevent cancer cell proliferation or induce cancer cell apoptosis.

2. Results:

2.1. Cytotoxicity

The prepared crude extracts were tested against different cancer cell lines: MCF-7, HCT-116, and HepG2. The results revealed that hexane and ethyl acetate extracts produced a significant impact compared to n-butanol extracts (Table 1). The ethyl acetate extract yielded the highest recorded values of IC$_{50}$ (11.22 ± 0.679 µg/mL) on the HepG2 cell line, while the hexane extract yielded 24.29 ± 0.85 µg/mL against the same cell line. However, the n-butanol extract presented an insignificant impact, showing <100 µg/mL on all cell lines. The results were confirmed by the cell viability curves (Figure 1).

| Extract      | MCF-7     | HCT-116   | HepG2     |
|--------------|-----------|-----------|-----------|
| Hexane       | 32.7 ± 0.58 | 27.79 ± 0.71 | 24.29 ± 0.85 |
| Ethyl acetate| 28.03 ± 0.98 | 34.6 ± 0.82  | 11.22 ± 0.679 |
| n-Butanol    | <100      | <100      | <100      |
| Doxorubicin  | 0.6 ± 0.022 | 0.45 ± 0.0516 | 0.42 ± 0.103 |
Figure 1. Dose-response curve of different extracts of *A. lagopoides* in solid tumor cell lines MCF-7, HCT-116, and HepG2. Cells were exposed to the extracts for 72 h. Cell viability was determined using SRB-U SulphoRhodamine-B assay and data are expressed as mean ± S.D. (n = 3).
2.2. Fluorescence Microscopic Analysis of Cell Viability and Apoptosis

The results obtained from the fluorescence microscope revealed that hexane and ethyl acetate extracts have a high ability to induce apoptosis. Cells exhibited different apoptotic morphological characteristics, such as membrane blebs, chromatin condensation, and the formation of apoptotic bodies (Figure 2).

![Figure 2](image)

**Figure 2.** Morphological changes of HCT-116, MCF-7, and HepG2 cells induced by the IC\textsubscript{50} concentration of hexane and ethyl acetate extracts of *A. lagopoides* for 48 h, stained with AO/EB. The images were taken using fluorescence microscopy at 20×. MB: membrane blebbing; CC: chromatin condensation; EA: early apoptosis; LA: late apoptosis; AB: an apoptotic body; N: necrosis. Scale bar: 2 \(\mu\)m.

2.3. Cell Cycle Analysis

To identify the phase of the cell cycle affected by crude hexane and ethyl acetate extracts of *A. lagopoides* plant, the current study used a flow cytometer. Results revealed that the hexane and ethyl acetate extracts exhibit similar impact mechanism on the G0/G1 phase of the different cancer cell types (Table 2 & Figure 3).
Table 2. Effects of hexane and ethyl acetate extracts of *A. lagopoides* on the cell cycle distribution of three tumor cell lines over 24 h, compared with control cells.

| Tumor Cell Line | Compound         | Cell Cycle Phase |
|-----------------|------------------|------------------|
|                 |                  | G0/G1           | S              | G2/M           |
| HCT-116         | Control          | 44.8 ± 0.85     | 51.79 ± 0.59   | 3.41 ± 0.29    |
|                 | Hexane extract   | 69.59 ± 0.38    | 26.28 ± 0.52   | 4.12 ± 0.89    |
|                 | Ethyl acetate    | 67.57 ± 0.58    | 26.6 ± 0.58    | 5.8 ± 0.89     |
| MCF-7           | Control          | 45.4 ± 0.97     | 46.2 ± 0.81    | 8.34 ± 0.93    |
|                 | Hexane extract   | 61.8 ± 0.9      | 25.91 ± 0.59   | 12.21 ± 0.31   |
|                 | Ethyl acetate    | 70.82 ± 0.49    | 25.3 ± 0.8     | 3.8 ± 0.75     |
| HepG2           | Control          | 50.48 ± 0.62    | 43.3 ± 0.71    | 6.21 ± 0.36    |
|                 | Hexane           | 72.79 ± 0.6     | 26.91 ± 0.9    | 0.3 ± 0.51     |
|                 | Ethyl acetate    | 78.3 ± 0.40     | 21.5 ± 0.5     | 0.19 ± 0.33    |

Figure 3. Effect of hexane and ethyl acetate fractions of *A. lagopoides* aqueous ethanol crude extract on the cell cycle distribution of different cancer cells. Cells were exposed to hexane extract (B) and ethyl acetate extract (C) for 48 h and compared with cell control (A). Cell cycle distribution was determined using DNA cytometry analysis, and different cell phases plotted. (D) Percent of total events (n = 3).
2.4. LC-MS/MS Profiling

2.4.1. LC-MS/MS of Hexane Extract

The analysis of hexane extract by LC-MS/MS showed that the non-polar chemical constituents of *A. lagopoides* leaves contained more than one class of natural product compounds, namely: phenolic compounds, monoterpenes, sesquiterpenes, terpenophenols, and steroids (Table 3).

Table 3. LC-MS/MS analysis of hexane crude extracts of *A. lagopoides* leaves.

| No | Rt  | Compound Name                | Compound Formula | [M + H]^+ Found for ms | [M + H]^+ Found for ms/ms | [M + H]^+ Calculated | Mass Error | Uncertainty in m/z | Ref. |
|----|-----|------------------------------|------------------|------------------------|---------------------------|----------------------|------------|-------------------|------|
| 1  | 7.42| Paracoumaryl alcohol         | C_9H_10O         | 151.0755               | 133.0564, 123.0438, 77.0397, 45.0342 | 151.0765             | −6.6192    | ±0.0010           |      |
| 2  | 5.41| Dihydrojasmone               | C_11H_{16}O      | 167.1432               | 79.0542, 59.0487, 31.01182 | 167.1441             | −5.3846    | ±0.0009           |      |
| 3  | 6.89| Iridodial                    | C_10H_{16}O      | 169.1223               | 95.0659, 67.0547, 57.0705 | 169.1234             | −6.5041    | ±0.0011           |      |
| 4  | 5.50| Jasmolone                    | C_11H_{16}O      | 181.1222               | 163.0389, 149.0236, 84.9599 | 181.1234             | −6.6253    | ±0.0012           |      |
| 5  | 6.81| Callicarpenal (terpenophenol) | C_{16}H_{26}O    | 235.2058               | 217.1991, 91.0538, 81.0693, 57.0696 | 235.2067             | −3.8264    | ±0.0009           |      |
| 6  | 6.33| Neoflavan                    | C_{13}H_{12}O    | 225.0911               | 105.0336, 77.0385         | 225.0921             | −4.8869    | ±0.0011           |      |
| 7  | 6.39| Bakuchiol                    | C_{18}H_{24}O    | 257.1898               | 121.1003, 95.0666, 43.01175 | 257.1910             | −4.6658    | ±0.0012           |      |
| 8  | 8.87| Desmosterol                  | C_{27}H_{44}O    | 385.3469               | 109.0649, 97.0648, 81.0704 | 385.3476             | −1.8165    | ±0.0007           |      |
| 9  | 8.20| Stigmasta-1,3,5-triene        | C_{29}H_{46}     | 395.3677               | 150.1160, 145.1011, 81.0699 | 395.3683             | −1.5176    | ±0.0006           |      |
| 10 | 8.27| Stigmastan-3,5-diene          | C_{29}H_{46}     | 397.3830               | 147.1180, 95.0672, 81.0707 | 397.3840             | −2.5165    | ±0.0010           |      |
| 11 | 9.01| Brassicasterol               | C_{28}H_{46}O    | 399.3622               | 109.0648, 97.0645, 81.0698 | 399.3632             | −2.5040    | ±0.0010           |      |
| 12 | 8.11| δ-tocopherol                 | C_{37}H_{46}O    | 403.3580               | 137.0604, 57.0700         | 403.3582             | −0.4958    | ±0.0002           |      |

2.4.2. LC-MS/MS of Ethyl Acetate Extract

LC-MS/MS profiles show that the semipolar compounds extracted by ethyl acetate are from four different natural product classes: monoterpenes, sesquiterpenes, isoflavones and flavones (Table 4).
Table 4. LC-MS/MS analysis ethyl acetate crude extracts of *A. lagopoides* leaves.

| No | Rt | Compound Name          | Compound Formula | [M + H]^+ Found for ms | [M + H]^+ Found for ms/ms | [M + H]^+ Calculated | Mass Error | Uncertainty in m/z | Ref. |
|----|----|------------------------|------------------|-------------------------|----------------------------|-----------------------|------------|-------------------|------|
| 1  | 1.79 | 7-Deoxyloganetic acid | C_{10}H_{14}O_{4} | 199.0967                | 149.0211, 98.9830, 69.0699 | 199.0976             | 4.5204    | ±0.0009           |      |
| 2  | 7.26 | Rodundone              | C_{15}H_{22}O_{5} | 219.1743                | 99.0080, 95.0859, 67.0538 | 229.1081             | 4.8012    | ±0.0011           |      |
| 3  | 5.04 | Loganetin              | C_{11}H_{16}O_{5} | 229.1070                | 286.0478, 258.0526        | 229.1081             | 3.6536    | ±0.0011           | [32] |
| 4  | 5.76 | Pratensein             | C_{16}H_{12}O_{6} | 301.0707                | 286.0478, 258.0526        | 301.0718             | 3.6536    | ±0.0011           |      |
| 5  | 5.91 | psi-Tectorigenin       | C_{16}H_{12}O_{6} | 301.0707                | 286.0478, 258.0526        | 301.0718             | 3.6536    | ±0.0011           |      |
| 6  | 6.7  | Cirsiliol              | C_{17}H_{14}O_{7} | 331.0813                | 109.1011, 95.0655, 81.0697| 331.0823             | 3.0204    | ±0.0010           | [33] |

3. Discussion

Control of biological processes within the cell, such as cell division, transcription–translation of proteins, or apoptosis, requires the activation of many biological pathways by a complicated enzyme system, which itself requires the presence of many cofactors and coenzymes [34]. Consequently, predicting the activation of pathways via pure compounds may not be accurate; hence, the use of crude extract may enhance synergistic impacts between secondary metabolites. Consequently, the current study inspected the ability of hexane, ethyl acetate, and *n*-butanol crude extracts of *A. lagopoides* to induce apoptosis where it is known to be the most promising pathway for a cancer therapy strategy [22]. Furthermore, the isolated active ingredients and secondary metabolites from *A. lagopoides* plant leaves were analysed using LC-MS/MS to determine the relationship between their cytotoxic, apoptotic, and antiproliferative activity and their chemical composition, allowing the mechanism of action to be investigated from different points of view.

Consequently, results showed that hexane (nonpolar) and ethyl acetate (semi-polar) extracts had the highest cytotoxic activity, compared to *n*-butanol (polar) extracts. These observations indicate that the non-polar and semi-polar components of *A. lagopoides* may have ingredients or molecules involved in the activation mechanisms of one or more antiproliferative pathways (Table 1). Moreover, results revealed that equipotent concentrations of hexane and ethyl acetate extract arrested the cell cycle in the G0/G1 phase, showing high values of almost 65 to 78 percent in all cancer cell lines. In light of the aforementioned observations, it can be concluded that the extracts have no clastogenic impact, because if such impacts are present, the cell cycle will be arrested in the S phase [23]. When the cell cycle is arrested in the G0/G1 phase, as in this case, cells will go on to activate one of the enrolled pathways such as apoptosis, necrosis, or differentiation. As the cancer cells cannot activate the differentiation mechanism, they will choose either apoptosis or necrosis. For that reason, the use of specific apoptotic and necrotic stains such as acridine orange and ethidium bromide were suitable to confirm the pathway that had been chosen. The current study depended on this to determine the mechanism of action for the different extracts’ constituents; the cellular morphology changes were screened by a mix of fluorescent dyes (ethidium bromide:acridine orange) (Figure 2). Based on the observeds morphological apoptosis signals, it appears that both extracts induce apoptosis rather than necrosis. Recent studies have revealed that the checkpoints in the G0/G1 phase are under the regulation of cyclin E and D; this encourages us to suggest that the active molecules or ingredients of the hexane and ethyl acetate extracts may have the ability to regulate cyclin E or D pathways [24,25]. It was therefore necessary to conduct a chemical analysis to reveal the molecules found in the extracts. LC-MS/MS profiling
of the active extracts (hexane and ethyl acetate) confirmed that there were two classes of compound responsible for the anticancer activity. The first class was phenolic compounds [35,36] which presented in the hexane extract as pure phenolic compounds, and in ethyl acetate as flavones and isoflavones. Phenolic compounds are reported as antioxidants in many documents [37]; this study reveals that they may act as cofactors or coenzymes in apoptotic activation pathways. The second compound class, monoterpenes, were detected in both hexane and ethyl acetate extract. These would not be expected to be constituents of butanol extract, since butanol extract does not show any anticancer activity; this is confirmed in the current study results. Therefore, the current study suggests that the monoterpene classes detected in *A. lagopoides* may exhibit anticancer activity by inducing apoptosis and blocking the cell cycle in different stages [37]. Interestingly, despite that both extracts being able to cross the cell membrane, the induction of apoptosis via ethyl acetate extract was greater than hexane extract. This may be due to the mechanisms of action of the two extracts: Ethyl acetate extract enhances the apoptosis induction pathway, meanwhile, hexane extract may follow another pathway, depending on gene repair mechanisms; the LC-MS/MS confirmed the presence of iridodial monoterpene, which acts as gene repair agent [38]. Expanding upon these observed results, the suggested mechanism of action of the hydrophobic molecules extracted from *A. lagopoides* is related to their capability to flow efficiently through the phospholipid bilayer membrane of the cell. This phenomenon changes the internal cellular homeostasis, which arrests cell division by a mechanism that requires further study. However, the interesting point here is that all different pathways will ultimately induce apoptosis, which is the preferred mechanism to eliminate cancer cells.

The LC-MS/MS of hexane extract resulted in the first reported LC-MS/MS data for paracoumaryl alcohol, dihydrojasmine, iridodial, jasmolone, callicarpenal, neo flavan, xibornol, stigmasta-1,3,5-triene, and stigmastan-3,5-diene. Meanwhile, the LC-MS/MS data for the other compounds detected in the hexane extract (bakuchiol, desmosterol, brassicasterol, and delt-tocopherol) are in good agreement with the reported MS/MS data for them [21–31].

The chemical analysis of the *A. lagopoides* ethyl acetate extract by LC-MS/MS revealed LC-MS/MS data for the first time for 7-deoxyloganetic acid, rodundone, loganinet, and psi-tectorigenin, but the MS/MS data for pratensein and cirsiliol have been previously reported in many documents [32,33].

4. Materials and Methods

4.1. Cell Lines, Chemicals, and Biochemicals

Ethanol, methanol, and SulphoRhodamine-B (SRB) stains were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All other chemicals were obtained from Life Technologies/Gibco Co. (Carlsbad, CA, USA) unless mentioned otherwise. Cell culture vessels usually replenished from Nunc Co. (Roskilde, Denmark). Human colon (HCT-116), human liver (HepG2) and human breast (MCF-7) cancer cell lines acquired from Vacsera (Giza, Egypt). Cells were routinely maintained in RPMI 1640 cell culture media and supplemented with 1 mM sodium pyruvate, 2 mM/L glutamine, 100 units/mL penicillin-streptomycin and 10% fetal bovine serum. Subsequently, they incubated in a humidified, 5% CO₂ at 37 °C.

4.2. Extraction and Crude Extracts Preparation

The fresh leaves of *Aeluropus lagopoides* were collected from Al-Hridha Beach at 17.7644488,41.9261616, Asser region, Saudi Arabia on 17 July 2017. For the crude extract preparation, 100 g of fresh leaves were washed with distilled water and ground by a grinder with 500 mL of 80% aqueous ethanol. The leaves were then immersed in 1L of 80% aqueous ethanol, left for seven days at room temperature (18–24 °C), and occasionally stirred. The ethanol extract was filtered using filter paper and concentrated to dryness under reduced pressure using a rotary evaporator at 37 °C (Ika, Deutschland, Germany). The concentrated crude extract weight was 10 g, and the extraction yield was 10%. The crude extract was then reconstituted in 400 mL of distilled water and extracted with different
solvents according to the polarity (hexane, ethyl acetate, and n-butanol were non-polar, semipolar, and polar, respectively) using the liquid–liquid extraction method. After that, the solvent phase was separated and re-evaporated using a rotary evaporator. The solvent was then left at room temperature (20–26 °C) for complete evaporation for five days. The crude hexane extract weighed 1.3721 g, and the extraction yield was 10% of the ethanol yield. The ethyl acetate weighed 0.9816 g, and the extraction yield was 9.8% of the ethanol yield. The n-butanol weighed 3.723 g, and the extraction yield was 30% of the ethanol yield. Additionally, 0.01 g of each crude extract was diluted in 1 mL of dimethyl sulfoxide (DMSO) as a stock solution for bioactivity assays. The crude extracts were stored for further studies at 4 °C.

4.3. Cytotoxic Activity of A. lagopoides Crude Extracts

The cytotoxicity and anticancer activities of crude extracts of prepared A. lagopoides leaves were tested against human breast (MCF-7), human colon (HCT-116), and human liver (HepG2) cancer cell lines using the SRB assay described by Skehan et al. [21]. Different cancer cell lines exposed to a range of concentrations (0.01 to 100 µg/mL) of hexane, ethyl acetate, and n-butanol crude extracts were then incubated in a 5% CO₂-humidified incubator at 37 °C for 72 h. Doxorubicin used as a positive control. Treated cells were fixed with TCA (10%) for 1 h at 4 °C. Subsequently, to remove TCA, cells were washed with water many times, and then a 0.4% SRB solution was used to stain cells in a dark place for 10 min. Stained cells washed with 1% glacial acetic acid. Finally, to dissolve SRB-stained cells, tris-HCl was used. After drying overnight, the color intensity of the remaining cells was measured at 540 nm by Elisa [34].

4.4. Detection Activity Signals of Apoptosis

For the detection of apoptotic bodies, the treated cells were washed using PBS washing buffer twice and then collected using 0.25% trypsin-EDTA. After that, the cells were stained using ethidium bromide (EtBr) and acridine orange (AO) in 1:1 concentration. Cells were then transferred to slides. Stained apoptotic bodies were detected and photographed under a Nikon Fluorescent microscope (Tokyo, Japan).

4.5. Cell Cycle Distribution Using DNA Flow-Cytometry

Adherent cancer cells were exposed to IC₅₀ equivalent concentrations of extract solutions for 48 h. Cells were then suspended using 0.25% trypsin-EDTA, washed with ice-cold PBS, and resuspended in 0.5 mL of PBS. Cells were then fixed in 70% ice-cold ethanol at 4 °C for 1 h before being transferred to −20 °C until they were required for analysis. Upon analysis, the fixed cells were washed with ice-cold PBS and resuspended in 1 mL of PBS containing 50 µg/mL RNase A and 10 µg/mL propidium iodide (PI). After 20 min incubation at 37 °C, cells were analyzed for their DNA contents with FACSVantageTM (Becton Dickinson Immunocytometry Systems, San Jose, CA, USA). For each sample, 10,000 events were acquired. Cell cycle distribution was calculated using CELLQuest software (Becton Dickinson Immunocytometry Systems, San Jose, CA, USA) [38].

4.6. Statistical Analysis

The IC₅₀ calculation was performed using Sigma Plot version 12.0 (San Jose, CA, USA).

4.7. LC/MS-MS for Plant Extracts

Analyses were performed on SCIEX X500R QTOF system (includes UPLC-MS/MS (Woodlands Central Indus. Estate., Singapore)). The separation was performed using Phenomenex Kinetex 2.6 µm Phenyl-Hexyl 100 A (50 × 4.6 mm, Phenomenex, Madrid Avenue, Torrance, CA, USA). The mobile phase consists of phase A (10 mM ammonium formate in water) and phase B (0.05% formic acid
in methanol). A variable gradient flow rate was used, which is described in Table 5. A positive non-targeted mode was used for the analyte.

### Table 5. LC gradient parameters.

| Time (min) | Flow Rate (mL/min) | A Conc. | B Conc. |
|------------|-------------------|---------|---------|
| 0.00       | 0.7               | 90.0    | 10.0    |
| 7.00       | 0.7               | 2.00    | 98.0    |
| 8.50       | 0.7               | 2.00    | 98.0    |
| 8.60       | 0.7               | 90.0    | 10.0    |
| 9.50       | Stop              |         |         |

5. Conclusions

In this study, the ethyl acetate fraction of the *A. lagopoides* leaves showed significant cytotoxicity with IC$_{50}$ under 20 in the HepG2 cancer cell line at 11.22 ± 0.679. This value was recommended by the NCI for further anticancer drug research. Also, the extract was shown to induce apoptosis and arrest the cell cycle at the G0/G1 phase. LC-MS/MS profiling indicated the presence of different bioactive compounds, such as flavonoids, which have a long history of use in anticancer drugs. In light of these data, hexane and ethyl acetate extracts contain apoptotic pathways-inducing molecules or cofactors.

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**Sample Availability:** Samples of the compounds are available from the authors.

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