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Research Article / Araştırma Makalesi

Cytotoxic Activities on Selected Lamiaceae Species from Turkey by Brine Shrimp Lethality Bioassay

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Abstract

In this study, an experiment for cytotoxic activity was carried out on the methanol and water extracts of three species belonging to the Lamiaceae family (*Thymus zygioides Griseb, Teucrium sandrasicum, Origanum sipyleum*) collected from Turkey. A brine shrimp (*Artemia salina* L.) lethality test was established for the present study and the cytotoxicity was reported in terms of 50 % lethal concentration (LC_{50}). The LC_{50} values of the extracts were calculated using an EPA Probit Analysis Program (version 1.5). The *A. salina* were hatched and the active shrimps were collected for use in the assay. Ten *A. salina* were added to different concentrations of the extracts, the surviving shrimps were counted after a period of 24 h and the lethality concentration LC_{50} was assessed. The water and methanol extracts of these plants showed cytotoxicity against brine shrimp. The cytotoxic activities of the extracts ranged from 34.66-643.652 µg/mL. The strongest cytotoxic activity were obtained from the methanol extract of *T. zygioides*. This significant lethality of the extracts from three species could be the source of potential cytotoxic components in these species but this will require further investigation.

Keywords: *Artemia salina*, Cytotoxic, *Origanum sipyleum, Thymus zygioides, Teucrium sandrasicum.*

Türkiye'den Seçilmiş Lamiaceae Türlerinin Brine Shrimp Letalite Yöntemiyle Sitotoksik Aktivitelerinin Tayini

Öz

Bu çalışmada, Türkiye'den toplanan Lamiaceae familyasına (*T. zygioides, T. sandrasicum, O. sipyleum*) ait üç türün metanol ve su ekstraktları üzerinde sitotoksik aktivite deneyi gerçekleştirilmiştir. Bu çalışma için brine shrimp (*Artemia salina* L.) letalite testi yapılmış ve sitotoksisite % 50 ölümcül konsantrasyon (LC_{50}) olarak bildirilmiştir. Ekstraktların LC_{50} değerleri EPA Probit Analiz Programı (versiyon 1.5) kullanılarak hesaplanmıştır. *A. salina* lar yumurtalarından çıkarılmış ve deneyde kullanılmak üzere aktif olanlar toplanmıştır. Ekstraktların farklı konsantrasyonlarına on adet *A. salina* ilave edilmiş, hayatta kalanlar 24 saat sonra sayılmış ve öldürücü konsantrasyon LC_{50} olarak değerlendirilmiştir. Bu bitkilerin su ve metanol ekstraktları tuzlu su karidesine karşı sitotoksisite göstermiştir. Ekstraktların sitotoksik aktiviteleri 34.66 - 643.652 µg / mL arasında değişmiştir. En güçlü sitotoksik aktivite *T. zygioides*'in metanol özünden elde edilmiştir. Üç türden elde edilen ekstraktların önemli ölçüde gösterdiği öldürücülük etkilerinin, bu türlerde bulunan potansiyel sitotoksik

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bileşenlerden kaynaklandığı düşünülmektedir, ancak bunun açığa çıkarılması için daha fazla araştırma yapılması gereklidir.

Anahtar Kelimeler: *Artemia salina*, Sitotoksik, *Origanum sipyleum, Thymus zygioides, Teucrium sandrasicum.*

1. Introduction

Plants are sources of functional compounds that are medically and biologically important. They can be used for several fields instead of synthetic ones, such as medicinal and pharmacological applications and particularly in order to find solutions for the side effects of a drug. For this reason, there has been an increase in recent years into the investigation of aromatic and medicinal plants relating to the designation of the biological activities of these compounds, such as antioxidant, anthelmintic, antimicrobial and cytotoxic activities (Nickavar & Esbati 2012; Al-Dabbas 2017).

Many species of the Lamiaceae family are considered to be extremely important due to their use in medicine, foodstuffs and cosmetics. The Lamiaceae family consists of approximately 230 genera and 7100 species, worldwide (Harley et al 2004). Some of the major genera belonging to the Lamiaceae family are *Teucrium*, *Origanum* and *Thymus*. These genus plants are well known for their antimicrobial, antispasmodic, cytotoxic and anthelmintic activities (Gharaibeh et al 1989; Sivropoulou et al 1996; Nickavar & Esbati 2012; Kaska et al 2018). In present study, in order to determine the cytotoxicity of these medicinal plants we conducted a brine shrimp lethality bioassay, which based on the ability to kill laboratory cultured brine shrimp.

Owing to the expense of these toxicological tests and the suffering they can cause to laboratory animals, there is an increasing trend these days to replace laboratory animals with invertebrates. These methods using invertebrates, including a range of alternative procedures utilized in a variety of experiments, thereby reduce the need for vertebrates and decrease the use of strategies that can adversely affect laboratory animals (Kanwar 2007).

Artemia salina is a simple invertebrate marine organism that has variety of significant features including its ease of hatching into larvae, its low cost, the availability of its eggs, the rapid growth of its nauplii and its commercial availability in dry cysts. As a result, the *Artemia salina* is acknowledged as a simple and effective test animal for bioassay and toxicology studies (Svoboda & Hampson 1999; Kanwar 2007).

The brine shrimp cytotoxic bioassay is considered to be useful tool for the preliminary assessment of general toxicity and for estimating the medium lethality concentration LC_{50}. It has also been used for the detection of fungal toxins and pesticides (Meyer et al 1982; McLaughlin et al1991) and universally as a test for detecting cytotoxic effects. Likewise, it is also frequently used in laboratories for the prescreening of plant extracts that have potential medicinal benefits, such as antimicrobial or antiparasitic (Adoum et al 1997; Mayorga et al 2010). In addition, the brine shrimp cytotoxic bioassay is highly sensitive to a variety of chemical substances and only a small amount of sample is required (Meyer et al 1982; Svoboda & Hampson 1999; Hossain et al 2009).

The objective of present study was to evaluate the cytotoxic activity of water and methanol extracts of three Lamiaceae species (*T. sandrasicum, O sipyleum and T. zygioides*) using brine shrimp lethality bioassay method.

2. Materials and Methods

2.1. Plant Materials and Preparation of The Plant Extracts

The aerial parts of the medicinal plants used were collected from different regions of Turkey (Table 1). The plant materials were identified and stored with voucher specimens at the private herbarium of Dr. Mehmet Cicek, plant taxonomist from Pamukkale University, Denizli, Turkey. The plant materials were air-dried, milled and extracted with methanol, and water. Each sample (20 g) were mixed with 200 mL of solvents and shaking at 50^{o} C for 6 h in a temperature and filtered through Whatman No.1 filter paper. The solvent was evaporated using a rotary evaporator under vacuum at 40-50^{o} C. The samples were lyophilized and kept at -20 ^{o}C.

Table 1. Collection Site, Date and The Herbarium Numbers of The Lamiaceae Plants

Plant	Collection Site	Collection Date (month/year)	Herbarium No
Teucrium sandrasicum	Sandras Mountain (between Denizli-Muğla)	July/2017	2017-145
Thymus zygioides	Honaz Mountain (Denizli)	June/2016	2016-996
Origanum sipyleum	Çamlık forest, old Denizli-Kızılcabölük road (Denizli)	June/2017	2017-4-92

2.2. Cytotoxic bioassay

The possible cytotoxic activity of *O. sipyleum, T. zygioides and T. sandrasicum* was evaluated using the brine shrimp lethality bioassay (Meyer et al 1982). The brine shrimps (*Artemia salina*) were hatched using *A. salina* eggs in a beher-glass (1 L), filled with air-bubbled sterile artificial seawater (3.8 g sea salt was dissolved in 100 ml water) and left to incubate under artificial light for 24-48 h at 28 ºC. In each experiment 0.5 mL of plant extract was mixed with 4.5 mL of brine solution and the sample was tested at 1000, 500, 100, 50 and 10ppm. Following incubation, active nauplii free from egg shells were collected and used for assay. Ten nauplii were drawn through a glass capillary and placed into test tubes containing different concentration of extracts and the control tubes. The extracts and controls tubes were maintained under artificial light for 24 h at 28 ºC. The experiments were conducted in a set of three tubes per concentration and the controls.

2.3. Lethality Concentration Determination

For each concentration of the all the extracts and controls, the number of dead shrimps were counted and recorded using an overhead projector. The larvae were regarded as dead if no activity of the appendage was seen within 10 sec. The data analysis were done using the EPA Probit Analysis Program.

3. Results and Discussion

The brine shrimp method is rapid (requires 24-48 hours), inexpensive, simple (aseptic technique is not required), needs no special equipment and only a relatively small amount of the sample is required (Hossain et al 2009). This bioassay can be employed to determine the cytotoxic activity of several plant extracts and it is a very useful tool for screening a wide range of chemical compounds for their various bioactivities.

An investigation was made *in vitro* against the brine shrimp to determine the cytotoxic activity of the water and methanol extracts of *T. zygioides, T. sandrasicum and O. sipyleum* plants from the Lamiaceae family. Meyer et al. (1982) reported that if LC_{50} values of extracts lower than 1000, they are toxic and If LC_{50} values of extracts higher than 1000, they are nontoxic. The results (LC_{50} values) were found to be lower than 1000 µg/mL as shown in Table 2, considered significantly active. The order at which the cytotoxic potential of the test samples decreased was as follows: methanol extract of *T. zygioides*> water extract of *T. sandrasicum*> water extract of *T. zygioides* > methanol extract of *O. sipyleum*> methanol extract of *T. sandrasicum* > water extract of *O. sipyleum.*

Table 2. The Lethal Concentration (LC_{50}) of Extracts From Lamiaceae Family Plants

Plant	Part used	Extract	Concentration (ppm)	LC_{50} (µg/mL)
T. sandrasicum	stem, flowers and leaves	methanol	10:50:100:500:1000	414.689
		water	10:50:100:500:1000	202.955
T. zygioides	stem, flowers and leaves	methanol	10:50:100:500:1000	34.66
		water	10:50:100:500:1000	327.41
O. sipyleum	stem, flowers and leaves	methanol	10:50:100:500:1000	389.661
		water	10:50:100:500:1000	643.652

Low LC_{50} value indicates high cytotoxic activity. It can therefore be stated that the methanol extracts of the *T. zygioides* plant has possess strongest cytotoxic activity on the *Artemia salina.*

Thymus, Origanum and *Teucrium* plants from Lamiaceae family are commonly used as herbal teas, flavoring agents, aromatic and medicinal plants due to their biological and pharmacological properties (Canadanovic-Brunet et al 2006; Nakipoglu et al 2007; Ozkan et al 2007; Nickavar & Esbati 2012). The most promising extract was the methanol extract of *T. zygioides* which has been used for the treatment of several diseases, such as gastrointestinal and respiratory tract problems (Sargın et al 2013) and the lethality (LC_{50}) values is 34.66 µg/mL. The another promising extract was the water extract of *T. sandrasicum* which is one of the endemic species of the *Teucrium* genus and the aerial parts of this plant are widely used in the daily diet (Aksoy-Sagirli et al 2015) and the lethality (LC_{50}) values is 202.955µg/mL. In addition, *Teucrium* plants are well known for their

hypoglycemic, antiseptic, antispasmodic and anthelmintic activities (Gharaibeh et al 1989; Aksoy-Sagirli et al 2015).

In the present study, each of the extracts was also subjected to the brine shrimp lethality bioassay for possible cytotoxic action and the methanol and water extracts of these three plants were found to be toxic to brine shrimp nauplii.

To date, a number of studies have been made of the biological activities of various extracts and phytochemical components from Lamiaceae plants by several different authors (Canadanovic-Brunet et al 2006; Ozkan et al 2007; Nickavar & Esbati 2012; Aksoy_Sagirli et al 2015; Rehman et al 2016; Kaska et al 2018) . These studies reported that these plants provide a rich source of phytochemical components, particularly phenolic compounds, which are considered to be the basis of various biological activities, including antioxidant, antibacterial and cytotoxic activity. Accordingly, it could be attributed that the presence of these active components are the basis of the cytotoxic activity.

The cytotoxic activity of these extracts from Lamiaceae plants as described in this study, suggest that they could be effective against toxicity, but further investigation is required to find and isolate the chemical compounds that provide the cytotoxic properties.

4. Conclusion

The brine shrimp lethality test is highly beneficial for assessing the bioactivity of plant extracts. In the present study, three species from the Lamiaceae family showed LC_{50} values of less than 1000 µg/mL and these results indicate that these plants possessed strong cytotoxic activities. Therefore, the water and methanol extracts of these three species from the Lamiaceae family warrant further phytochemical screening in order to determine their active cytotoxic components. This will in turn increase information on the usability of the plant for the pharmacological applications.

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