Brine Shrimp Lethality Bioassay of *Fumaria Densiflora* Dc. and *Fumaria Officinalis* L. Extracts

**Tuğçe Fafal Erdoğan**

*Introduction*

*Fumaria* species have been used in traditional medicine as antihypertensives, diuretics, hepatoprotectants and laxatives (to treat gastrointestinal disorders), as well as in the treatment of raches and conjunctivitis. The biological activity of *Fumaria* is mostly associated with the presence of isoquinoline alkaloids in the plant. In the last few years, a large number of scientific reports have been described the properties of *Fumaria*. There are seventeen wild-growing species belonging to this genus in Turkey.

The extracts of *F. officinalis* L. have been used in traditional medicine for varied purposes treatment of digestive problems, certain metabolic diseases, liver disorders and to purify blood.

Phytochemical investigation revealed the presence of several alkaloids such as adlumidine, coticine, fumariline, perfumine, protopine, fumaranine, fumarline, paprafumicin and paprarine. The plant has also been evaluated pharmacologically and shown to possess antihelmintic, antipyretic and hypoglycemic properties.

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The studies on the activity of some *Fumaria* species are shown in Table I.

<table>
<thead>
<tr>
<th>SPECIES</th>
<th>PHARMACOLOGICAL ACTIVITIES</th>
<th>NUMBER OF LITERATURE</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Fumaria indica</em> Pugsley</td>
<td>Central nervous system depressant</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td>Hypoglycemic</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td>Antiparasitic</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td>Antihepatotoxic</td>
<td>25, 26</td>
</tr>
<tr>
<td></td>
<td>Antispasmodic</td>
<td>26</td>
</tr>
<tr>
<td><em>F. parviflora</em> Lam.</td>
<td>Antispasmodic, antihypertensive, antiarrhythmic</td>
<td>23</td>
</tr>
<tr>
<td><em>Fumaria sp.</em></td>
<td>Antimicrobial</td>
<td>28</td>
</tr>
<tr>
<td><em>F. vaillantii</em> Loisel.</td>
<td>Antihypertensive</td>
<td>29</td>
</tr>
<tr>
<td></td>
<td>Hepatoprotective</td>
<td>30</td>
</tr>
</tbody>
</table>

TABLE I
Some studies on the activity of *Fumaria* species
In this study, the cytotoxic activity of the extracts of Herba Fumariae densiflorae and Herba Fumariae officinalis were evaluated by the *Artemia salina* (brine shrimp) lethality bioassay\(^9,10\).

**Material and Methods**

**Plant Materials**

The *Fumaria densiflora* DC. was collected from West Anatolia in İzmir-Kemalpaşa, in April 1999 and the *F. officinalis* L. was collected from middle Anatolia in Burdur-Bağsaray, in May 1999, and identified by M. Ali Önür. Voucher specimens *F. densiflora* DC. (No. 1239), *F. officinalis* L. (No. 1242) are deposited in the herbarium of the Department Pharmacognosy of the Faculty of Pharmacy in Ege University in İzmir.

**Preparation of Plant Extracts**

Air-dried and powdered plant materials were extracted with n-hexane, ethyl acetate, ethanol, methanol, and water (infusion) at room temperature; the extracts were evaporated to dryness in vacuo (60 °C) and weighed.

**Cytotoxic Studies**

Cytotoxicity was evaluated by the brine shrimp lethality bioassay (24). Sea salt (3.8 g) was dissolved in 100 ml water and filtered. Brine shrimp (*Artemia salina*) (San-Fransisko Bay Brand Inc., Newark, CA 94560, USA) eggs were placed into the water and left to incubate for 48 h at 28° in a small tank (Otsuka Pharmaceutical Co. Ltd., Tokyo, Japan). Each extract was tested at 1000, 100 and 10 ppm. Then 20 mg of plant extract was dissolved in 2 ml chloroform (20 mg/2 ml). From this solution 500, 50 or 5 μl was transferred to vials corresponding to 1000, 100 or 10 ppm, respectively. Vials including chloroform and extraction solvents (500μl) were prepared as controls. After incubation, 10 brine shrimp larvae (nauplii) were introduced into vials containing graded concentrations (ranging from 10 to 1000 ppm) of the extracts.
After 24 h, the number of surviving shrimps at each concentration of the extracts were counted and data analyzed with the Finney computer program to determine the LC$_{50}$ at a 95 % confidence interval. Sea salt (Sigma 9883) was used in activity tests. The cytotoxic activity of all extracts was compared with umbelliferone and colchicine as the activity cytotoxic substances$^{25, 26}$.

**Results and Discussion**

The cytotoxic activity of n-hexane, ethanol, methanol, ethyl acetate and water extracts of Herba *Fumariae densiflorae* and Herba *Fumariae officinalis* were investigated *in vitro* against the brine shrimp. The results are given in Table II.

**TABLE II**

<table>
<thead>
<tr>
<th>PLANT</th>
<th>EXTRACTS</th>
<th>CONCENTRATION (ppm)</th>
<th>LC$_{50}$ (μg/ml)</th>
<th>SD (%) (n=3)</th>
<th>% Capacity</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>F. densiflora</em> DC.</td>
<td>n-hexane</td>
<td>1000:100:10</td>
<td>741.61</td>
<td>0.88</td>
<td>% 0.93</td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>1000:100:10</td>
<td>854.41</td>
<td>0.41</td>
<td>% 1.41</td>
<td></td>
</tr>
<tr>
<td>Ethanol</td>
<td>1000:100:10</td>
<td>&gt;1000</td>
<td>0.39</td>
<td>% 3.12</td>
<td></td>
</tr>
<tr>
<td>Methanol</td>
<td>1000:100:10</td>
<td>&gt;1000</td>
<td>0.01</td>
<td>% 4.13</td>
<td></td>
</tr>
<tr>
<td>Water</td>
<td>1000:100:10</td>
<td>&gt;1000</td>
<td>0.05</td>
<td>% 2.89</td>
<td></td>
</tr>
<tr>
<td><em>F. officinalis</em> L.</td>
<td>n-hexane</td>
<td>1000:100:10</td>
<td>901.24</td>
<td>0.08</td>
<td>% 0.79</td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>1000:100:10</td>
<td>&gt;1000</td>
<td>0.67</td>
<td>% 2.05</td>
<td></td>
</tr>
<tr>
<td>Ethanol</td>
<td>1000:100:10</td>
<td>&gt;1000</td>
<td>0.46</td>
<td>% 4.02</td>
<td></td>
</tr>
<tr>
<td>Methanol</td>
<td>1000:100:10</td>
<td>&gt;1000</td>
<td>0.54</td>
<td>% 5.21</td>
<td></td>
</tr>
<tr>
<td>Water</td>
<td>1000:100:10</td>
<td>&gt;1000</td>
<td>0.98</td>
<td>% 4.75</td>
<td></td>
</tr>
<tr>
<td><em>Umbelliferon</em></td>
<td>500:50:5</td>
<td>377.02</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Kolsispin</em></td>
<td>500:50:5</td>
<td>0.0009</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
The brine shrimp bioassay was used as an indicator for general toxicity and also as a guide for the detection of antitumor and pesticidal compounds. Protopine, the most important alkaloid of *Fumaria* species, which was previously found to be cytotoxic, could be responsible for the observed brine shrimps lethality activities of the n-hexane and ethyl acetate extracts.

The n-hexane and ethyl acetate extracts of *Fumaria densiflora* DC. and the n-hexane extract of *F. officinalis* L. showed cytotoxic activity against the brine shrimp. These extracts were toxic (LC$_{50}$ < 1000) in the brine shrimp bioassay. Water extracts showed no cytotoxic activity. The obtained results provide the safety of the water extracts of Herba Fumariae officinalis and Herba Fumariae densiflorae in traditional uses.

**Summary**

**Brine Shrimp Lethality Bioassay of Fumaria densiflora DC. and Fumaria officinalis L. Extracts**

The cytotoxic activities of Herba Fumariae officinalis and Herba Fumariae densiflorae prepared from *Fumaria officinalis* L., growing wildly in Bağsaray (Burdur) and *Fumaria densiflora* DC., growing wildly in Kemalpaşa town Uluçak village (İzmir), respectively were determined. LC$_{50}$ value of n-hexane, ethyl acetate, ethanol, methanol and water extracts of the plant materials were determined using brine shrimp (*Artemia salina*) lethality bioassay. The n-hexane and ethyl acetate extracts of *Fumaria densiflora* DC. and the n-hexane extract of *F. officinalis* L. showed cytotoxic activity against the brine shrimp.

**Key Words:** Fumaria officinalis L., Fumaria densiflora DC., Brine Shrimp, Cytotoxic activity
Özet

**Fumaria densiflora DC. ve Fumaria officinalis L. Ekstrelerinin Brine Shrimp Yöntemiyile Sitotoksik Aktivite Çalışmaları**

Bağsaray (Burdur)'dan toplanan *Fumaria officinalis* ten hazırlanan Herba Fumariae officinalis ve Kemalpaşa ilçesi Ulucak köyü (İzmir)'den toplanan *Fumaria densiflora* DC. bitkisinden hazırlanan Herba Fumariae densiflorae droglarından hazırladığımız n-hegzan, etil asetat, etanol, metanol ve su ekstreleri üzerinde brine shrimp (*Artemia salina*) yöntemi ile sitotoksik aktivite tayini yapılmış ve LC50 değerleri hesaplanmıştır. *F. densiflora* DC.’dan elde edilen n-hegzan ve etil asetat ekstreri ile *F. officinalis* L.’den elde ettğimiz n-hegzan ekstresi sitotoksik aktivite göstermiştir.

*Anahtar Kelimeler:* Fumaria officinalis L., Fumaria densiflora DC., Brine Shrimp, Sitotoksik aktivite

**REFERENCES**

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