Evaluation of Antiproliferative Activity of Some Traditional Anticancer Herbal Remedies from Jordan

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Abstract

Purpose: To evaluate the in vitro antiproliferative activity of the extracts of three plants from Jordan flora against a panel of human tumor cell lines representing the most common types of cancer in Jordan, breast, colorectal and skin cancers.

Methods: The methanol extracts of the aerial parts of the three plants (Arbutus andrachne L., Chrysanthemum coronarium L., and Teucrium polium L.) were prepared and assessed for antiproliferative activity against six human tumor cell lines (A375.S2, WM1361A, CACO-2, HRT18, MCF-7, T47D) using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide MTT cell proliferation assay.

Results: C. coronarium extract, at the concentration range of 25 to 400 µg/mL, significantly inhibited (10 – 50 %) the proliferation of the 6 cell lines in a dose-dependent manner, whilst the extracts of the other two plants exhibited weak antiproliferative activity (2 – 10 % inhibition). The half-maximal inhibitory concentration (IC50) values of C. coronarium extract against the six cell lines were in the range of 75.8 to 138.5 µg/mL.

Conclusion: The methanol extract of the aerial parts C. coronarium possesses a relatively potent antiproliferative activity and therefore might be a potential source of natural compounds that can be developed into new antineoplastic agents.

Keywords: Antiproliferative, Arbutus andrachne L., Chrysanthemum coronarium L., Teucrium polium L. Jordan flora, Medicinal plants, Cancer, Antineoplastic

INTRODUCTION

Cancer is one of the leading causes of death worldwide. The global incidence of cancer is in continuous rise in concomitance with the presence of cancer therapy-related problems. The emergence of resistance, the high toxicity and the high cost of many anticancer agents are some of the reasons that limited their use in clinical settings [1]. Therefore, large bulk of research is now focused on finding new anticancer agents to address these issues.

Screening plants for anticancer activity has been a historically important field of research that has resulted in the introduction of several anticancer drugs to the market. Some of these are vinca alkaloids (vinblastine and vincristine) that were isolated from Catharanthus roseus and taxol...
(paclitaxel) that was isolated from Taxus brevifolia in the 1970s [2].

Plant-derived natural products are a potential source of cheap antineoplastic agents. Currently, this is of particular importance in developing countries where cancer-related economic burden is high [3]. Therefore, various research groups in these counties are investigating medicinal plants for cytotoxic activities [4-5].

In Jordan, one of the developing countries, cancer is the second leading cause of death after heart diseases, and the resources assigned for cancer treatment are limited [6]. Hence, searching for medicinal plants with antineoplastic activity is becoming popular among the scientific community in Jordan [7-9]. According to the cancer registry in Jordan, breast cancer is the most common type of cancer followed by colorectal carcinoma [6]. Therefore, it is important to find cheaper alternative to the conventional anticancer agents used for the treatment of these types of cancer. Skin cancer, for example, despite its low incidence in Jordan, is traditionally treated with topical herbal preparations as they provide a relatively safe and readily available choice of therapy for this type of cancer [6].

**EXPERIMENTAL**

**Plant materials**

Aerial parts of the plants under investigation (Arbutus andrachne L., Chrysanthemum coronarium L., and Teucrium polium L.) (Table 1) were collected from Northern Jordan. Verification of the taxonomic identity of each plant was performed by Dr. Khaled Tawaha (Faculty of Pharmacy, Amman, Jordan) and voucher specimens of the plants were deposited there (Table 1).

<table>
<thead>
<tr>
<th>Plant scientific name (voucher code)</th>
<th>Family</th>
<th>Common name</th>
<th>Local Arabic name (Jordan)</th>
<th>Part used</th>
<th>Reported pharmacological activities</th>
<th>Ethnopharmacological uses</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chrysanthemum coronarium L. (CC-MJ)</td>
<td>Asteraceae</td>
<td>Crown daisy or Garland</td>
<td>Besbas or Bassoum</td>
<td>Aerial parts</td>
<td>Antioxidant; antiangiogenic action [11]; cytotoxic activity (volatile oils) [14]; activation of type-1 immunity [12]; Xanthine oxidase inhibitory effect [15]; antihypercholesterolemic and antihyperglycemic activities in rats [16]; Antispasmodic, edible (cheese preparation) [10]; diuretic [14]</td>
<td></td>
</tr>
</tbody>
</table>
Plant extraction

The plant materials were air-dried under shade at room temperature (22 – 23 °C), and then separately homogenized into fine powder. The powdered materials were stored in airtight amber glass containers prior to extraction. Ten grams of the plant powder were soaked in 500 mL of methanol, in a shaking water bath, at 37 ºC (Labtech, Korea) for 5 days. The extract was then centrifuged at 1500 g for 10 min and the solvent in the recovered supernatant was evaporated under reduced pressure using a rotary evaporator (Heidolph Laborota, Germany). The solid residues were then dissolved in DMSO to prepare stock solutions and stored at -20 ºC for further use.

Cell cultures

Six solid human tumor cell lines (summarized in Table 2) were cultured in RPMI-1640 medium (Lonza) supplemented with 10 % fetal bovine serum (Gibco), 10 mM HEPES buffer (pH 7.3), 2 mM L-glutamine, 50 µg/mL gentamicin, 100 U/mL penicillin and 100 µg/mL streptomycin sulfate (all from Lonza). The cultures were maintained at 5 % CO₂ in a humid environment at 37 ºC. Viable cell count was determined by trypan blue dye exclusion method.

Cytotoxicity and MTT cell viability assays

Cells in their log phase of growth were used to prepare the cultures in a flat-bottomed 96-well microplate. Based on optimization experiments, the two malignant melanoma cell lines were seeded at a final count of 4 x 10⁴ cells/200 µl/well while the rest of the cell lines were seeded at a count of 2 x 10⁴ cells/200 µl/well. Cultures were then incubated for 24 h before they were left untreated (complete growth media only) as a cell growth control, treated with 6.25, 12.5, 25, 50, 100, 200, 400 µg/mL of the plant extracts (test samples), or treated with the corresponding final concentration of DMSO only (0.04, 0.08, 0.16, 0.32, 0.65, 1.3, 2.6 %) as a solvent control. For the screening test, cultures were treated with 100 µg/mL of the plant extract or 0.65 % of DMSO as described above. Each treatment group was prepared in triplicate wells. Cultures were then incubated for another 72 h before measurement of cell viability using MTT (3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay (sigma). Twenty microliters of 5 mg/mL MTT solution in PBS were added to each well and incubated for 3 h at 37 ºC to allow the formation of the purple formazin crystals within the viable cells. The supernatants were discarded and the crystals were then left to dissolve in 200 µL of DMSO for 10 min at 37 ºC, giving a purple-colored solution of formazin. Absorbance was measured at 570 nm using a microplate reader (Biotek, USA). Cell viability (Cv, %) was determined as in Eq 1.

\[ Cv(\%) = \frac{(At/Ac)}{100} \] (1)

where At and Ac are the absorbance of the test sample and control (blank solvent), respectively.

IC₅₀, defined as the concentration at which 50 % of cell viability was inhibited, was computed.

Statistical analysis

Data are expressed as mean ± standard deviation SD (n = 3). Statistical analysis of data using one-way ANOVA was done with Prism 5 software (GraphPad, USA). Differences between means were considered significant at p < 0.05.

RESULTS

Antiproliferative activity

As shown in Figure 1, C. coronarium extract significantly reduced cell viability of the 6 cell lines, particularly after incubation with 100, 200 or 400 µg/mL of the extract. IC₅₀ was in the range of 75.7 to 138.5 µg/mL. WM1361A and T47D cell lines, amongst the tested cell lines, were the most sensitive to the cytotoxic action of C. coronarium (IC₅₀ of 75.7 and 79.8 µg/mL, respectively).

As shown in Table 3, C. coronarium consistently reduced cell viability in all the cell lines, to varying extent, except CACO-II cells. In contrast,

<table>
<thead>
<tr>
<th>Table 2: Cell lines used in this study</th>
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<tbody>
<tr>
<td>Cell type</td>
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<tr>
<td>-----------</td>
</tr>
<tr>
<td>Epithelial-like</td>
</tr>
<tr>
<td>Epithelial</td>
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<tr>
<td>Epithelial</td>
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<tr>
<td>Epithelial</td>
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<tr>
<td>Epithelial-like</td>
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<tr>
<td>Melanocyte</td>
</tr>
</tbody>
</table>
Table 3: Cell viability of six tumor cell lines after treatment with 100 µg/mL of the methanol extract of the investigated plants for 72 h

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Arbutus andrachne L.</th>
<th>Chrysanthemum coronarium L.</th>
<th>Teucrium polium L.</th>
</tr>
</thead>
<tbody>
<tr>
<td>MCF-7</td>
<td>97.79 ± 6.45</td>
<td>91.45 ± 2.33</td>
<td>118.5 ± 11.28</td>
</tr>
<tr>
<td>T47D</td>
<td>108.8 ± 33.44</td>
<td>22.62 ± 12.23</td>
<td>98.5 ± 2.99</td>
</tr>
<tr>
<td>CACO-2</td>
<td>121.2 ± 3.00</td>
<td>94.75 ± 3.12</td>
<td>102.2 ± 1.82</td>
</tr>
<tr>
<td>HRT18</td>
<td>90.1 ± 6.3</td>
<td>44.37 ± 3.39</td>
<td>89.78 ± 7.44</td>
</tr>
<tr>
<td>A375.S2</td>
<td>40.56 ± 5.99</td>
<td>79.83 ± 2.63</td>
<td>61.1 ± 4.46</td>
</tr>
<tr>
<td>WM1361A</td>
<td>115.5 ± 2.96</td>
<td>70.97 ± 3.19</td>
<td>99.64 ± 3.53</td>
</tr>
</tbody>
</table>

Data are presented as mean ± SD (n = 3)

Figure 1: In vitro antiproliferative activity of the methanol extract of C. coronarium against 6 tumor cell lines. Cells were left untreated, treated with various concentrations of C. coronarium extract (6.25, 12.5, 25, 50, 100, 200 or 400 µg/mL), or treated with the corresponding concentration of DMSO as a solvent control. Cultures were left for 72 hours before determination of cell viability using MTT assay. (a) Cell viability of two human breast tumor cell lines. (b) Cell viability of two human rectal tumor cell lines. (c) Cell viability of two human malignant melanoma cell lines. Data are presented as normalized mean values ± SD (n = 3). *, p < 0.05; **, p < 0.01; ***, p < 0.001
both A. andrachne and T. polium did not exhibit antiproliferative activity against any of the cell lines except for A375.S2 malignant melanoma cell line (~40 and 60 % inhibition, respectively), which is attributed to the variable responsiveness of the different cell lines. C. coronarium was therefore considered to have a potential antiproliferative effect compared to the other two plants.

DISCUSSION

In Jordan, the search for medicinal plants with antitumor activity is becoming popular. This is in keeping with the increasing demand for a relatively cheaper and safer substitute to the established anticancer agents. To our knowledge, little evidence is known about the antiproliferative effect of A. andrachne, C. coronarium and T. polium, in spite of their use in traditional medicine for cancer treatment. In the present study, there is evidence of the antiproliferative activity of C. coronarium extract against three types of human tumor cell lines (breast cancer, colorectal cancer and skin cancer). However, the methanolic extract of A. andrachne and T. polium did not exhibit significant effects (except for A375.S2 cell line) at the concentration used.

Based on the screening test, C. coronarium extract consistently inhibited the proliferation of the six cell lines at 100 µg/mL. Variable degrees of antiproliferative activity was achieved against the different cell lines. The highest activity was demonstrated against malignant melanoma cell line (WM1361A) and ductal epithelial breast tumor cell line (T47D) with IC50 of 75.7 and 79.8 µg/mL, respectively. Whilst MCF-7 breast carcinoma cell line was least sensitive (IC50 = 138.5 µg/mL). In addition to the antiproliferative activity of the plant extract we show here, several previous reports demonstrated multiple pharmacological activities of C. coronarium extracts that support its potential anticancer activity. Choi et al. had demonstrated antiangiogenic effect of campesterol, a phytosterol isolated from C. coronarium, which inhibited fibroblast growth factor-mediated endothelial cell proliferation in vitro [11]. In addition, C. coronarium extract resulted in the activation of type-1 immunity in vitro through induction of IFN-γ production by murine natural killer cells in vitro [12]. Considering the volatile oil extract of C. coronarium, Marongiu et al. had demonstrated cytotoxic activity of this extract against Hela and Vero cells [14]. Therefore, C. coronarium might be of a promising therapeutic value in the treatment of cancer. This, however, requires further investigation of the anticancer activity of some known chemical constituents of the plant such as quinic acid derivatives and sesquiterpene lactones [18].

In literature, few species of the genus Chrysanthemum are reported to have anticancer activity. C. montifolium, for example, was reported to exhibit anti-mutagenic activity due to its flavonoids contents [19], while, cytotoxic activity was observed for C. segetum and C. flosculosus [14].

With respect to the antiproliferative activity of T. polium and A. andrachne, the methanolic extracts of both plants exhibited weak antiproliferative effect against the tumor cell lines (at 100 µg/mL). This is in keeping with the results obtained by another study in Jordan, which showed only a relatively weak antiproliferative effect of the ethanolic extracts of either plants [7]. However, considering T. polium, other studies in Iran demonstrated an in vitro cytotoxic effect of the methanol and the ethanol extracts of this plant against a range of human tumor cell lines (Skmel-3, Saos-2, SW480, MCF-7, KB, EJ and A431, A549, BT20, and PC12) [20]. Besides, the essential oils of T. polium ssp. capitatum (from Greece) exhibited in vitro antiproliferative effect against CACO-2 cell line [34]. This discrepancy might be attributed to the possible chemical polymorphism (chemotypes) among T. polium plants growing in different countries, and to the possible variable responses of the different tumor cell lines used in various studies.

CONCLUSION

The data obtained in this study suggest that the methanol extract of C. coronarium has potential antiproliferative activity against several human tumor cell lines. This new insight into the anticancer activity of this plant positions it as a source of lead compounds which are promising for the development of new treatments for some cancer types.

ACKNOWLEDGEMENT

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CONFLICT OF INTEREST

We declare that we have no competing interests.
CONTRIBUTION OF AUTHORS

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Eman Y Abu-rish, Mohammad K Mohammad and Yasser K Bustanji performed experimental design. Mohammad M Hudaib and Khaled A Tawaha were responsible for plants collection and extracts preparation. Sundus H Mashalla and Loay H AlAlawi performed cell cultures and cytotoxicity assays. Violet N Kasabri and Yehia S Mohamed performed data analysis. Eman Y Abu-rish and Yasser K Bustanji were responsible for writing the paper.

REFERENCES