

In vitro Cytotoxic Activity of *Sternbergia sicula*, *S. lutea* and *Pancratium maritimum* Extracts

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Introduction

Sternbergia Waldst & Kit. (winter daffodil) is a genus of bulbous monocotyledons belonging to the family Amaryllidaceae ¹. The taxonomical classification of the genus *Sternbergia* has not been very well clarified in the literature. *S. lutea* (L.) Ker-Gawl ex Sprengel was first described as *Amaryllis lutea* by Linnaeus, and then reassigned to *Sternbergia* by Sprengel. *S. sicula* was described by Tineo ex Guss. ^{1,2}. Based on the high level of morphological similarities between these two species *S. sicula* is recorded as *S. lutea* subsp. *sicula* in Flora Europea ³. Duman et al. ⁴, also, stated that *S. sicula* Tineo ex Guss. should be regarded as a subspecies of *S. lutea*. However, in the CITES bulb checklist ² and Flora of Turkey ³ *S. sicula* and *S. lutea* are classified as two different species. Regardless of whether *S. sicula* is a species or a subspecies of *S. lutea*, in this paper it will be referred as *S. sicula*. This species is widespread throughout Italy, Sicily, Greece, Aegean and East Mediterranean. *S. lutea* grows wildly in the Mediterranean area, Iran, Iraq and Russia.¹ Also, *S. schubertii* Schenk, known only from the type,

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is recorded as a synonym of *S. lutea*.⁴ In this context, six wild-growing species of this genus, *S. lutea*, *S. sicula*, *S. fischeriana* (Herbert) Rupr., *S. colchiciflora* Waldst & Kit, *S. clusiana* (Ker-Gawl.) Ker-Gawl. ex Sprengel and an endemic species, *S. candida* B. Mathew & T. Baytop, are distributed in Turkey^{1,2}.

The genus *Pancratium* L. includes about 15 species distributed throughout the Mediterranean, tropical Asia and tropical Africa⁵. *Pancratium maritimum* L., with white flowers and very large bulbs, is the only wild growing species of this genus in Turkey⁶.

Studies on *Sternbergia* and *Pancratium* species yielded compounds belonging to the skeletally different groups of Amaryllidaceae alkaloids⁷⁻¹¹. Moreover, a new mannose-specific lectin was isolated from *S. lutea* bulbs¹². Amaryllidaceae alkaloids have been shown to possess important biological activities including antitumor, antiviral and acetylcholinesterase inhibitory activity¹³⁻¹⁵. Among these alkaloids, galanthamine is used in the treatment of Alzheimer's Disease¹⁶. Also, analgesic and antimicrobial activities have been reported for extracts and alkaloids from *S. clusiana*, *S. sicula* and *S. lutea*^{17,18}.

In the present study, the cytotoxic activity of the extracts prepared from *S. sicula*, *S. lutea* and *P. maritimum* were determined by the brine shrimp (*Artemia salina* Leach.) lethality bioassay.

Material and Methods

Plant Material

S. lutea was collected from Çine (Aydın), *S. sicula* from Söke (Aydın) and *P. maritimum* from Pamucak-Kuyucak (Aydın) during flowering season. The plants were identified by Prof. M. Ali Onur from the Department of Pharmacognosy, Faculty of Pharmacy, Ege University, Izmir (Turkey). Voucher samples of *S. lutea* (No. 1292), *S. sicula* (No. 1388) and *P. maritimum* (No. 1294) are deposited in the Herbarium of the Department of Pharmacognosy, Faculty of Pharmacy, Ege University.

Preparation of Plant Extracts

n-Hexane, ethyl acetate, ethanolic and aqueous extracts were separately prepared from 20 g batches of the air-dried and powdered aerial

parts of the plant by percolation at room temperature. The same procedure was repeated for the preparation of the corresponding extracts from the bulbs. The evaporation of the solvents *in vacuo* furnished twenty-four different extracts from three species (Table I).

Brine shrimp Lethality Bioassay

The bioassay was conducted following the procedure published previously^{19, 20}. Brine shrimp (*Artemia salina* Leach) eggs (San Francisco Bay Brand, Inc. Newark, CA94560 USA) were used. Seawater was prepared by dissolving 3.8 g sea salt (Sigma-9883) in 100 ml of distilled water and put in a small plastic hatching container with perforated dividing dam (Otsuka Pharmaceutical Co. Ltd., Tokyo, Japan). A 40-W lamp was positioned near the container to provide direct light and heat (~27-28 °C). Brine shrimp eggs were placed in seawater and 48h was allowed for the shrimp to mature as nauplii. 10 mg of extract was dissolved in 2 ml of the solvent used in the extraction to prepare a 5 mg/ml stock solution. Then, 500, 50 and 5 ppm solutions were prepared by dilution and placed in vials. Also, a vial containing only the solvent was prepared for control. The solvents of the extracts were allowed to evaporate. Then, a suspension of nauplii was removed and 10 nauplii were transferred into each vial and the volume was adjusted to 5 ml by adding the same saline solution. Vials were incubated for 24h at room temperature under illumination. Three replicates were prepared for each concentration and experiments were performed in duplicate. After 24h, numbers of live nauplii were counted. LC₅₀ values after 24h exposure and 95 % confidence intervals were determined by using the Finney Computer programme. Colchicine was used as a reference substance at the concentrations of 500, 50 and 5 ppm.

Results and Discussion

The cytotoxic activity of n-hexane, ethyl acetate, ethanolic and aqueous extracts of bulbs and aerial parts of *S. sicula*, *S. lutea* and *P. maritimum* were investigated *in vitro* against the brine shrimp. Results are reported in Table I.

Some of the extracts (LC₅₀ < 1000) were found to be active in the brine shrimp lethality bioassay. All of the ethanolic extracts of the bulbs showed significant activity. However, none of the extracts prepared with

Table I
LC50 values for the extracts of bulbs and aerial parts of *S. sicula*, *S. lutea* and *P.maritimum*

PLANT	MATERIAL	EXTRACTS (yield %)	CONCENTRATION (ppm)	LC ₅₀ (µg/ml)
<i>S. sicula</i>	Bulbs	<i>n</i> -hexane (0.7)	500:50:5	574.66
		ethyl acetate (1.2)	500:50:5	182.06
		ethanol (6.9)	500:50:5	148.97
		Water (26.5)	500:50:5	212.51
	Aerial Parts	<i>n</i> -hexane (1.1)	500:50:5	>1000
		ethyl acetate (2.4)	500:50:5	>1000
		ethanol (8.6)	500:50:5	>1000
		Water (38.6)	500:50:5	>1000
<i>S.lutea</i>	Bulbs	<i>n</i> -hexane (0.6)	500:50:5	>1000
		ethyl acetate (1.2)	500:50:5	>1000
		ethanol (5.1)	500:50:5	126.84
		water (22.8)	500:50:5	>1000
	Aerial Parts	<i>n</i> -hexane (1.1)	500:50:5	>1000
		ethyl acetate (2.1)	500:50:5	>1000
		ethanol (12.1)	500:50:5	>1000
		water (69.9)	500:50:5	415.02
<i>P. maritimum</i>	Bulbs	<i>n</i> -hexane (0.9)	500:50:5	>1000
		ethyl acetate (1.5)	500:50:5	>1000
		ethanol (11.7)	500:50:5	482.00
		water (39.3)	500:50:5	360.85
	Aerial Parts	<i>n</i> -hexane (1.5)	500:50:5	>1000
		ethyl acetate (2.5)	500:50:5	>1000
		ethanol (10.5)	500:50:5	>1000
		water (33.7)	500:50:5	725.82
Colchicine			500:50:5	0.30

n-hexane, ethyl acetate and ethanol from the aerial parts of the three plant species, were active against *Artemia salina* Leach. Aqueous of *S. sicula* bulbs, *S. lutea* aerial parts and both parts of *P. maritimum* possessed significant activity. Among the investigated plant species, only all of the extracts prepared from the bulbs of *S. sicula* were shown to be active.

Brine shrimp lethality bioassay is a fast, simple and widely used method to determine the preliminary cytotoxicity of crude extracts and pure compounds. It is used for testing general toxicity and may be a predictor of effects on cancer cells.^{21,22} Many reports concerning the cytotoxic activity of Amaryllidaceae alkaloids are recorded in the literature^{23,24}. Lycorine, a widely distributed and a major alkaloid in Amaryllidaceae plants, has been proven to have various biological properties including cytotoxic activity^{25,26}. It has been isolated previously from *Sternbergia*^{9,10,27,28} and *P. maritimum*^{29,30}. Recently, the presence of this alkaloid has been shown in the tested species³¹. Therefore, together with other Amaryllidaceae alkaloids lycorine, may be responsible for the cytotoxic activity of the extracts. In addition, cytotoxic phenolic compounds (phenolic acids and flavonoids)^{32,33} isolated from *Sternbergia* species and from *P. maritimum*^{34,35} may contribute to the observed activity of the extracts.

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Summary

The cytotoxic activity of the n-hexane, ethyl acetate, ethanolic and aqueous extracts of the bulbs and aerial parts of *S.lutea* (L.) Ker-Gawl ex Sprengel, *S sicula* Tineo ex Guss. and *P. maritimum* L. were determined using the brine shrimp (*Artemia salina* Leach.) lethality bioassay. As a result, all of the ethanolic extracts of the bulbs showed significant activity. However, none of the n-hexane, ethyl acetate, ethanolic extracts of the aerial parts were active against *Artemia salina* Leach. The aqueous extracts of bulbs and aerial parts of *P. maritimum* were found to be active whereas aqueous extracts prepared from *S. sicula* bulbs and aerial parts of *S. lutea* showed significant activity.

Key Words: *S.lutea*, *S sicula*, *P. maritimum*, Brine Shrimp, Cytotoxic activity

Özet

***Sternbergia sicula*, *S. lutea* ve *Pancreatium maritimum*
Ekstrelerinin *In vitro* Sitotoksik Aktiviteleri**

S.lutea (L.) Ker-Gawl ex Sprengel, *S sicula* Tineo ex Guss. ve *Pancreatium maritimum* L. bitkilerinin soğanları ve toprak üstü kısımlarına ait n-hekzan, etilasetat, etanol ve su ekstrelerinin sitotoksik aktiviteleri brine shrimp (*Artemia salina* Leach.) yöntemi ile tayini edilmiştir. Sonuç olarak bütün bitkilerin soğanlarından hazırlanan etanol ekstreleri belirgin aktivite göstermiştir. Ancak bitkilerin toprak üstü kısımlarından hazırlanan n-hekzan, etilasetat ve etanol ekstrelerinin hiçbirinde aktivite bulunmamıştır. *P. maritimum* bitkisinin soğan ve toprak üstü su ekstreleri aktif çıkarken, *S. sicula* soğanları ve *S. lutea* toprak üstü kısımlarından hazırlanan su ekstreleri belirgin sitotoksik aktivite göstermiştir.

Anahtar Kelimeler: *S.lutea*, *S sicula*, *P. maritimum*, Brine shrimp, Sitotoksik aktivite

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