

BRINE SHRIMP LETHALITY BIOASSAY ON SOME *RANUNCULUS* SPECIES

BAZI *RANUNCULUS* TÜRLERİ ÜZERİNDE BRINE SHRIMP YÖNTEMİYLE SİTOTOKSİK AKTİVİTE ÇALIŞMALARI

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ABSTRACT

The cytotoxic activity of *Ranunculus pedatus* Waldst.&Kit. subsp. *pedatus* growing wildly in *Spil* (Manisa), *R. constantinopolitanus* DC. growing wildly in Yamanlar (İzmir), *R. isthmicus* Boiss. subsp. *tenuifolius* growing wildly in Bozdağ (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, ethyl acetate, methanol and water extracts of *R. pedatus* Waldst.&Kit. subsp. *pedatus*, the *n*-hexane, ethyl acetate and water extracts of *R. constantinopolitanus* DC. and the *n*-hexane, ethanol and water extracts of *R. isthmicus* Boiss. subsp. *tenuifolius* showed cytotoxic activity against the Brine shrimp.

Key Words: *Ranunculus pedatus* Waldst.&Kit. subsp. *pedatus*, *R. constantinopolitanus* DC., *R. isthmicus* Boiss. subsp. *tenuifolius*, Brine shrimp, Cytotoxic activity

ÖZET

Spil (Manisa)' dan toplanan *Ranunculus pedatus* Waldst.&Kit. subsp. *pedatus*, Yamanlar (İzmir)' den toplanan *R. constantinopolitanus* DC. ve Bozdağ (İzmir)' den toplanan *R. isthmicus* Boiss. subsp. *tenuifolius* bitki örneklerinden hazırlanan *n*-hekzan, etil asetat, metanol, etanol ve su ekstreleri üzerinde Brine shrimp (*Artemia salina*) yöntemi ile sitotoksik aktivite tayini yapılmış ve LC_{50} değerleri hesaplanmıştır. *R. pedatus* Waldst.&Kit. subsp. *pedatus*' un *n*-hekzan, etil asetat, metanol ve su ekstreleri, *R. constantinopolitanus* DC.' nin *n*-hekzan, etil asetat ve su ekstreleri ve *R. isthmicus* Boiss. subsp. *tenuifolius*' un *n*-hekzan, etanol ve su ekstreleri sitotoksik aktivite göstermiştir.

Anahtar Kelimeler: *Ranunculus pedatus* Waldst.&Kit. subsp. *pedatus*, *R. constantinopolitanus* DC., *R. isthmicus* Boiss. subsp. *tenuifolius*, Brine shrimp, Sitotoksik aktivite

INTRODUCTION

Ranunculus species have been used in traditional medicine as wound healing (1), antihemoroidal (2, 3, 4), maturation of abscess (5, 6), treatment of jaundice (6) and against rheumatism (5). *Ranunculus*, with some 600 species, is the genus with the greatest richness within the Ranunculaceae family (7). There are 85 wild-growing species belonging to this genus in Turkey (8, 9). *Ranunculus* species are known as “basur otu, düğün çiçeği, katırnalı” in our country (10).

This genus have previously been investigated from its flavonoids, saponins and alkaloids (11, 12, 13, 14). Phytochemical investigation revealed the presence of several flavonoids such as quercetin, kaempferol, vitexin, isovitexin, orientin, isoorientin (15), alkaloids such as berberine, columbamine, magnoflorine, palmatine (11), anthocyanins such as delphinidin and cyanidin (16), organic acids such as lactic acid (17), methylparaben, *p*-coumaric acid (18) and triterpenes such as betulinic acid, oleanolic acid and ursolic acid (19).

The studies on the activity of some *Ranunculus* species are shown in Table 1.

Table 1. Some studies on the activity of *Ranunculus* species

SPECIES	PHARMACOLOGICAL ACTIVITIES	NUMBER OF LITERATURE
<i>R. ficaria</i> L.	Cytotoxic	20
"	Antihemoroidal, antibacterial, antifungal	3
<i>R. sceleratus</i> L.	Antienflamatuar	14
"	Antifungal	21
"	Antiparasitic	22
<i>R. bulbosus</i> L.	Antifungal	22
<i>R. tricophyllus</i> Tausch.	Antiprotozoal	23

In this study, the cytotoxic activity of extracts of some *Ranunculus* species were evaluated by *Artemia salina* Leach. (Brine shrimp) lethality bioassay (24, 25).

MATERIALS AND METHODS

Plant Materials

The *R. pedatus* Waldst.&Kit. subsp. *pedatus* was collected from Manisa-Spil, the *R. constantinopolitanus* DC. was collected from Yamanlar-İzmir and *R. isthmicus* Boiss. subsp. *tenuifolius* was collected Bozdağ-İzmir. All of them were collected in May 2005 and identified by M. Ali Önür. Voucher specimens *R. pedatus* Waldst.&Kit. subsp. *pedatus* (No. 1364), *R. constantinopolitanus* DC. (No. 1349), *R. isthmicus* Boiss. subsp. *tenuifolius* (No. 1345) 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 then weighed.

Cytotoxic Studies

Cytotoxicity was evaluated by the Brine shrimp lethality bioassay (25). 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 °C 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 (500 µl) 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 (26, 27).

RESULTS AND DISCUSSION

The cytotoxic activity of n-hexane, ethanol, methanol, ethyl acetate and water extracts of *R.pedatus* Waldst.&Kit. subsp. *pedatus*, *R. constantinopolitanus* DC. and *R. isthmicus* Boiss. subsp. *tenuifolius* were investigated *in vitro* against the brine shrimp. The results are given in Table2.

Table 2. LC₅₀ values of extracts of *R. pedatus* Waldst.&Kit. subsp. *pedatus*, *R. constantinopolitanus* DC. and *R. isthmicus* Boiss. subsp. *tenuifolius* on brine shrimp lethality bioassay

PLANT	EXTRACTS	CONCENTRATION (ppm)	LC ₅₀ (µg/ml)	SD (%) (n=3)	% CAPACITY
<i>R. pedatus</i> Waldst.&Kit. subsp. <i>pedatus</i>	n-hexane	1000:100:10	185.84	0.98	% 0.92
	Ethyl acetate	"	741.61	0.57	% 2.97
	Ethanol	"	>1000	0.72	% 5.12
	Methanol	"	362.77	0.67	% 9.32
	Water	"	395.16	0.46	% 11.07
<i>R. constantinopolitanus</i> DC.	n-hexane	"	485.49	0.54	% 0.89
	Ethyl acetate	"	951.08	0.48	% 2.10
	Ethanol	"	>1000	0.87	% 6.42
	Methanol	"	>1000	0.77	% 10.07
	Water	"	178.23	0.35	% 18.77
<i>R. isthmicus</i> Boiss. subsp. <i>tenuifolius</i>	n-hexane	"	524.98	0.42	% 0.68
	Ethyl acetate	"	>1000	0.64	% 1.49
	Ethanol	"	756.39	0.36	% 5.29
	Methanol	"	>1000	0.58	% 13.64
	Water	"	287.09	0.47	% 16.25
<i>Umbelliferon</i>		500:50:5	377.02		
<i>Colchicine</i>		"	0.0009		

The phytochemical studies on various species of genus *Ranunculus* have reported that they contain flavonoids (quercetin, kaempferol, vitexin, isovitexin, orientin, isoorientin (15)), alkaloids (berberine, palmatine, columbamine, magnoflorine (11)), triterpenes (ursolic acid, oleanolic acid, betulonic acid (19)).

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 (24). Vitexin and isovitexin were previously found to be cytotoxic (28). Cytotoxic effect of berberine was determined against human tumor cell lines and it presented interesting cytotoxicity (29). Ursolic acid showed moderate cytotoxicities against human cancer lines (30).

Vitexin, isovitexin, ursolic acid or berberine could be responsible for the observed Brine shrimp lethality activities of some extracts. The n-hexane, ethyl acetate, methanol and water extracts of *R. pedatus* Waldst.&Kit. subsp. *pedatus*, the n-hexane, ethyl acetate and water extracts of *R. constantinopolitanus* DC. and the n-hexane, ethanol and water extracts of *R. isthmicus* Boiss.

subsp. *tenuifolius* showed cytotoxic activity against the Brine shrimp. These extracts were toxic ($LC_{50} < 1000$) in the Brine shrimp bioassay. The lethality of these extracts to Brine shrimp is indicative of the presence in these plants of a potent cytotoxic component which warrants further investigation.

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