

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

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Introduction

Fumaria species have been used in traditional medicine as anti-hypertensives, diuretics, hepatoprotectants and laxatives (to treat gastrointestinal disorders), as well as in the treatment of rashes and conjunctivitis^{1,2}. 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*^(3,4). There are seventeen wild-growing species belonging to this genus in Turkey^{5,6}.

The extracts of *F. officinalis* L. have been used in traditional medicine for varied purposes treatment of digestive problems⁷, certain metabolic diseases⁸⁻¹¹, liver disorders^{12,13} and to purify blood¹⁴⁻¹⁶.

Phytochemical investigation revealed the presence of several alkaloids such as adlumidicine, copticine, fumariline, perfumine, protopine¹⁷, fumaranine, fumaritine, paprafumicin and paprarine¹⁸. The plant has also been evaluated pharmacologically and shown to possess antihelmintic¹⁹, antipyretic²⁰ and hypoglycemic²¹ properties.

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

TABLE I
Some studies on the activity of *Fumaria* species

SPECIES	PHARMACOLOGICAL ACTIVITIES	NUMBER OF LITERATURE
<i>Fumaria indica</i> Pugsley	Central nervous system depressant	22
"	Hypoglycemic	21
"	Antiparasitic	22
"	Antihepatotoxic	25, 26
"	Antispasmodic	26
<i>F. parviflora</i> Lam.	Antispasmodic, antihypertensive, antiarthmic	23
<i>Fumaria</i> sp.	Antimicrobial	28
<i>F. vaillantii</i> Loisel.	Antihypertensive	29
"	Hepatoprotective	30
<i>F. asepele</i> Boiss., <i>F. capreolata</i> L., <i>F. cilicica</i> Hauskkn., <i>F. densiflora</i> DC., <i>F. judaica</i> Boiss., <i>F. kralikii</i> Jordan, <i>F. parviflora</i> Lam.	Acetylcholinesterase inhibitory activity	31
<i>F. vaillantii</i> Loiss., <i>F. capreolata</i> L., <i>F. kralikii</i> Jordan, <i>F. asepele</i> Boiss., <i>F. densiflora</i> DC., <i>F. macrocarpa</i> Boiss., <i>F. parviflora</i> Lam., <i>F. judaica</i> Boiss.	Antiviral, antimicrobial	32

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 LC₅₀ 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

LC₅₀ values of extracts of *Fumaria densiflora* DC. and *F. officinalis* L. on brine shrimp lethality bioassay

PLANT	EXTRACTS	CONCENTRATION(ppm)	LC ₅₀ (µg/ml)	SD (%) (n=3)	% Capacity
<i>F. densiflora</i> DC.	n-hexane	1000:100:10	741.61	0.88	% 0.93
	Ethyl acetate	1000:100:10	854.41	0.41	% 1.41
	Ethanol	1000:100:10	>1000	0.39	% 3.12
	Methanol	1000:100:10	>1000	0.01	% 4.13
	Water	1000:100:10	>1000	0.05	% 2.89
<i>F. officinalis</i> L.	n-hexane	1000:100:10	901.24	0.08	% 0.79
	Ethyl acetate	1000:100:10	>1000	0.67	% 2.05
	Ethanol	1000:100:10	>1000	0.46	% 4.02
	Methanol	1000:100:10	>1000	0.54	% 5.21
	Water	1000:100:10	>1000	0.98	% 4.75
<i>Umbelliferon</i>		500:50:5	377.02		
<i>Kolşisin</i>		500:50:5	0.0009		

The brine shrimp bioassay was used as an indicator for general toxicity and also as a guide for the detection of antitumor and pesticidal compounds³³. Protopine, the most important alkaloid of *Fumaria* species, which was previously found to be cytotoxic³⁶, could be responsible for the observed brine shrimp 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 Ulucak 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 Yöntemiyle Sitotoksik Aktivite Çalışmaları**

Bağsaray (Burdur)' dan toplanan *Fumaria officinalis*' ten hazırlanan Herba Fumariae officinalis ve Kemalpaşa ilçesi Ulucak köyü (İzmir)' nden 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 ekstresi ile *F. officinalis* L.' den elde ettiğimiz n-hegzan ekstresi sitotoksik aktivite göstermiştir.

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

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