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Evaluation of the Toxicity Activity of Bioactive Compounds of Some Geophytes against Brine Shrimp (*Artemia salina* L.)

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Research Article

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Abstract

Artemia salina L.is an important model organism for the world ecosystem, which is very sensitive, easily available, allows to work in the laboratory environment, is used in toxic effect studies, and gives safe results. This study was designed to evaluate the toxic effect of water extract of fresh and underground parts of some species of five geophyte genera (*Arum rupicola* var. *virescens* (Stapf) P.C. Boyce, *Cyclamen cilicium* Boiss. & Heldr, *Gagea bohemica* (Zauschn.) Schult. & Schult. f., *Narcissus tazetta* subsp. *tazetta* L., *Paeonia kesrouanensis* J. Thiébaut) against *A. salina*. All taxa were found to be more toxic in the fresh part than in the underground part. The fresh part of *P. kesrouanensis* was shown the highest toxic effect with 44.44 ± 2.78% (1.56 ± 0.13 mg/mL, LC_{50}) and the underground part of *N. tazetta* subsp. *tazetta* was shown the lowest toxic effect with 8.33 ± 0.00% (294.68 ± 1.54 mg/mL, LC_{50}). The underground part of *N. tazetta* subsp. *tazetta* and *Gagea bohemica* were not found toxic with > 1000 mg/ml, LC_{90}. Based on the studies showing that *A. salina* toxic effect studies are related to insecticide and anticancer studies, it is seen that especially the fresh part of *P. kesrouanensis* has potential insecticide and anticancer properties, but more studies are needed.

Keywords: Artemia salina (L.), Toxicity effect, *Narcissus tazetta* subsp. *tazetta, Paeonia kesrouanensis*

Bazı Geofit Türlerin Biyoaktif Bileşiklerinin Tuzlu Su Karidesi (Artemia salina)'ne Karşı Toksisite Aktivitesinin Değerlendirilmesi

Özet

Artemia salina L. çok hassas, kolay ulaşılabilir, laboratuvar ortamında *in vivo* çalışmaya olanak sağlayan, toksik etki çalışmalarında kullanılıp güvenli sonuçlar veren, dünya ekosistemi için önemli bir model organizmadır. Bu çalışma, geofit olan beş cinse ait bazı türlerin (*Arum rupicola* var. *virescens* (Stapf) P.C. Boyce, *Cyclamen cilicium* Boiss. & Heldr, *Gagea bohemica* (Zauschn.) Schult. & Schult. f., *Narcissus tazetta* subsp. *tazetta* L., *Paeonia kesrouanensis* J. Thiébaut) toprak üstü ve toprak altı kısımlarının su ekstraktının *A. salina*'ya karşı toksik etkisini değerlendirmek için tasarlanmıştır. Bütün taksonlarda toprak üstü kısım, toprak altı kısıma göre daha toksik olduğu bulunmuştur. *P. kesrouanensis*'in toprak üstü kısmı 44.44 ± 2.78% (1.56 ± 0.13 mg/mL, LC_{50}) değeri ile en yüksek toksik etkiyi gösterirken *N. tazetta* subsp. *tazetta*'nın toprak altı kısmı 8.33 ± 0.00% (294.68 ± 1.54 mg/mL, LC_{50}) değeri ile en düşük toksik etkiyi göstermiştir. *N. tazetta* subsp. *tazetta* ve *G. bohemica*'nın toprak altı kısmı > 1000 mg/ml, LC_{90} ile toksik bulunmamıştır. *A. salina* toksik etki çalışmalarının insektisit ve antikanser çalışmaları ile ilgili olduğunu gösteren çalışmalara dayanarak, özellikle *P. kesrouanensis*'in yer üstü kısmının potansiyel insektisit ve antikanser özelliklere sahip olduğu ancak daha fazla çalışmaya ihtiyaç duyulduğu görülmüştür.

Anahtar Kelimeler: Artemia salina (L.), Toksik etki, *Narcissus tazetta* subsp. *tazetta, Paeonia kesrouanensis*

INTRODUCTION

Bioactive phyto-compounds found in plants include anthocyanins, phenolic acids, catechins, tannins, etc. compounds with complex structures. These compounds, which are necessary for the reactions in the metabolism of the living organism, can cause adverse problems when poorly applied and the plant used may have a toxic effect and have a mutagenic or genotoxic potential (Tülay and Özlem, 2007; Almeida et al., 2020). Various experiments are used to investigate the potential toxicity of herbal extracts with *in vivo*

tests on laboratory animals designated as models (Carballo et al., 2002; Mayorga et al., 2010; Veni and Pushpanathan, 2014). Recently, numerous studies have focused on both the pharmacology and toxicity of the herbs used (Parra et al., 2001).

Artemia is a genus that can survive in high salinity rates (4.5-340%) belonging to the Artemiidae family (Başbuğ, 1999; GBIF, 2019[a]). It can survive and improve, especially in a hypertonic environment, and can be found all over the world. It is one of the few organisms that can survive in the world's most saltwater lakes with microalgae and certain types of bacteria (Asem et al., 2010; Johari et al., 2019; Sellami et al., 2020). It is highly preferred both scientifically and in aquaculture due to its structures that have good nutritional value, can be readily available, low cost, high reproduction rate, easy to cultivate, and are not easily affected by environmental conditions (McLaughlin et al., 1991; Yun et al., 2020). Brine shrimp (*Artemia salina*) Toxicity Assay (BSTA) method, which uses *A. salina* larvae as test animals, is a competent, fast, and low-cost simple biological bioassay test that requires a minimal amount of sample created by exposing the larvae to the test sample in saline solution (McLaughlin et al., 1991; Montanher et al., 2002; Hamrun et al., 2020). The method is easily understood and uses small amounts to analyze the content (Pisutthanan et al., 2013). This bioassay is a widely used method to search for novel anticancer compounds derived from plants, and it has been shown that toxicity test results are associated with the cytotoxic compounds of the anticancer with this method. This bioassay suitable correlates with its cytotoxic activity in some human solid tumors and pesticide, insecticidal activity (McLaughlin et al., 1998; Hamrun et al., 2020; Mughni and Yusop, 2020). BSTA made from plant extracts has been successfully used to biomonitor the isolation of cytotoxic (Siqueira et al., 1998), antimalarial (Perez et al., 1997), insecticidal (Oberlies et al., 1998), and antifeedant (Labbe et al., 1993) compounds. This bioassay also provides the forefront screen that can be backed up with a more detailed assay once the active compound has been isolated (Pisutthanan et al., 2013). In this study, the results of a screening of water extracts of five geophyte used for toxicity against *A. salina* larvae are presented.

MATERIALS and METHODS

Plant Materials and Extract Preparation

The plants were collected in 2018-2019 during the flowering period and were identified by Dr. Olcay Düşen and stored with herbarium number at PAMUH in Pamukkale University, Denizli, Turkey (Table 1). At room temperature, dried fresh part (leaves and fruits), underground part (bulb and root) cut into 5 mm x 5 mm cubes. 100 g of the cut samples and the solvent was added (acetone, methanol, or water). It was kept in a shaking water bath for 6 hours and filtered through Whatman paper, and the solvent was added again. After filtration, the alcohol and water were evaporated. Extracts were kept at -20 ^{o}C (Turan and Mammadov, 2020).

Table 1. Features of collected species of geophyte genera.

Plant Name	Locality	Altitude (m)	Date	Herbarium Number
Arum rupicola var. *virescens* (Plant List, 2012[a])	Tunceli Province	1230	June, 2019	PAMUH 1003 M. Turan
Cyclamen cilicium (Plant List, 2012[b])	Antalya Province	938	December, 2019	PAMUH 1004 M. Turan
Gagea bohemica (Zauschn.) (Plant List, 2012[c])	Denizli Province	777	March, 2019	PAMUH 1002 M. Turan
Narcissus tazetta subsp. *tazetta* (GBIF, 2019[b])	Muğla Province	6	February, 2019	PAMUH 1001 M. Turan
Paeonia kesrouanensis. (GBIF, 2019[c])	Burdur Province	1649	May, 2018	PAMUH 1700 L. Sevim

Brine Shrimp (*Artemia salina*) Toxicity Assay (BSTA)

The assay of investigating the toxic effects of extracts against *A. salina* was determined by modifying the Krishnaraju et al. (2005) method. Water extracts of plants were used in the experiment. Two days before the experiment, *Artemia* eggs sold commercially (Rotifish Artemia Mix, 18g) put in a jar

containing 500-600 mL of distilled water, and an air hose connected to the air motor is placed inside the jar for continuous air ventilation. The jar is placed in the aquarium filled with 25% water. The aquarium was kept bright at 28-29 ^{o}C. *Artemia* eggs were expected to hatch for two days. After two days, 0.5 M sea saline solution was prepared. The extract solution prepared with extracts in 4 different concentrations as 0.1, 0.25, 0.5 and 1 mg/mL was added to each test tube. By shining light from the bottom, 12 live *Artemia* were added to the test tubes, and test tubes are placed in a tube holder. The tube holder was placed in the aquarium kept lighting at a temperature of 28-29 ^{o}C. After 24 hours, each test tube was poured into a petri dish, and the *Artemia* that remained still for a long time and was certain to be lifeless were counted and noted. The test was considered valid if less than 10% of the control nauplii were immobile.

Statistical Analysis

All assays were performed in 3 replicates. The mean ± standard error was analyzed using Microsoft Excel, and the results were analyzed using the Statistical Package for Social Sciences (SPSS) statistical software (2017). Significant differences among groups were identified by one-way analysis of variance (ANOVA) with Duncan's multiple range test, setting $p < 0.05$ as the level of significance. $LC_{50(min)}$, LC_{50}, $LC_{50(max)}$, LC_{90}, and x^2 were made by Probit Analysis in STATPLUS (2015) program in larvicidal activity assays.

RESULTS and DISCUSSION

The use of *A. salina* as an animal model for the assessment of acute toxicity is increasing because this micro-shellfish is characterized by high sensitivity, ease of use, low cost, and visualization with the naked eye (Demarchi et al., 2020). The average mortality rates (%) and statistical results of the *A. salina* after 24 h of exposure are shown in Table 2. The LC_{50} values of the plant extracts were obtained by a plot of the percentage of the shrimp nauplii killed against the concentrations of the extracts. The best-fit line was obtained from the data through regression analysis in Figure 1.

The LC_{50} value is the concentration value that kills 50 % of a population, and the LC_{90} value kills 90 percent of a population, and the lower this value, the more toxic the substance used. When the toxic effect value of the underground and fresh parts of the five taxons against *A. salina* is compared, the fresh part extract of the *P. kesrouanensis* has the highest toxicity with the 44.44 ± 2.78% (1.56 ± 0.13 mg/mL, LC_{50}) value, while the underground part of the *N. tazetta* subsp. *tazetta* species with the least toxicity is shown with the 8.33 ± 0.00% (294.68 ± 1.54 mg/mL, LC_{50}) value. In the study of Turan and Mammadov (2018), *C. alpinum* was studied in ethanol extract of underground and aboveground parts and found that it was more active in the underground part with the value of 0.257 mg/mL, LC_{50}.

Table 2. Average mortality rates (%) of the concentrations of the genera studied at 24 h exposure to *A. salina* and statistical values.

Plant \ Solvent	24 h of exposure (1 mg/mL, %)	$LC_{50\ (min)}$ (mg/mL)	LC_{50} (mg/mL ± std error)	$LC_{50\ (max)}$ (mg/mL)	LC_{90} (mg/mL ± std error)	x^2
Fresh Part						
A. rupicola var. *virescens*	33.33 ± 4.81 ab^x	1.23	1.81 ± 0.12	3.67	13.03 ± 0.27	0.73
C. cilicium	27.78 ± 2.78 a	1.84	3.77 ± 0.27	21.26	65.11 ± 0.60	0.05
G. bohemica	30.56 ± 5.56 ab	1.41	2.24 ± 0.15	5.52	18.69 ± 0.33	0.75
N. tazetta subsp. *tazetta*	36.11 ± 5.56 ab	1.36	2.19 ± 0.16	5.52	21.00 ± 0.35	1.39
P. kesrouanensis	44.44 ± 2.78 b	1.03	1.56 ± 0.13	3.44	19.66 ± 0.35	2.45
Underground Part						
A. rupicola var. *virescens*	22.22 ± 2.78 ab^x	3.92	21.70 ± 1.24	>10000	2443.69 ± 2.69	0.60
C. cilicium	13.89 ± 2.78 ac	4.04	17.40 ± 0.87	>10000	451.20 ± 1.64	0.02
G. bohemica	13.89 ± 2.78 ac	7.60	134.16 ± 9.70	>10000	>10000	0.068
N. tazetta subsp. *tazetta*	8.33 ± 0.000 c	0.28	294.68 ± 1.54	>10000	>10000	0.41
P. kesrouanensis	30.56 ± 5.56 b	2.16	5.34 ± 0.38	68.40	148.06 ± 0.86	2.62
Negative Control (Distilled water)	00.00 ± 00.00					

a^x: If the lower cases in the column are the same, there is no statistical difference in Duncan's multiple range test ($p \leq 0.05$). Each part was statistically calculated among themselves.

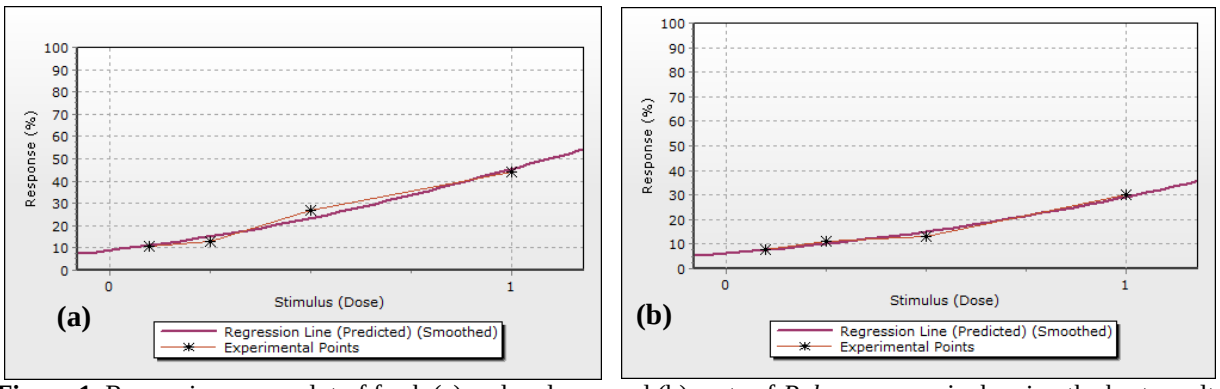

Figure 1. Regression curve plot of fresh (a) and underground (b) parts of *P. kesrouanensis* showing the best results

Umaru et al. (2020) studied stem-bark extracts of *Barringtonia asiatica* (L.) Kurz and found the best result with an LC_{50} of 34.059 (µg/mL) in methanol extract. Itam and Anna (2020) found the best result in ethyl acetate extract with 70.03 (µg/mL, LC_{50}) in their study with *Syzygium malaccense* (L.) Merr. & L. M. Perry leaves. Ogbole et al. (2020) studied the toxicity of the peptide-rich and methanol crude extracts against brine shrimp, and the crude methanol extract of *Calliandra portoricensis* Benth. was more toxic (5.13 µg/ mL, LC_{50}) compared to the Arginine-rich Peptide extract (6.12 µg/mL, LC_{50}). Rosyadi et al. (2020) found the 67.17 (µg/mL, LC_{50}) value in their study with methanolic extract of *Piper crocatum* Ruiz & Pav. leaf. In the study of Hamrun et al. (2020), the most significant number of larvae mortalities was seen at a concentration of 1000 ppm and found the 58.82 ppm, LC_{50} value with methanolic extract of *Eucheuma spinosum* J.Agardh red algae extract. Osamudiamen et al. (2020) investigated the anticancer and brine shrimp toxic activity of hexane, dichloromethane, ethyl acetate, and methanol extracts of *Mezoneuron benthamianum* Baill species, the results show that dichloromethane and hexane extracts had the highest activity against the *A. salina*, with a lethal concentration of 29.29 mg/mL, LC_{50} and 99.96

mg/mL, LC_{50} values, respectively. Ara et al. (1999) studied six seaweed and found the best results in aqueous extracts with *Stokeyia indica* Thivy & Doshi value of 64 µg/mL, LC_{50} after 24 hours. Padmaja et al. (2002) found the best result after 24 hours with 6.9 µg/mL, LC_{50} value in the fruit of *Piper longum* L. in their study with India medicinal herbs. Santos Pimenta et al. (2003) studied eighteen extracts of Brazilian medicinal plants and found the lowest LC_{50} value in the ethanolic extract of the *Annona nutans* R.E.Fr. seed with the 0.20 µg/mL, LC_{50} value.

A good correlation between larvicidal activity against *Aedes aegypti* L. and brine shrimps was also found in Luna et al. (2005) studies, indicating general toxicity of the active components. Especially *A. aegypti* and *Culex pipiens* L. are a mosquito species responsible for the transmission of yellow fever, dengue fever, chikungunya, and zika virus, which has become a matter of public health since the last three decades (Carvalho and Moreira, 2017). Of the studied extracts, *P. kesrouanensis* will likely increase toxicity to *A. aegypti* larvae since its cytotoxic effect against brine shrimp is significantly increased, and more studies are needed to prove this when there are not many studies in the literature. As the studies on this subject increase, it has been reported that the increase in toxic effect activity leads to an increase in larvicidal activity and can assist public health programs against mosquito reproduction (Maia-Neto et al., 2020). The results of our study are consistent with all other studies reported in the literature, especially *P. kesrouanensis*, with its toxic effect, which can potentially have anticancer and insecticidal properties.

CONCLUSION

Especially the fresh part has toxic activity against brine shrimp (*A. salina*) according to the BSTA method. The *P. kesrouanensis* showed the highest activity both fresh and underground. With this feature, *P. kesrouanensis* can be said to have potential anticancer and insecticidal properties. Based on the possible relationship between brine shrimp lethality, cancer, and insecticide, this study could serve further ethnobotanical, phytochemical, agricultural, medical, pharmaceutical research.

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