Ethnomedical Survey and Cytotoxic Activity of Medicinal Plant Extracts Used in Kohgiluyeh and Boyerahmad Province in Iran

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Ethnomedical Survey and Cytotoxic Activity of Medicinal Plant Extracts Used in Kohgiluyeh and Boyerahmad Province in Iran

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Methanol extracts of selected medicinal species used in Kohgiluyeh and Boyerahmad (Iran) were evaluated in vitro for their cytotoxic activity against MCF7, HepG2, MDBK, and A549 cells through MTT assay. The extract from Dorema aucheri demonstrated cytotoxicity with IC50 of 20.09 and 48.65 µg.mL−1 against HepG2 and A549 cells, respectively, which was further fractionated and subjected to MTT assay. The results may support the non-cytotoxicity of most plant species used traditionally as natural remedies.

KEYWORDS Ethnobotany, ethnopharmacology, MTT assay

INTRODUCTION

The potential of bioactive plants or their extracts to provide new and novel products for disease treatment and prevention is enormous (35). Indigenous
populations use a wide range of plants to maintain their health, and there is great promise for new drug discovery based on traditional plant uses for therapeutic purposes (10).

Kohgiluyeh and Boyerahmad is a mountainous region located in southwest Iran, with a population of 634,299, according to the 2006 census (32). Despite the diversity and great potential of the medicinal herbs of this province, little scientific work has been done. Traditionally, many of the species have been used as infusions, decoctions, or other forms of preparations to treat illnesses but have not been investigated sufficiently for safety and cytotoxicity. This study evaluated the antiproliferative effects of 16 plant extracts and 3 fractions of species selected based on information collected by interviewing local healers of the province (Table 1).

MATERIAL AND METHODS

Plant Material

Sixteen plant species were collected from Kohgiluyeh and Boyerahmad province, Iran (2009–2010) and were authenticated by botanists at the Traditional Medicine and Materia Medica Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran. All voucher specimens were deposited at the Traditional Medicine and Materia Medica Research Center herbarium for future reference. The local names and traditional use, time of collection, mode of administration, part of the plants listed as being used, and parts were collected and subjected to the cytotoxicity assay are presented (Tables 1 and 2).

Extraction

Dried powdered plants (10 g) were macerated with MeOH at room temperature for 24 h. Where the upper part of the plant (flowers or leaves) was reported as being used, the entire aerial part including flowers and leaves were extracted. In some cases, as in Gentiana olivieri and Haussknechtia elymaitica, although only the underground parts were used traditionally, both underground and aerial parts were extracted separately and examined. The extracts were dried using a rotary evaporator at temperature not exceeding 40°C and tested for cytotoxicity.

Fractionation

Dorema aucheri that demonstrated cytotoxic activity was selected for fractionation. Then 30 g of dried powdered plant was macerated with petroleum ether at room temperature; after 24 h, it was filtered, and the
<table>
<thead>
<tr>
<th>No.</th>
<th>Species</th>
<th>Family</th>
<th>Local name</th>
<th>Traditional use / mode of preparation*</th>
<th>Time of traditional collection*</th>
<th>Parts Traditionally used*</th>
<th>Parts extracted in this study</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>Achillea wilhelmsii</em></td>
<td>Asteraceae</td>
<td>Berenjas /berendʒas/</td>
<td>bleeding, high blood sugar and pressure/infusion</td>
<td>spring</td>
<td>flowers</td>
<td>aerial parts</td>
</tr>
<tr>
<td>2</td>
<td><em>Arctium minus</em></td>
<td>Asteraceae</td>
<td>Babaadam /babaadam/</td>
<td>rheumatoid complaints, kidney stones (leaves), skin diseases (roots)/ infusion</td>
<td>spring</td>
<td>leaves and root</td>
<td>aerial parts, Tuberous roots</td>
</tr>
<tr>
<td>3</td>
<td><em>Daphne mucronata</em></td>
<td>Thymelaceae</td>
<td>Keshk /keʃk/</td>
<td>joint pain/ ointment made from concentrated decoction</td>
<td>spring, summer</td>
<td>stem bark</td>
<td>aerial parts</td>
</tr>
<tr>
<td>4</td>
<td><em>Descurainia sophia</em></td>
<td>Brassicaceae</td>
<td>Khak-shir /kʰakʃir/</td>
<td>stomach disorders, constipation/ macerate constipation/ cooked</td>
<td>late spring, summer</td>
<td>seeds</td>
<td>aerial parts</td>
</tr>
<tr>
<td>5</td>
<td><em>Dorema aucheri</em></td>
<td>Apiaceae</td>
<td>Bilhar /bilhar/</td>
<td>constipation/ cooked</td>
<td>late winter, summer</td>
<td>aerial parts</td>
<td>tuber</td>
</tr>
<tr>
<td>6</td>
<td><em>Gentiana olivieri</em></td>
<td>Gentianaceae</td>
<td>Malandar /malandar/</td>
<td>for disinfecting wounds/ externally: powdered drug</td>
<td>spring</td>
<td>root</td>
<td>aerial parts, underground part</td>
</tr>
<tr>
<td>No.</td>
<td>Species</td>
<td>Family</td>
<td>Local name</td>
<td>Traditional use / mode of preparation*</td>
<td>Time of traditional collection*</td>
<td>Parts Traditionally used*</td>
<td>Parts extracted in this study</td>
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</tr>
<tr>
<td>7</td>
<td>Haussknechtia elymaitica</td>
<td>Apiaceae</td>
<td>Kelos/ke'leus/</td>
<td>high blood sugar and pressure/ cooked</td>
<td>spring, summer</td>
<td>aerial parts</td>
<td>basal leaves, Tuber</td>
</tr>
<tr>
<td>8</td>
<td>Helichrysum oligocephalum</td>
<td>Asteraceae</td>
<td>Darameye karimkhani /'darameye 'karim'kñìni/</td>
<td>cold (in combination with other herbs)/ powder in combination with other herbs</td>
<td>spring</td>
<td>aerial parts</td>
<td>whole plant</td>
</tr>
<tr>
<td>9</td>
<td>Lepidium draba</td>
<td>Brassicaceae</td>
<td>Sozah /se'zah/</td>
<td>fever, colds/ infusion esophagus wounds/ decoction</td>
<td>spring</td>
<td>aerial parts</td>
<td>aerial parts</td>
</tr>
<tr>
<td>10</td>
<td>Lonicera nummularifolia</td>
<td>Caprifoliaceae</td>
<td>Shen /'/jen/</td>
<td>colds, dry cough/ cooked in combination with other herbs</td>
<td>spring</td>
<td>leaves</td>
<td>whole plant</td>
</tr>
<tr>
<td>11</td>
<td>Salvia sclarea</td>
<td>Lamiaceae</td>
<td>Marvareshk /'marvareʃk/</td>
<td>menorrhagia after child birth /inhalation menstrual disorders, in particular amenorrhea/ decoction</td>
<td>spring</td>
<td>aerial parts</td>
<td>whole plant</td>
</tr>
<tr>
<td>12</td>
<td>Scrophularia striata</td>
<td>Scrophulariaceae</td>
<td>Bonje-mari/'bondʒemari/</td>
<td></td>
<td>spring</td>
<td>aerial parts</td>
<td>aerial parts</td>
</tr>
<tr>
<td>13</td>
<td>Stachys pilifera</td>
<td>Lamiaceae</td>
<td>O-lile /oli'le/</td>
<td></td>
<td>spring</td>
<td>aerial parts</td>
<td>aerial parts</td>
</tr>
</tbody>
</table>

*Unpublished data.
<table>
<thead>
<tr>
<th>No.</th>
<th>Species</th>
<th>Effects</th>
<th>Previously isolated compounds</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>Achillea wilhelmsii</em></td>
<td>Anti-oxidant (31) stimulant of humoral and cellular immune functions in mice (30) total cholesterol and LDL-cholesterol, triglyceride lowering effect decrease was observed in diastolic and systolic blood pressure after 2 and 6 months, respectively (5).</td>
<td>2-methyl butanal, alpha-pinene, alpha-thujene, camphene, hexanal, beta-pinene and 1,8-cineol (9), camphor, borneol, linalool, 1,8-cineole, chrysanthemol acetate, and carvacrol (1)</td>
</tr>
<tr>
<td>2</td>
<td><em>Arctium minus</em></td>
<td>Antihypertensive activity (8)</td>
<td>peptides (8)</td>
</tr>
<tr>
<td>3</td>
<td><em>Daphne mucronata</em></td>
<td>Gnidilatimonein (diterpene): cytotoxic (39) Gnidilatimonein (diterpene): apoptotic (17)</td>
<td>Daphnechin, aquillochin, umbelliferone, and coumarin (27) gnidilatimonein (39)</td>
</tr>
<tr>
<td>4</td>
<td><em>Descurainia sophia</em></td>
<td>Relieve cough, prevent asthma, reduce edema, promote urination, cardiotonic effect (33)</td>
<td>cis-beta-ocimene, menthol, neoisomenthyl acetate, alloaromadendrene, longicyclene (16),descurainoside, sinapic acid (33), descurainolide A,B, descurainin, strophantilin,isorhamnetin, descuraic acid (34), descurainin A and descurainoside B, quercetin-3-O-beta-D-glucopyranosyl-7-O-beta-gentiobioside, kaempferol-3-O-beta-D-glucopyranosyl-7-O-beta-gentiobioside,isorhamnetin-3-O-beta-D-glucopyranosyl-7-O-beta-gentiobioside, quercetin-7-O-beta-gentiobioside, kaempferol-7-O-beta-gentiobioside,isorhamnetin-7-O-beta-gentiobioside, quercetin-3,7-di-O-beta-D-glucopyranoside, kaempferol-3,7-di-O-beta-D-glucopyranoside,isorhamnetin-3, 7-di-O-beta-D-glucopyranoside, kaempferol-3-O-beta-D-glucopyranosyl-7-O-[(2-O-trans-sinnapoyl)-beta-D-glucopyranosyl(1-&gt;6)]-beta-D-glucopyranoside, sinapic acid ethyl ester and 3, 4, 5-trimethoxy-cinnamic acid (36)</td>
</tr>
<tr>
<td>5</td>
<td><em>Dorema aucheri</em></td>
<td>Analgesic, anti-inflammatory (19)</td>
<td>Flavonoids (7,19), Coumarines (7)</td>
</tr>
<tr>
<td>No.</td>
<td>Species</td>
<td>Effects</td>
<td>Previously isolated compounds</td>
</tr>
<tr>
<td>-----</td>
<td>-----------------------</td>
<td>------------------------------------------------------------------------</td>
<td>-------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>6</td>
<td><em>Gentiana olivieri</em></td>
<td>Hypoglycaemic, antihyperlipidemic (29), hepatoprotective (2,23)</td>
<td>Isoorientin (23)</td>
</tr>
<tr>
<td>7</td>
<td><em>Haussknechtia elymaitica</em></td>
<td>Immunomodulatory effect (4)</td>
<td>—</td>
</tr>
<tr>
<td>8</td>
<td><em>Helichrysum oligocephalum</em></td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>9</td>
<td><em>Lepidium draba</em></td>
<td>—</td>
<td>glucoraphanin (25)</td>
</tr>
<tr>
<td>10</td>
<td><em>Lonicera nummulariifolia</em></td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>11</td>
<td><em>Salvia sclarea</em></td>
<td>Antibacterial (15,36) anti-inflammatory, analgesic (essential oil) (24)</td>
<td>Scareol, manool, salvipisone, ferruginol, microstegiol, candsisioi, 7-oxoroyleanone, 2,3-dehyrodsalvipisone, 7-oxoferruginol-18-al, as well as two sesquiterpenes, caryophyllene oxide and spathulenol, alpha-amyrin, beta-sitosterol, apigenin, luteolin, 4’-methylapigenin, 6-hydroxyluteolin-6,7,3,4’-tetramethyl ether, 6-hydroxy apigenin-7,4’-dimethyl ether (13,37,38) essential oil: beta-myrcene, linalool, linalyl acetate, linalyl formate, trans-caryophyllen, alpha-terpineol, geranyl formate, germacrene, neryl acetate, geranyl acetate, neryl alcohol, geraniol, caryophyllene oxide, spathulenol (12), 1-oxoethiopinone (13)</td>
</tr>
<tr>
<td>12</td>
<td><em>Scrophularia striata</em></td>
<td>Anti-inflammatory (6) matrix metalloproteinases inhibitor (11)</td>
<td>Cinnamic acid, three flavonoids (quercetine, isorhamnetin-3-O-rutinoside and nepitrin) and one phenyl propanoid glycoside (acteoside 1) (20)</td>
</tr>
<tr>
<td>13</td>
<td><em>Stachys pilifera</em></td>
<td>—</td>
<td>trans- verbenol, cis- chrysanthemyl acetate (18)</td>
</tr>
</tbody>
</table>
filtrate was concentrated, the residue of the plant was treated with chloroform for another 24 h for the chloroform fraction to be prepared (CF), and the steps were repeated for methanol fraction. The concentrated fractions were then subjected to the cytotoxicity assay.

Preparation of Extracts and Fractions for MTT Assay

The extracts and fractions were dissolved in dimethyl sulfoxide (DMSO): 10 mg.mL$^{-1}$ of each sample to make stock solutions. Serial dilutions were prepared accordingly from the above stock solution to get final concentrations with DMSO not exceeding 1%.

Cell Lines

MCF7 (human breast adenocarcinoma), HepG2 (hepatocellular carcinoma), MDBK (bovine kidney cells), and A549 (non-small-cell line carcinoma) cells were obtained from Pasteur Institute, Tehran, Iran. MCF7 cells were cultured in Dulbecco’s modified eagle medium (DMEM; Gibco) with 5% fetal bovine serum (FBS; Gibco) while the other three cell lines were cultured in RPMI 1640 medium (Sigma) with 10% FBS to maintain the desired growth. All cell lines were treated with 1% penicillin-streptomycin (Sigma) in a humidified incubator at 37°C in an atmosphere of 5% CO$_2$. The growth curve of each cell line was assessed.

MTT Assay

Cell viability was assessed in a micro-culture tetrazolium/formazan assay (MTT assay), as given earlier (3) with some modifications (21,22,26) in the absence and presence of different concentrations of the compounds. The cells were seeded in 96-well plates at $11 \times 10^3$ for MDBK cells, $9 \times 10^3$ for MCF7, $15 \times 10^3$ for HepG2, and $8 \times 10^3$ for A549 cells and incubated at 37°C. After 24 h, the medium was replaced with fresh medium containing concentrations of compounds to be tested at (in µg.mL$^{-1}$) 100, 50, 25, 12.5, 6.25, and 3.125. After 72-h exposure of cells at 37°C to each extract, the medium was replaced with fresh medium containing MTT ([3-(4,5-dimethylthiazol-2-yl)-2,4-diphenyltetrazolium bromide]; Sigma), with a final concentration of 0.5 mg.mL$^{-1}$. The cells were incubated for another 4 h in a humidified atmosphere at 37°C, then the medium containing MTT was removed, and the remaining formazan crystals were dissolved in DMSO. The absorbance was recorded at 570 nm with an enzyme-linked immunosorbent assay reader (TECAN), using Tamoxifen (22) as positive control.

The relative cell viability (%) related to control wells was calculated by $[A]_{\text{samples}} / [A]_{\text{control}} \times 100$ where $[A]_{\text{samples}}$ is the absorbance of test sample and
\[ A_{\text{control}} \] is the absorbance of wells containing cells, cell culture medium, and DMSO 1%. The dose-response curves were graphed by the Microsoft Excel program, and IC$_{50}$ values were assessed.

**RESULTS**

Nineteen samples (16 plant extracts and 3 fractions of *Dorema aucheri*) were evaluated for cytotoxic activity. Extracts with IC$_{50}$ > 100 µg.mL$^{-1}$ in MTT assay were considered inactive (all but *D. aucheri* extract). *In vitro* bioassay evaluation demonstrated that *D. aucheri* MeOH extract exhibited cytotoxic activity in HepG2 (IC$_{50}$ = 20.09 µg.mL$^{-1}$) and A549 cells (IC$_{50}$ = 48.65 µg.mL$^{-1}$); fractions of *D. aucheri* (petroleum ether fraction and chloroform fraction) showed cytotoxic activity in all four cell lines while fractionation with methanol had no cytotoxic activity against any of the cell lines (Figure 1).

**DISCUSSION**

Of all the plant species examined, *D. aucheri* tuber extract and fractions demonstrated cytotoxic activity. Although cytotoxic diterpenes have been reported from *Daphne mucronata* (17,39), its extract showed no cytotoxic activity with the concentrations tested for MTT assay in this study. More investigations with higher concentrations of the extract might show cytotoxic activity. The essential oil of *Salvia sclarea* had been reported to have cytotoxic activity (14) but the extract was not cytotoxic in this study. This

**FIGURE 1** The IC$_{50}$ values of *Dorema aucheri* methanol extract and fractions in petroleum ether (PF), chloroform (CF), and methanol (MF) against MCF7, HepG2, MDBK and A549 cells.
study identified non-cytotoxicity of a majority of plant extracts used in traditional healing in the Kohgiluyeh and Boyerahmad province in Iran and suggested groundwork for evaluating the biologic activity of the traditional use of plant species.

REFERENCES


