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Assessment of cytotoxic activity on alkaloid of *Amaryllidaceae* and *Papaveraceae* species

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Abstract:

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Eight species of *Amaryillidaceae* (*Stenbergia colchiciflora W. et K., Pancratium maritimum L.)* and *Papaveraceae (Corzdalis solida (L.) Swartz, Chelidonium majus L., Corydalis bulbosa (L.) DC, Fumaria officinalis L., Fumaria vaillanttiLoisel.)* were examined for cytotoxic activity. Twelve alkaloid fractions of mentioned above species were evaluated for cytotoxic action using brine shrimp (*Artemia salina*) lethality assay. Among the plants tested the extracts of S. colchiciflora had the highest activity. The order examined extracts based on their cytotoxic activity, could be ordered as follows: *C. bulbosa* (*bulbs*), *P. maritimum*, *C. solida* (*herba*), *G. flavum* (*root*), *Ch. majus* (*herba*), *C. solida* (*bulbs*). The rest extracts did not shou toxicity to brine shrimp.

Key words: *Amaryllidaceae*, *Papaveraceae*, cytotoxic activity, *Artemia salina*

Introduction

The species of the Amaryllidaceae and Papaveraceae biosynthesize pharmacologically active alkaloids, which are known to exhibit different pharmacological effects such as antitumor, anticancer, acetylcholinesterase inhibitory and cytological activities (Sener and Orhan 2005, Houghton et al., 2006). In the Bulgarian flora Amaryllidaceae family is represented by five genera from which *Galanthus* species and *Leucojum aestivum L.* are the most popular and used plants in the treatment of different neurological disorders such as Parkinson disease and etc. (Jordanov, 1964, Nikolov, 2006). Family Papaveraceae comprises seven genera with more than 30 species distributed in Bulgaria (Kuzmanov, 1970). Many of these species have been investigated in the search for novel cytotoxic compounds, but there is still a demand to find more information concerning the cytotoxic potential of the species (Colombo and Bosisio, 1996, Camacho et al., 2003, Sener and Orhan, 2005).

A number of studies have demonstrated the use of the brine shrimp larvae *Artemia salina* (nauplii) assay to screen plants having cytotoxic activity. The metod appears to be convenient, rapid, inexpensive and low toxic amounts are suffucuent to perform the test (Quignard et al., 2003, Wanyoike et al., 2004, Krishnaraju et al., 2005).

In the present study we assess the cytotoxic activity of twelve alkaloid extracts of eight species of *Amaryllidaceae* and *Papaveraceae* using Brine shrimp (*Artemia salina*) lethality assay.

Different plant parts – overground and underground of some species were tested separately for cytotoxic activity.

Materials and Methods

Plant material

Plants of *Amarylliadaceae* and *Papaveraceae* families were collected from different locations as indicated in Table 1.

Isolation of total alkaloid fractions

The dried and powdered plant material was extracted with 2 % H_2SO_4. After removing of the neutral compounds with basified with 25 % ammonia and the alkaloids were extracted with EtOAc tree times. The organic solvent was evaporated and the dry alkaloid fraction was dissolved in MeOH to check for occurrence on alkaloids.

Sample preparation

Total alkaloid fractions (50 mg) of examined species were first dissolved in 1 ml dimethylsulphoxide (DMSO). Serial dilutions were made to obtain four concentrations – 1000, 100, 10 and 1 µg/ml.

Preparation of saline solution

Artificial sea water (saline solution) was prepared by dissolving 20 g of sea salt in 1 l of destillated water.

Hatching of brine shrimp larvae

Brine shrump eggs (*Artemia salina*) were incubated at 27 ± 1˚C in a conical shaped vessel (1L), containing saline solution under constant aeration and an incandescent lamp for 24 h.

Brine shrimp lethality assay

After hatching, active brine shrimp larvae (nauplii) free from egg shell were collected with a pipette. Ten nauplii were introduced into vial containing 5 ml saline solution and graded concentrations (ranging from 1 to 1000 µg/ml) of the tested alkaloid fractions. The experiments were conducted along with control contain 5 ml saline solution, 0.1 ml DMSO and 10 nauplii. After 24 h, the number of affected or dead shrimps was counted at each concentration of the fractions. The data were analyze.

Results and discussion

Brine shrimp (*Artemia salina*) lethality assay of the twelve extracts revealed the occurrence of alkaloids with cytotoxic activity in the most samples. The values for cytotoxicity were expressed as LD_{50} (Table 2). The extracts of S. colchiciflora and C. bulbosa (bulbs) showed considerable toxicity against brine shrimp with LD_{50} values 120 µl/ml and 220 µl/ml respectively.

The alkaloid fractions of *P. maritimum*, *C. bulbosa* (bulbs) and *C. solida* (herba) exhibited moderate brine shrimp lethality / up to 500 µg/ml. Low cytotoxicity was shown by extracts of *Fumaria ssp., G. flavum* (herba), *Ch. majus* (root) and *C. bulbosa* (herba) did not show toxicity. The values of LD_{50} for these species are over 1000 µg/ml.

In the comparative analysis of the overground and underground parts of *C. bulbosa* considerable differences in the cytotoxic action were established. The extract of tuber possesses stronger cytotoxicity in comparison with the extract of the aerial parts. Miyazawa et al. (1998) who have examined insecticidal properties on extracts of aerial and tuber parts of C. bulbosa have found too that the extract of tuber has considerably stronger activity. May be are significant differences of the alkaloid composition in the different parts of this species and the alkaloids of the tubers possess higher toxicitu. It will be interesting to examine alkaloid composition in the different parts of C. bulbosa and this will be object of a future study.

The extracts of aerial and root parts of *Ch. majus* also exhibited differences in their activity but not as much as the extracts of *C. bulbosa*. There are data that the alkaloid fraction from the roots of Ch. majus differ from that of the aerial part both in total content and in the relative proportions of individual constituents. Chelidonine hass been isolated as the main component of the roots (up tp 1.4 % dry weight) together with sanguinarine and chelerythrine. The major alkaloid in the aerial part is comptisine (up to 1.10 % dry weight), while there is 20 times less sanguinarine and chelidonine than in the roots (Colmbo and Bosisio, 1996). This different chemical composition could be the reason for the different activity of the extracts. Similar is the situation with the extracts of G. flavum, but in this case more cytotoxic compounds are found in the roots. Calo et al. (1998) has found that the extract obtained from rest parts (stem, leaf and fruit pericarps). These data together with our results give us a reason to suppose that the most biological active compounds in *G. flavum* are found in the roots.

Conclusion

The significant lethality expressed by alkaloid fractions of *S. colchiciflora*, and *C. bulbosa* (bulbs) on brine shrimp is an indicator of the presence of potent cytotoxic component wich has to be investigated in the future.

Table 1. Plant species, location and voucher specimen

Plant species	Family	Location, district and year of collection	Voucher specimen, Herbarium; SOM
Sternbergia colchiciflora W. et K.	Amaryllidaceae	Ioglav, Lovech, Bulgaria (2005)	Co 3998
Pancratium maritimum L.	Amaryllidaceae	Sinemorets, Burgas, Bulgaria (2006)	Co 3997
Corydalis solida (L.) Swartz	Papaveraceae	Ljulin Mountain, Sofia region, Bulgaria (2006)	Co 1154
Chelidonium majus L.	Papaveraceae	Ljulin Mountain, Sofia Region, Bulgaria (2006)	Co 1152
Corydalis bulbosa (L.) DC	Papaveraceae	Lozenska Mountain, Sofia region, Bulgaria (2006)	Co 1156
Fumaria officinalis L.	Papaveraceae	Vitosha Mountain, Sofia, Bulgaria (2006)	Co 3996
Fumaria vaillantii Loisel.	Papaveraceae	Sofia, Bulgaria (2006)	Co 2999
Glaucium flavum Crantz	Papaveraceae	Sinemorest, Burgas, Bulgaria (2006)	Co 4000

Table 2. The mean LC_{50} values ± S.D. for alkaloid fractions screened against brine shrimp larvae

Sample No	Plant species	Organ	LC_{50}[a] ± S.D[b]. (µg/ml)
1	*Sternbergia colchiciflora*	herba	120±12
2	*Pancratium maritimum*	herba	448±9
3	*Corydalis solida*	herba	513±18
4		bulbs	895±21
5	*Chelidonium majus*	herba	870±12
6		radix	>1000
7	*Corydalis bulbosa*	herba	>1000
8		bulbs	220±10
9	*Fumaria officinalis*	herba	>1000
10	*Fumaria vaillantii*	herba	>1000
11	*Glaucium flavum*	herba	>1000
12		radix	717±16

a Lethal concentration for 50% of *Artemia salina* nauplii.
b mean of two measurements (10 nauplii per concentration; dead nauplii were counted).

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