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Anthelmintic Activities of Methanol Extracts of Five Different Geophyte Plant Species

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ABSTRACT

The purpose of this study was to look into the *in vitro* anthelmintic activities of methanol extracts of *Hyacinthella lineata* (Steud. ex Schult. & Schult.f.) Chouard, *Ornithogalum umbellatum* L., *Allium reuterianum* Boiss., *Cyclamen coum* Mill., and *Sternbergia clusiana* (Ker Gawl.) Ker Gawl. ex Spreng.. The paralysis and death times of the Indian earthworm, Cosmocerca ornata, were used to assess anthelmintic activity. Three different concentrations, (5,10 and 20 mg mL^{-1}) of methanol extracts were used to determine the time required to paralyze and to induce death in the earthworms. Albendazole (15 mg mL^{-1}) was used as reference standard drug. Plant extracts at higher concentrations (20 mg mL^{-1}) produced faster paralytic effects and a shorter time to death. The results revealed that *S. clusiana* killed all of the test worms within 20 ± 0.6 min of exposure and was 100% effective. Among all five plants extracts tested, *S. clusiana* was found to be highly effective and significant against *C. ornata*. The findings will aid in understanding the significance and application of these medicinal plant species in the food industry, traditional medicine, and pharmaceutical applications. This anthelmintic study of the plant extracts revealed that traditional medicine may be as effective as modern medicine in combating pathogenic micro-organisms.

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Beş Farklı Geofit Bitki Türünden Elde Edilen Metanolik Ekstraktların *in vitro* Antihelmint Aktivitesinin Araştırılması

ÖZET

Bu çalışma *Hyacinthella lineata, Ornithogalum umbellatum, Allium reuterianum, Cyclamen coum, Sternbergia clusiana* türleri metanol ekstraktlarının in vitro antihelmintik aktivitelerini araştırmak için tasarlanmıştır. Hint toprak solucanı *Cosmocerca ornata*'nın paraliz ve ölüm zamanının hesaplanması ile antihelmint aktivite değerlendirilmesi yapılmıştır. Paraliz ve ölüme yol açan süreyi belirlemek için üç farklı konsantrasyon (5,10 ve 20 mg mL^{-1}) kullanılmıştır. Referans standart olarak Albendazol (15 mg mL^{-1}) kullanılmıştır. Bitki özütleri yüksek konsantrasyonlarda (20 mg mL^{-1}) daha hızlı paralitik etkiler ve daha kısa zamanda ölüm göstermiştir. Sonuçlar göstermiştir ki, *Sternbergia clusiana* türüne maruz kalan test solucanlarının tümü 20±0.6 dakika içinde ölüm göstererek %100 etkili olmuştur. Beş bitki türü arasında *S. clusiana*'nın solucanlara (*Cosmocerca ornata*) karşı oldukça etkili olduğu ve önemli sonuçlar verdiği bulunmuştur. Sonuçlar, bu tıbbi türlerin gıda endüstrisindeki, geleneksel tıptaki ve farmasötik uygulamalardaki önemini ve kullanımını anlamamıza yardımcı olacaktır. Bitki özleri üzerinde yapılan bu antelmintik çalışma, geleneksel tıbbın patojenik mikroorganizmaların üstesinden gelmek için modern tıp kadar etkili olabileceğini ortaya koydu.

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INTRODUCTION

Helminthes infections are among the most common infections in humans. Human intestinal parasitic worms are vectored through air, food, and water, causing disease state, secreting toxins, and stealing vital nutrients from host bodies. The current treatment regimens for these diseases have limitations because the anthelmintic drugs used are primarily microfilaricidal, with little effect on the adult worms; thus new drugs are urgently needed. In this regard, natural products have made and continue to make important contributions to this therapeutic area. The drugs currently used for helminthes infections include combinations of DEC (diethylcarbamazine) and albendazole, ivermectin and albendazole or the use of DEC fortified salt, which has also been described in (Murugamani et al., 2012).

The plant mediated drugs may be a potential alternative to synthetic anthelmintic drugs. The resistance against the synthetic anthelmintic results in the search of alternative natural resources to overcome the drug resistance (Thooyavan et al., 2018).

The plants are known to provide a rich source of botanical anthelmintics. A number of medicinal plants have been used to treat parasitic infections in man and animals. In traditional system of medicine the practitioners use various indigenous plants for the treatment of anthelmintic (Vidyadhar et al., 2010). Secondary metabolites isolated from plants by different isolation and purification techniques play major role as alternative to synthetic drugs.

To eliminate the harmful side effects of the synthetic anthelmintic drugs and to provide newer and cheaper alternative, it is important for us to promote the studies of traditionally used anthelmintic plants, which will lead to the development of new anthelmintic substances with ease of availability and lesser side-effects.

Hyacinthella Schur (Liliaceae) is a genus of 17 species distributed in mainly Mediterranean regions.*Hyacinthella* genus is constantly changing place between families (Liliaceae, Hyacinthaceae, recently Asparagaceae). Genus represented 12 species, in which 10 of them are endemic, in Turkey. Recently, several scientific studies on the floristic, morphology, and anatomy of this genus have been performed (Aydin and Mammadov, 2017).

The genus *Ornithogalum* (family: Liliaceae) comprises 150 species, distributed in temperate Europe, Asia, and Africa. Some *Ornithogalum* plants are known to be poisonous; of these, several cardenolide glycosides have been isolated and identified (Ghannamy *et al.*, 1987). Phytochemical studies revealed that the bulbs of some species contain a variety of steroidal compounds and steroidal glycosides such as cholestane glycosides, acylated cholestane bisdesmosides, saponins, and spirostanol glycosides, some of which exhibit significant cytotoxic activities against cultured tumor cells and have anticancer potential (Delazar et al., 2009). Some *Ornithogalum* species (sp.) have been reported to exhibit a wide range of biological activities such as anticancer, anti-inflammatory, antimicrobial, antioxidant and antitumor (Plančić et al., 2014).

The species of *A. reuterianum* are members of the *Alliaceae* family. *Allium* L., which is a genus, is important because it creates a group of natural antioxidants. Since ancient times, many *Allium* species, such as onion, garlic, leek, and chives, have been used as foods, spices, and herbal remedies in widespread areas of the world, especially in the northern hemisphere. The *Allium* genus is one of the major sources of polyphenolic compounds and the antioxidative activity of some *Allium* species has been reported and has been mainly attributed to a variety of organo-sulfurous compounds as well as their precursors.

The genus *Cyclamen*, which possesses geofit plant species, belongs to the family of Primulaceae (Davis,1978). In Turkey, this genus is represented by 12 taxa, 5 of which are endemic (Guner et al., 2000). *Cyclamen* plants are used as medicinal plants and also as ornamental plants (Mathew et al., 2001). Pharmacological investigations into the extracts or isolated saponins of *Cyclamen* spp. tubers exhibited in vitro cytotoxic, antimicrobial, analgesic, and anti-inflammatory activities. Also, analgesic, anti-inflammatory, and antimicrobial activities of some *Cyclamen* species such as *C. repandum* and *C. mirabile* have been reported (Dall'acqua et al., 2010). *C. coum* is used in traditional medicine for the treatment of hemorrhages, ecchymosis, inflammations, hemorrhoids, and cancer (Jaradat et al., 2017). The tubers were used in Turkish folkloric medicine for infertility treatment in women. Recent studies have shown that *C.coum* can be used as a perspective medicinal plant in clinical practice (Bokov et al., 2020).

Sternbergia Waldst & Kit. (winter daffodil) is a genus of bulbous monocotyledons belonging to the family Amaryllidaceae (Mathew and Davis, 1999). The species of *Sternbergia* always takes the interest of scientists; therefore, several studies have been done on this species (Mammadov et al., 2011). Plants of the family Amaryllidace are well known not only for their ornamental value but also for the alkaloids they produce. Studies on *Sternbergia* species yielded compounds belonging to the basically different groups of Amaryllidaceae alkaloids (Kıvcak and Gözler, 1993).

Amaryllidaceae is a family of monocotyledonous plants with significant economic and medical value. Amaryllidaceae alkaloids have been shown to possess important biological activities, including antitumor, antiviral, and acetylcholinesterase inhibitory activity (Kaya et al., 2010; Berkov et al., 2009). Also, analgesic and antimicrobial activities have been reported for extracts and alkaloids from S*. clusiana, S. sicula,* and *S. lutea* (Unver et al., 2005).

The purpose of this study is to evaluate the anthelmintic activity of methanol extracts of bulbs and leaves of *H. lineata*, *O. umbellatum, A. reuterianum, C. coum*, and *S. clusiana.*

MATERIALS and METHODS

Preparation of plant extracts

H. lineata species were collected in the spring of 2020 from the Honaz locality, near Denizli province, in Turkey and identified from the book of Flora of Turkey. The fresh bulbs and leaves of the plant samples were cleaned and dried in the shadow for extraction. The voucher specimen was deposited at the herbarium of Pamukkale University the Laboratory of Botanics, under the "PAU2020-2023" number.

O.umbellatum species were collected in April 2020 from the Honaz locality, near Denizli province in Turkey and identified from the book of Flora of Turkey. The fresh bulbs and leaves of the plant samples were cleaned and dried in the shadow for extraction. Dried plant parts (bulbs and leaves) were pulverized. Each ground sample was transferred into a beaker. The voucher specimen was deposited at the herbarium of Pamukkale University the Laboratory of Botanics, under the "PAU2020-2024" number.

S.clusiana species were collected in the spring of 2019 from the Kavaklıdere locality, near Muğla province, in Turkey and identified from the book of Flora of Turkey. The fresh bulbs and leaves of the plant samples were cleaned and dried in the shadow for extraction. The voucher specimen was deposited at the herbarium of Pamukkale University the Laboratory of Botanics under the "PAU219-2021" number.

A. reuterianum species were collected in the spring of 2019 from the Kötekli locality, near Muğla province, in Turkey and identified from the book of Flora of Turkey. The fresh bulbs and leaves of the plant samples were cleaned and dried in the shadow for extraction. The voucher specimen was deposited at the herbarium of Pamukkale University the Laboratory of Botanics, under the "PAU219-2022" number.

C. coum species were collected in the spring 2019 from Kötekli locality, near Muğla province, in Turkey and identified from the Flora of Turkey book. Each part (tubers and leaves) was dried in the shadow at room temperature and low humidity. The voucher specimen was cataloged as "PAU219-2023" in the Pamukkale University the Laboratory of Botanics.

Each material (Table1) was thoroughly cleaned with water and dried (bulbs and leaves) under shade for seven days. All samples were kept at 25˚C until extraction. In our laboratory, the dried materials were ground and powdered before being stored separately for future use. Dried plant parts are kept in tightly-sealed dark containers until they are needed. About 200 g of each plant material was used for extraction. Methanol (100mL) was added in the ratio of 1:10 and each plant material was put in a water bath at 55 ºC for 6 h . The extraction mixture was separated from the residue by filtration through filter paper (Whatman No. 1). The plant residue was re-extracted twice with methanol. After the filtration, the two extracts were combined. The residual solvent of methanol extracts of samples was removed under reduced pressure at 48 - 49°C using a rotary evaporator (IKA RV10D, Staufen, Germany). Extracts were produced in duplicates and used to assay the biological activity (Aydin and Mammadov, 2017).

Table 1. *Plant materials evaluated for their anthelmintic activity*
Çizelge 1. Antihelmintik aktiviteleri açısından değerlendirilen bitki materyalleri

Botanical name	*Family*	*Parts used*
H. lineata	*Asparagaceae*	Bulb, leaf
O. umbellatum	*Asparagaceae*	Bulb, leaf
A. reuterianum	*Amaryllidaceae*	Bulb, leaf
C. coum	*Primulaceae*	Bulb, leaf
S. clusiana	*Amaryllidaceae*	Bulb, leaf

Evaluation of the in vitro anthelmintic activities

The anthelmintic activities were performed according to the method mentioned in Ghosh et al. (2005). The adult *Cosmocerca ornata* (of the *Cosmocercidae* family) resembles intestinal round worm parasites in terms of anatomy and physiology. *C. ornata* was placed in a petridish containing three different concentrations (5, 10, and 20 mg mL^{-1}) each of plant methanol extracts. Each petridish was placed with six worms and observed for paralysis (or) death. The mean time for paralysis was noted when no movement of any sort could be observed except when the worm was shaken vigorously; the time of death of the worm (min) was recorded after ascertaining that worms neither moved when shaken nor when given external stimuli. In the same manner, albendazole was included as a reference compound. The test results were compared to samples that had been treated with the reference substance albendazole (15 mg mL-1).

RESULTS and DISCUSSION

The results in Table 2 show the anthelmintic activities of the extracts obtained from each plant species. The present study also shows that there was an increase in the anthelmintic activities as the concentrations increased. The extracts were effective in causing the death of the worms as well as promoting paralysis. All the doses of the extracts of each plant showed better anthelmintic activity than the standard. *S. clusiana* was the most potent, requiring less time for paralysis and death of the worms. It showed a concentration-dependent anthelmintic property (Table 2). *S. clusiana* bulb extract was the most active against *C. ornata* with paralysis time of 12 ± 0.6 and death time of 20 ± 0.6 in minutes at 20 mg mL^{-1},while *O. umbellatum* leaves extract was the least active with paralysis time of 98 ± 0.3 and death time of 105 ± 0.5 in minutes at 20 mg mL^{-1} (Table 2). The strong anthelmintic activity of the extract of *S. clusiana* may be due to the presence of rich polyphenolic compounds in its essential oil, which are monoterpenoid phenols and alkaloids (Berkov et al., 2009; Kaya et al., 2010).

Table 2. *Anthelmintic activity of plant materials*
Çizelge 2. Bitki materyallerinin antihelmintik aktivitesi

Plant species		*Concentration (mg mL^{-1})*	*Time taken for paralysis (min)*	*Time taken for death (min)*
dH_2O control			-	-
Albendazole (Reference)		15	39±0.5	58±0.4
A. reuterianum	*Bulb*	5	46±0.3	57±0.4
		10	26±0.2	38±0.7
		20	19±0.4	30±0.4
	Leaves	5	52±0.6	66±0.8
		10	37±0.7	45±0.5
		20	26±0.3	35±0.3
H.lineata	*Bulb*	5	115±0.3	120±0.7
		10	89±0.2	100±0.7
		20	73±0.6	80±0.2
	Leaves	5	86±0.7	98±0.3
		10	83±0.2	95±0.8
		20	79±0.4	90±0.4
O. umbellatum	*Bulb*	5	100±0.3	115±0.7
		10	95±0.4	105±0.5
		20	91±0.2	100±0.6
	Leaves	5	116±0.5	130±0.2
		10	108±0.4	120±0.7
		20	98±0.3	105±0.5
S. clusiana	*Bulb*	5	33±0.6	48±0.6
		10	22±0.7	35±0.4
		20	12±0.6	20±0.6
	Leaves	5	38±0.4	55±0.4
		10	27±0.6	40±0.4
		20	24±0.5	30±0.3
C.coum	*Bulb*	5	65±0.7	78±0.2
		10	48±0.6	60±0.7
		20	36±0.6	47±0.5
	Leaves	5	50±0.5	65±0.7
		10	47±0.4	58±0.5
		20	35±0.3	46±0.4

The anthelmintic activities of the species investigated in this study wereilluminated for the first time. Studies have shown that phenolic and alkaloids possess plants to produce anthelmintic activities because alkaloids chemically belong to polyphenolic compounds. Phenolics and alkaloids are known to interfere with the generation of energy in helminth parasites by uncoupling the oxidative phosphorylation and also bind to free proteins in the GIT of the host animal or glycoprotein on the parasite cuticle, leading to death (Mali and Wadekar, 2008).

It is possible that phenolic compounds and alkaloids also present in *S. clusiana* extracts may be responsible for the anthelmintic activity. *S. clusiana* shows moderate anthelmintic activity comparable to other plants.

All values are present as mean+SD; n=6 in each group. Comparisons made between standard versus treated groups.

Results obtained from the anthelmintic assay indicated that higher concentrations of plant extracts produced faster paralytic effects and showed a shorter time of death. Methanol extracts of plant species demonstrated paralysis as well as death of worms, especially at a higher concentration of 20 mg mL^{-1}, while a 5 mg concentration also showed activity but at a longer time. An *in vitro* assay was used in this present investigation in agreement with the findings. This provides a cheaper, more economical and rapid turnover in contrast to *in vivo* assays as far as anti-parasitic properties of plants and plant extracts are concerned (Blessing et al., 2019).

Iqbal *et al.* (2001), has been reported that the alcoholic extract of the bulb of *A. sativum* shows in vitro anthelmintic activity against human *Ascaris lumbricoldes*. *A. sativum*'s alcoholic extract has demonstrated anthelmintic activity in vitro against *Heterakis gallinae* and *Ascaridia galli*, *Haemonchus contortus*, a free-living worm of *Rhabditis sp.*, larvae of *Nippostrongylus brasiliensis*, and eggs of *Ascaris summ* (Chybowski, 1997; Nagaich, 2000).Singh et al. (2008) reported the alcoholic extract of *A. sativum* causes a paralytic effect on liver amphistomes *Gigantocotyle explanatum*.

It has been shown that the ethanolic extracts of the tuber and leaf parts of the *Cyclamen alpinum* have anthelmintic activity. After 6 minutes, it was seen that the tuber part (LC_{50}: 0.52 mg mL^{-1}) is more toxic than the leaf part (LC_{50}: 1.32 mg mL^{-1}) with an LC_{50} value (Turan and Mammadov, 2018).

The phytochemical constituents showing anthelmintic effects include alkaloids, saponins, polyphenols, tannins, etc. Alkaloids suppress the transfer of sucrose from the stomach to the small intestine, diminish the support of glucose to the helminths, and act on the CNS(Central Nervous System) causing paralysis. Synthetic anthelmintic drugs are usually associated with various side effects. More attention is attracted by the increasing problems of resistance development in helminthes against synthetic anthelmintics. However, plants are the richest source of bioactive compounds. The best alternative to modern synthetic drugs is plant-derived medicine. Many investigators have worked on similar aspects, and their reports support this investigation, revealing that plants are potent anthelmintic agents.

CONCLUSION

It is concluded that all possess a varying degree of anthelmintic activity. Ultimately, it is possible that methanol extracts of *Sternbergia clusiana* bulb possess significant anthelmintic activity.The results suggest the presence of biologically active components in *S. Clusiana,* which may be worth further investigation. These findings of this geophyte species will be the basis for future work on the purification and identification of the active compounds, which may be useful for medicinal purposes, and on the potential use of this plant for nutrition. This is the first comprehensive study on the anthelmintic activities of species. These results indicated that extracts have strong anthelmintic activities, and that these extracts can be used as a food additive in food processing and pharmaceutical industries.

Author's Contributions

The contribution of the authors is equal.

Statement of Conflict of Interest

Authors have declared no conflict of interest.

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