Süleyman Demirel Üniversitesi
Fen Bilimleri Enstitüsü Dergisi
Cilt 22, Sayı 2, 562-568, 2018

Süleyman Demirel University
Journal of Natural and Applied Sciences
Volume 22, Issue 2, 562-568, 2018

DOI: 10.19113/sdufbed.65047

Radical Scavenging Activity and Antibacterial Effect of Three *Cyclamen* L. Tuber Extracts on Some Fish Pathogens

Cennet ÖZAY[1], Gülşen ULUKÖY[*2], Ramazan MAMMADOV[1], Zeynep SAYIN[2]

[1] Pamukkale Üniversitesi, Fen-Edebiyat Fakültesi, Biyoloji Bölümü, 20070, Denizli
[2]Muğla Sıtkı Koçman Üniversitesi, Su Ürünleri Fakültesi, Su Ürünleri Yetiştiriciliği Bölümü, 48000, Muğla

(Alınış / Received: 29.01.2018, Kabul / Accepted: 28.05.2018, Online Yayınlanma / Published Online: 19.06.2018)

Keywords
Cyclamen L.,
ABTS,
DPPH,
NO,
Fish pathogen,
Disc diffusion

Abstract: *Cyclamen* L. is a member of Primulaceae family, a geophyte, which have been utilized for their biological activities in folk medicine. The aim of this study is to investigate the antibacterial activity of three *Cyclamen* species on some fish pathogens as well as their radical scavenging activity potentials. *C. cilicium* Boiss. & Heldr., *C. pseudibericum* Hildebr. and *C. hederifolium* Aiton were collected from different regions in Turkey. *Cyclamen* tubers were extracted in ethanol and then DPPH, ABTS and NO methods were utilized for the assurance of radical scavenging activities of the extracts. Antibacterial activity of the extracts were determined *in vitro* against five bacterial fish pathogens, namely, *Vagococcus salmoninarum, Staphylococcus epidermidis, Lactococcus garvieae, Vibrio anguillarum* and *Yersinia ruckeri*. All the tested tuber extracts exhibited moderate antibacterial activity against four bacteria and the radical scavenging activity in the order of potency: *C. cilicium*>*C. pseudibericum*>*C. hederifolium*. In conclusion, this study showed that the *Cyclamen* species have moderate antibacterial activity against fish pathogenic bacteria except *V. anguillarum*.

Üç Sıklamen Yumru Ekstraktının Radikal Süpürme Aktivitesi ve Bazı Balık Patojenleri Üzerindeki Antibakteriyel Etkisi

Anahtar Kelimeler
Sıklamen,
ABTS,
DPPH,
NO,
Balık patojeni,
Disk difüzyon

Özet: Sıklamen (*Cyclamen* L.) Primulaceae familyasının bir üyesi olup, geofit bitki olarak biyolojik aktivitelerinden dolayı halk arasında tıbbi amaçlı kullanılmaktadır. Bu çalışmanın amacı, üç sıklamen türünden elde edilen ekstraktların balık patojenleri üzerindeki antibakteriyel aktivitelerini ve radikal süpürme aktivite potensiyellerini belirlemektir. Bu çalışmada kullanılan türler; *C. cilicium* Boiss. & Heldr., *C. pseudibericum* Hildebr. ve *C. hederifolium* Aiton, Türkiye'nin farklı bölgelerinden toplanmıştır. Sıklamen yumruları etanolde ekstrakte edilmiş ve daha sonra ekstraktlardaki radikal süpürme aktivitelerinin belirlenmesi için DPPH, ABTS ve NO metodları kullanılmıştır. Sıklamen bitki ekstraktlarının antibakteriyel aktivitesi *in vitro* yöntemle beş bakteriyel balık patojenine (*Vagococcus salmoninarum*, *Staphylococcus epidermidis*, *Lactococcus garvieae*, *Vibrio anguillarum* ve *Yersinia ruckeri)* karşı belirlenmiştir. Yumrulardan elde edilen ekstraktlarının test edilen tüm bakterilere karşı *V. anguillarum* hariç antibakteriyel aktivite sergilediğini ve radikal süpürme aktivitesinin en yüksekten en düşüğe sırasıyla *C. cilicium*>C. *pseudibericum*>*C. hederifolium* olduğu belirlenmiştir. Sonuç olarak bu çalışma, test edilen Sıklamen türlerinin su ürünleri yetiştiriciliğinde görülen patojen bakteriler üzerinde *V. anguillarum* hariç orta düzeyde antibakteriyel etkinliğe sahip olduğunu göstermiştir.

1. Introduction

The diminishing of fish from natural resources and expanding need for food are the two principle factors for the demand of developing aquaculture sector [1]. To be able to meet the consumers demand for fish, aquaculture has been continued to spread out very rapidly. In cultured fish, the diseases are playing one of the major roles as a limiting factor in production and causing high mortality rates in farms by affecting

* Corresponding author: gulukoy@mu.edu.tr

economic income negatively [2]. The microorganisms turn into a serious problem for the health of aquatic organisms especially bacteria [3]. In farms, bacterial diseases in fish caused by mainly; *Vibrio anguillarum*, *Yersinia ruckeri, Lactococcus garvieae, Vagococcus salmoninarum and Staphylococcus epidermidis* have been described as the etiological agents [2, 4, 5]. The using of antibiotics for the treatment of diseases generates some reactions such as; toxic effect onto fish and the residue deposition into the aquatic environment. These residues could accumulate and influence health of organisms [6]. It was also expressed that some bacterial pathogens have gained antibacterial resistance against the antibiotics [7]. These issues have urged to look for other antibacterial substances that can be applicable as much as a drug [8]. It has been demonstrated earlier that the phytomedicines may serve as an effectively therapeutic agents against antimicrobial resistant bacteria [9, 10, 11]. Hence, natural products are of great interest as a promising source to control disease problems in aquaculture [12].

The recent investigations have been demonstrated that the potential power of plant extracts against diseases by taking a part to remove free radicals from organism [13, 14]. Free radical is a molecule with an unpaired electron and is played a vital role in an oxidative stress related diseases such as rheumatoid arthritis, cardiovascular disorders and neurological degenerative diseases [15]. It is important to know that the antioxidant properties of materials are playing very critical role in prevention of disease by its capacity of the searching free radicals in the organisms. Antioxidants show endurance against oxidative stress by scavenging the free radicals and by many other mechanisms [16, 17].

Cyclamen L. belongs to the family of Primulaceae and is a tuberous perennial geophyte plant which is represented by 21 species on the Earth. In Turkey, this genus is represented with 12 taxa, 5 of them are endemic species [18]**.** *Cyclamen* is a medicinal plant containing so many ingredients such as triterpenoidal saponins which is demonstrated to have antimicrobial features [19, 20]. *Cyclamen* tubers have been widely used by people in old-times as the purgative, sedative, laxative, antihelmintic and abortive properties [21]**.** Previously, the antimicrobial properties of *Cyclamen* tuber extracts were explained [22, 23] but there hasn't been found research on antibacterial activity of the *Cyclamen* extracts on bacterial fish pathogens in the literature.

The aim of this study was to determine the radical scavenging activity of three *Cyclamen* species (*C. cilicium, C. pseudibericum*, *C. hederifolium*) ethanolic extracts and antibacterial activity of these extracts against some bacterial fish pathogens.

2. Material and Method

2.1. Plant material and extraction

C. pseudibericum, C. hederifolium and *C. cilicium* were collected in 2015 from Hatay-Turkey (600 m), Aydın-Turkey (30 m) and Antalya-Turkey (700 m), respectively and authenticated in the Department of Biology, Pamukkale University, Denizli-Turkey. The tubers of plants were air-dried over shadow at 25 °C, powdered to as a fine grain. Then ethanolic extracts were obtained by following the method by Handa et al [24]. The extracts were lyophilized and stored at -20 °C until use.

Radical Scavenging Activity

2.2. DPPH method

When the DPPH radical gets an electron from the antioxidant compound, the color changes from violet to yellow which is detected colorimetrically. The 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity of the ethanol extracts was determined as specified by Wu et al [25]. 1 mL of concentrations (200 to 1000 µg/mL) of the extracts was added to 4 mL of metanolic solution of DPPH radical. This solution was incubated at darkroom (25 °C, 30 min) and then the absorbances were measured at 517 nm. Butylated hydroxytoluene (BHT) was used as a positive control. There were three replicates in each assay. The inhibition of activity was calculated;

$$I\,(\%) = (A_0 - A_1)\,/A_0 \times 100 \qquad (1)$$

A_0: the absorbance value of the control,
A_1: the absorbance value of the *Cyclamen* extracts.

2.3. ABTS radical scavenging assay

2,2 Azino-bis (3-ethylbenzothiazloine-6-sulfonic acid) ($ABTS^{\cdot+}$) radical solution was prepared and diluted with methanol in order to fix the absorbance at 734 nm as 0.700. Briefly, $ABTS^{\cdot+}$ solution were mixed with *Cyclamen* extracts (150-600 µg/mL) and the absorbances were read after 15 min at 734 nm [26]. There were three replicates in each assay. The radical scavenging activities of the extracts was obtained:

$$\text{ABTS radical scavenging activity (\%)} = [(\text{Ac-At}) / \text{Ac}] \times 100 \qquad (2)$$

Ac: the absorbance value of the control,
At: the absorbance value of the *Cyclamen* extracts.

2.4. Nitric oxide scavenging activity

Nitric oxide (NO) was produced from sodium nitroprusside (SNP) which measured by using the

Griess reaction as described by Balakrishnan et al. [27]. At different concentrations (125-1000 µg/mL), the mixture containing SNP (5mM) in pH 7.3, PBS with the extracts were prepared and incubated for 3 hours at 25 °C. The absorbance value was determined at 546 nm. The positive control was ascorbic acid. There were three replicates in each assay. The NO scavenging activity was calculated:

$$\text{NO scavenging activity (\%)} = [(Ac-At) / Ac] \times 100 \quad (3)$$

Ac: the absorbance value of the control,
At: the absorbance value of the *Cyclamen* extracts.

2.5. Antibacterial activity

2.5.1. Fish pathogens

Three Gram positive (*V. salmoninarum, S. epidermidis* and *L. garvieae*) and two Gram negative bacteria (*V. anguillarum* and *Y. ruckeri*) were used. *V. salmoninarum, V. anguillarum, L. garvieae and Y. ruckeri* isolated from rainbow trout while *S. epidermidis* isolated from gilthead seabream. The strains were obtained during disease outbreak from cultured fish. The strains brought from the microorganisms collection (Table 1) of Eğirdir Fisheries Faculty in Isparta-Turkey.

Table 1. Bacterial fish pathogens used in the study

Collection No	Bacteria	Origins	Regions
V1	*V.salmoninarum*	Rainbow trout	Isparta/ Turkey
S1	*S. epidermidis*	Gilthead sea bream	Muğla/ Turkey
M1	*L. garvieae*	Rainbow trout	Muğla/ Turkey
A4	*V. anguillarum*	Rainbow trout	Muğla/ Turkey
V0018	*Y. ruckeri*	Rainbow trout	Muğla/ Turkey

2.5.2. Disc diffusion assay

The antibacterial activity was determined *in vitro* by using the disc diffusion technique (Kirby-Bauer) [28]. Shortly, inocula of each strain was suspended in sterile physological saline solutions and homogenized by vortexing until the density of the test suspension match the turbidity standart of $1x10^8$ cfu/ml (0.5 Mc Farland standard). The extracts were applied in certain concentration which was determined in a preliminary study. The extracts from tuber of three *Cyclamen* species prepared as 2, 4, 6, 8 and 10% concentrations and solutions had been absorbed into the blank discs (6mm). The 0.1 ml of the broth cultures of test bacteria was inoculated onto Mueller Hinton Agar (MHA) medium. Only for Vibrio species 2% NaCl added into mediums. The discs were placed on the inoculated agar plate. The plates were incubated at 25°C for 24h. Then, the formed inhibition zones around the discs were measured as in mm. The control discs were impregnated in the same amount (25 µl) of ethanol which was used as negative control while, oxytetracycline (30 µg) and streptomycin (10 µg) discs were used as positive control. Studies were carried out at the same temperature and design also repeated three times. The measured inhibition zones were expressed as average values. The antibacterial activity of plant extracts (mean ± standard deviation (SD)) was interpreted as proposed by Inhibition zones >15 mm were categorized as strong activity, from 8 to 15 mm as moderate activity, and from 1 to 8 mm as weak activity according to Bansemir et al. [8].

2.6. Statistical analysis

Statistical analysis was conducted by using the SPSS software (Version 22, SPSS Inc., Chicago, IL, USA).The statistical significance between plant species and concetrations was evaluated with one-way ANOVA followed by Duncan test. All experimental data are expressed as mean ± standard deviation. Values of P less than 0.05 were considered to be statistically significant.

3. Results

3.1. Radical scavenging activity

The tuber extracts from *C. cilicium*, *C. hederifolium* and *C. pseudibericum*in ethanol were examined for their radical scavenging activity. The free radical scavenging activity of the extracts is associated with hydrogen atom or electron donation capabilities and the structures of the antioxidant compounds of the extracts. The free radical scavenging activity values of *Cyclamen* species were determined by using the DPPH, ABTS, and NO radical scavenging assays. The results of the DPPH radical scavenging activity of three *Cyclamen* tuber extracts were shown in Figure 1. It can be clearly seen that DPPH scavenging activity of three *Cyclamen*L. species significantly ($P<0.05$) increased with rising concentration from 200 to 1000 mg/mL. It was observed that the extracts showed concentration dependent free radical scavenging activity. The highest scavenging activity was detected on extract of *C. cilicium* (55.80 ± 0.65%), in 1000 µg/mL concentration.

The decrease levels in ABTS radical was distinguished by the reducing valueat 734 nm by antioxidants. The ABTS values in the extract of *C. cilicium* at the concentrations from 150 µg/mL to 600 µg/mL were obtained as 17.98-54.30%. The positive control (BHT) showed to generate a dose dependent decrease of ABTS radicals at concentrations from 150 µg/mL to 600 µg/mL (Figure 2).

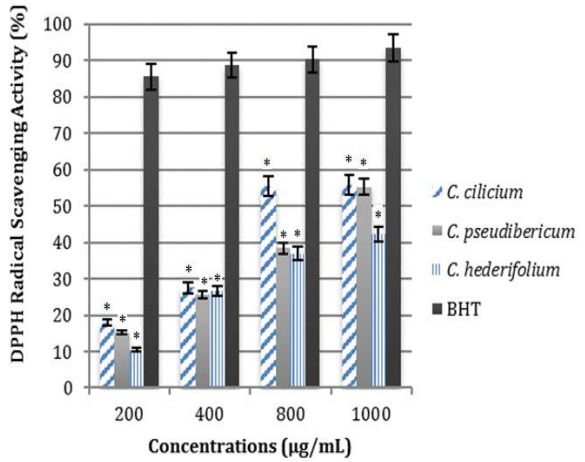

Figure 1. DPPH radical scavenging activity of *Cyclamen* extracts. Data are presented as the mean value ± SD. *P < 0.05 versus BHT

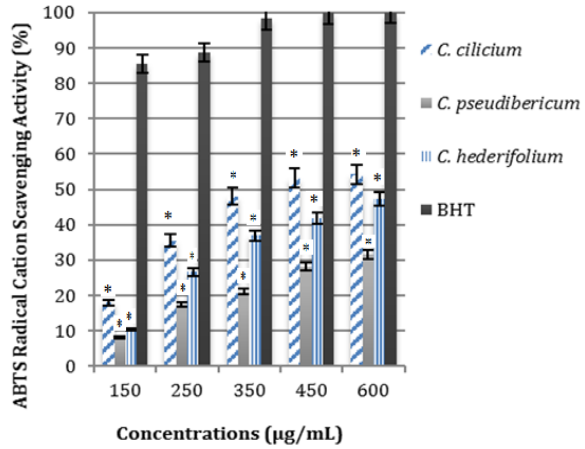

Figure 2. ABTS radical cation scavenging activity of *Cyclamen* extracts. Data are presented as the mean value ± SD. *P < 0.05 versus BHT

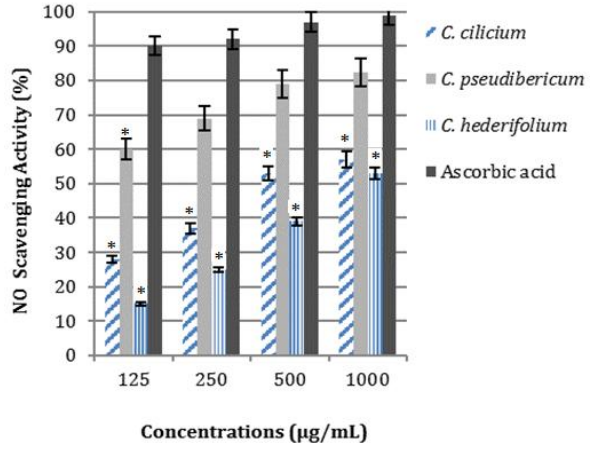

Figure 3. NO scavenging activity of *Cyclamen* extracts. Data are presented as the value ± SD. *P < 0.05 versus ascorbic acid

The NO scavenging activity in tuber extract of *C. pseudibericum* was 60.00% at the 125 µg/mL, concentration whereas the maximum activity was obtained as 82.30% at 1000 µg/mL. Figure 3 demonstrates the inhibition level was expanded with rising of the concentration of the extracts (P < 0.05). Nevertheless, the activity of ascorbic acid was more pronounced than that of the extracts of three *Cyclamen* L. species.

3.2. Antibacterial activity

Ethanolic *Cyclamen* tuber extracts were tested against five bacterial fish pathogens by the means of disc diffusion assay. Results of five concentration for each plant extract were evaluated but there wasn't statiscally significant value depending on concentrations. Three *Cyclamen* L. tuber extracts at 10% concentration had been found to have moderate antibacterial activity on all tested pathogenic bacteria, except *V. anguillarum* (Table 2). The highest inhibition zone of *C. hederifolium* tuber extract measured as 10 mm against *V. salmoninarum, Y. ruckeri* and *L. garvieae*, *C. pseudibericum* showed 12 mm inhibition zone against *S. epidermidis.* The results showed that all the *Cyclamen* tuber extracts exhibited moderate antibacterial activity against all tested bacteria except *V. anguillarum*.

4. Discussion and Conclusion

Plants are one of the most known medicinal source which have been used in treating all kind of diseases since ancient times. They also have important metabolites which could be discovered as a candidate for the development of new chemotherapeutics agents. Their active secondary metabolites are not definitely required for the normal plant growth but responsible for a lot of bioactive compounds used in health of organisms. The most important of secondary metabolites can be classified as alkaloids, phenols, terpenoids, glicosides and saponins [29]. Saponins are a class of secondary metabolites which have been accepted as the most characteristic and important chemical compounds in *Cyclamen* species. Tubers of *Cyclamen* species are particularly rich in triterpene saponins [30, 31]. It is generally believed that plants have more saponin content which show antibacterial activity [23].

In the present study, tubers of the three *Cyclamen* species were analyzed for their free radical scavenging activities. Ethanolic extracts of the tubers were also evaluated for antibacterial activity on some fish pathogens. Assessment of antioxidant properties of plants can't be done precisely by any single strategy due to complex nature of phytochemicals [32]. Therefore, the antioxidant activity of tubers of *Cyclamen* species was obtained by using three different methods (ABTS, DPPH and NO assays). ABTS measure is not just applicable to both lipophilic and hydrophilic systems/antioxidants in addition a dependable and fast test to obtain the total antioxidant activity. Furthermore, proton radical scavenging is a significant attribute of antioxidants. $ABTS^{+}$ has a characteristic absorbance maximum at 734 nm that declines with the scavenging of proton radicals [33].

Table 2. Obtained inhibition zones (Mean ± SD) in *Cyclamen* tuber extracts (10%) against tested bacteria

Plant extracts and positive controls	Bacterial fish pathogens				
	V. anguillarum	*Y. ruckeri*	*L. garvieae*	*V. salmoninarum*	*S. epidermidis*
C. cilicium	-	$9.0^{c} \pm 0.10$	$11.0^{c} \pm 0.13$	$10.1^{b} \pm 0.12$	$10.0^{b} \pm 0.11$
C. hederifolium	-	$10.1^{c} \pm 0.15$	$10.2^{c} \pm 0.12$	$10.2^{b} \pm 0.12$	$9.1^{c} \pm 0.10$
C. pseudibericum	-	$10.2^{c} \pm 0.12$	$8.3^{c} \pm 0.07$	$9.0^{b} \pm 0.08$	$12.2^{b} \pm 0.12$
Oxytetracycline (+)	$30.0^{a} \pm 0.19$	$27.3^{a} \pm 0.16$	$23.4^{a} \pm 0.14$	$23.2^{a} \pm 0.12$	-
Streptomycin (+)	$20.0^{b} \pm 0.17$	$19.0^{b} \pm 0.18$	$13.0^{b} \pm 0.11$	-	$14.4^{a} \pm 0.13$

Different letters within the given column are significantly different at $P < 0.05$

The 2,2-diphenyl-1-picrylhydrazyl- DPPH free radical scavenging ability assay is depend on the scavenging activity of stable DPPH. It had been reported the DPPH radical scavenging activity of ethanolic tuber extracts of *Cyclamen graecum* as 89.6 ± 3.70% [34]. Compared to other two *Cyclamen* extracts, *C. cilicium* extract exhibited the highest ABTS and DPPH radical scavenging activity. These results are consistent with Zengin and Aktumsek [35] and Wang et al [36] who found a powerful correlation between ABTS and DPPH assay. Among all the extracts tested, *C. pseudibericum* extract showed the highest NO scavenging activity.

In the other study [37], it was determined that extracts (using four different solvent) from different parts of *Cyclamen mirabile* (tubers and leaves) possessed high antioxidant activity *in vitro*. *Cyclamen mirabile* leaves showed higher antioxidant activities than that of positive controls. The crude extracts of *C. africanum* possessed antioxidant activity, the results indicate that the extraction yield and the antioxidant potential depend on the polarity of the solvent. However, they could obtain antibacterial activity [38]. We had found that the tubers of the *Cyclamen* species which had been investigated in this study had antioxidant activity but less than the positive control.

Occurrence of bacterial diseases is very much threatening to the intensive fish farming systems. Many countries banned the use of antibiotics since their prolonged exposure to environment is associated with harmful side effects and is a concern of public health [39]. Since plant based drugs cause much lower incidence of adverse reactions compared to synthetic pharmaceutical [40], researchers felt the urgency to develop an alternative approach of herbal medication towards management of diseases. It has been shown that some *Cyclamen* tuber extracts have the antibacterial features [23, 41] and may be important for the prevention from bacterial diseases.

This study aimed to check the antibacterial activity of the three *Cyclamen* species against several fish pathogenic bacteria. Two groups (Gram negative and Gram positive) bacteria were used in order to evaluate their reaction to ethanolic *Cyclamen* exracts.

The *Cyclamen* tuber extracts were exhibited an inhibitory activity to the growth of mostly Gram-positive bacteria but not to the growth of the Gram-negative bacteria, although some researchers reported that *Cyclamen* tubers extracts had antimicrobial activities against *Pseudomonas aeruginosa* bacteria [42]. The obtained results of this research were in agreement with many other researchers whom explaining that Gram negative bacteria had shown more resistant to many plant extracts previously studied probably their complex cell membrane structure. Similarly, the antibacterial activities of *Cyclamen persicum* and *Cyclamen mirabile* tuber extracts were found to be effective on Gram positive bacteria but noton Gram negative ones [23, 43]. There are several reports on *Cyclamen* species have antibacterial activity on some pathogens of human. But there hasn't been found any report on antibacterial activity of extracts of *Cyclamen* on fish pathogens.

Tuber of *Cyclamen* species contains several saponins as the major antimicrobial agents. These compounds have shown inhibitory effect against many bacteria and fungi [41]. In our findings, this saponin content might cause the moderate antibacterial activity on fish pathogens. Further studies are required to determine the content of tuber extracts.

In conclusion, antibacterial activity of the ethanolic tuber extracts of *C. cilicium*, *C. pseudibericum* and *C. hederifolium* was investigated on some fish pathogens. Also, the radical scavenging activities of the *Cyclamen* extracts were detected by applying three different assays. The results from the study suggest that *C. cilicium* and *C. pseudibericum* extracts can be utilized as a decent source of natural antioxidants and they have moderate antibacterial activity against four fish pathogens except *V. anguillarum*.

Acknowledgment

The authors sincerely thanks to Eğirdir Fisheries Faculty Members in Fish Diseases Division for providing bacterial strain samples.

References

[1] Dogruez, N., Zeybek, Z., Karagoz, A. 2008. Antibacterial activity of some plant extracts. IUFS Journal of Biology, 67(1), 17-21.

[2] Toranzo, A. E., Magariños, B., Romalde, J. L. 2005. A review of the main bacterial fish diseases in mariculture systems. Aquaculture, 246, 37-61.

[3] Cavallo, R. A., Stabili, L. 2002. Presence of vibriosis in seawater and *Mytilus galloprovincialis* (Lam.) from the Mar Piccolo of Taranto (Ionian Sea). Water Research, 36, 3719-3726.

[4] Austin, B., Austin, D. A. 2007. Bacterial Fish Pathogens: Diseases of Farmed and Wild Fish, 4th ed. Praxis Publ. Ltd., Chichester, 552p.

[5] Radjasa, O. K. S., Sabdono, A., Prayitno, S. B., Hutabarat, S. 2009. Phylogenetic diversity of the causative agents of vibriosis associated with groupers fish from Karimunjawa Islands, Indonesia. Current Research in Bacteriology, 2, 14-21.

[6] Cabello, F. C. 2006. Heavy use of prophylactic antibiotics in aquaculture: a growing problem for human and animal health and for the environment. Environmental Microbiology, 8, 1137-1144.

[7] Immanuel, G., Vincybai, V. C., Sivaram, V., Palavesam, A., Marian, M. P. 2004. Effect of butanolic extracts from terrestrial herbs and seaweeds on the survival, growth and pathogen (*Vibrio parahaemolyticus*) load on shrimp *Penaeus indicus* juveniles. Aquaculture, 236, 53-65.

[8] Bansemir, A., Blume, M., Schroder, S., Lindequist, U. 2006. Screening of cultivated seaweeds for antibacterial activity against fish pathogenic bacteria. Aquaculture, 252, 79-84.

[9] Cowan, M. M. 1999. Plant products as antimicrobial agents. Journal of Clinical Microbiology, 12, 564-582.

[10] Abdallah, E. M. 2011. Plants: An alternative source for antimicrobials. Journal of Applied Pharmaceutical Science, 01(06), 16-20.

[11] Gupta, P. D., Birdi, T. J. 2017. Development of botanicals to combat antibiotic resistance. Journal of Ayurveda and Integrative Medicine, 8, 266-272.

[12] Emeka, U., Iloegbunam, N. G., Gbekele-Oluwa, A. R., Bola, M., 2014. Natural Products and Aquaculture Development. IOSR Journal of Pharmacy and Biological Sciences. 9(2), 70-82

[13] Ramalingum, N., Mahomoodally, M. F. 2014. The Therapeutic Potential of Medicinal Foods. Advances in Pharmacological Sciences, 2014, 354264.

[14] Arulselvan, P., Fard, M. T., Tan, W. S., Gothai, S., Fakurazi, S., Norhaizan, M. E., Kumar, S. S. 2016. Role of Antioxidants and Natural Products in Inflammation. Oxidative Medicine and Cellular Longevity, 2016, 5276130.

[15] Pham-Huy, L. A., He, H., Pham-Huy, C. 2008. Free Radicals, Antioxidants in Disease and Health. International Journal of Biomedical Science, 4(2), 89-96.

[16] Domej, W., Oettl, K., Renner, W. 2014. Oxidative stress and free radicals in COPD – implications and relevance for treatment. International Journal of Chronic Obstructive Pulmonary Disease, 9, 1207-1224.

[17] Lobo, V., Patil, A., Phatak, A., Chandra, N. 2010. Free radicals, antioxidants and functional foods: Impact on human health. Pharmacognosy Reviews,4(8), 118-126.

[18] Guner, A., Aslan, S., Ekim, T., Vural, M., Babac, M. T. 2012. List of Turkish Plants (Vascular Plants). Istanbul: Flora Researches Association and Nezahat Gokyigit Botanical Garden Publishing, Istanbul, 1290p.

[19] Calis, I., Yuruker, A., Tanker, N., Wright, A. D., Sticher, O. 1997. Triterpene saponins from *Cyclamen coum* var. coum. Planta Medica, 63(2), 166-70.

[20] Sajjadi, S., Saboora, A., Mohammadi, P. 2016. Comparison of aglycon and glycosidic saponin extracts of *Cyclamen coum* tuber against *Candida* spp. Current Medical Mycology, 2(2), 40-44.

[21] Speroni E., Cervellati, R., Costa, S., Dall'Acqua, S., Guerra, M. C., Panizzolo C., Utan A., Innocenti G. 2007. Analgesic and antiinflammatory activity of *Cyclamen repandum* S. et S. Phytotherapy Research, 21, 684-689.

[22] Mahasneh, A. M., El-Oqlah, A. A. 1999. Antimicrobial activity of extracts of herbal plants used in the traditional medicine of Jordan. Journal of Ethnopharmacology, 64, 271-276.

[23] Alkowni, R., Jodeh, S., Hussein, F., Jaradat, N. 2018. Phytochemical screening and antibacterial activity of (*Cyclamen persicum* Mill) tuber extracts. Pakistan Journal of Pharmaceutical Sciences, 31(1), 187-192.

[24] Handa, S. S., Khanuja, S. P. S., Longo, G., Rakesh, D. D. 2008. Extraction Technologies for Medicinal and Aromatic Plants, United Nations Industrial Development Organization and the International Centre for Science and High Technology no. 66. Trieste, Italy, 266p.

[25] Wu, C., Chen, F., Wang, X., Kim, H. J., He, G., Haley-Zitlin, V., Huang, G. 2006. Antioxidant Constituents in Feverfew (*Tanacetum parthenium*) Extract and Their Chromatographic Quantification. Food Chemistry, 96,220-227.

[26] Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., Rice-Evans, C. 1999. Antioxidant

activity applying an improved ABTS radical cation decolorization assay. Free Radical Biology and Medicine, 26(9-10), 1231-1237.

[27] Balakrishnan, N., Panda, A. B., Raj, N. R., Shrivastava, A., Prathani, R. B. 2009. The evaluation of nitric oxide scavenging activity of *Acalypha indica* Linn Root. Asian Journal Research Chemistry, 2(2), 148-150.

[28] CLSI (Clinical and Laboratory Standards Institute). 2008. Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated from Animals; Approved Standard. (Third Ed.), CLSI, Wayne, PA, USA. http://clsi.orgaccessed 26 March 2015; CLSI document VET01-A3 (formerly M31-A3)

[29] Taiz, L., Zeiger, E. 1998. Plant Physiology. 2nd Edition, Sinauer Associates Publishers, Massachusetts, 778p.

[30] Altunkeyik, H., Gulcemal, D., Masullo, M., Alankus-Calışkan, O., Piacente, S., Karayıldırım, T. 2012. Triterpene saponins from *Cyclamen hederifolium*. Phytochemistry, 73(1),127-133.

[31] Mihci-Gaidi, G., Pertuit, D., Miyamoto, T., Mirjolet, J. F., Duchamp, O., Mitaine-Offer, A-Claire.; Lacaille-Dubois, M-Aleth. 2010. Triterpene Saponins from *C. persicum*. Natural Product Communications, 5, 1023-1025.

[32] Du, G. R., Li, M. J., Ma, F. W., Liang, D. 2009. Antioxidant capacity and the relationship with polyphenol and Vitamin C in Actinidia fruits. Food Chemistry, 113, 557-562.

[33] Mathew, S., Abraham, T. E. 2006. In vitro antioxidant activity and scavenging effects of *Cinnamomum verum* leaf extract assayed by different methodologies. Food and Chemical Toxicology, 44, 198-206.

[34] Metin, H., Aydin, C., Ozay, C., Mammadov, R. 2013. Antioxidant activity of the various extracts of *Cyclamen graecum* Link tubers and leaves from Turkey. Journal of the Chemical Society of Pakistan, 35, 1332-1336.

[35] Zengin, G., Aktumsek, A. 2014. Investigation of antioxidant potentials of solvent extracts from different anatomical parts of *Asphodeline anatolica* E. Tuzlacı: an endemic plant to Turkey. African Journal of Traditional, Complementary and Alternative Medicines, 11(2), 481-488.

[36] Wang, L., Wang, Z., Li, X. 2013. Preliminary phytochemical and biological activities study of solvent extracts from a cold-field fruit *Malus baccata* (Linn.) Borkh. Industrial Crops and Products, 47, 20-28.

[37] Sarıkurkcu, C. 2011. Antioxidant activities of solvent extracts from endemic *Cyclamen mirabile* Hildebr. tubers and leaves. African Journal of Biotechnology, 10(5), 831-839.

[38] Mazouz, W., Djeddi, S. 2014. Biological Properties of Algerian *Cyclamen africanum* Extracts. Advancesin Environmental Biology, 8(4), 900-903.

[39] Cuhna, B. A. 2000. The diagnostic significance of relative bradycardia in infectious disease. Clinical Microbiology and Infection, 6(12), 633-634.

[40] Shariff, N., Sudarshana, M. S., Umesha, S., Hariprasad, P. 2006. Antimicrobial activity of *Rauvolfia tetraphylla* and *Physalis minima* leaf and callus extracts. African Journal of Biotechnology, 5, 946-950.

[41] Calış, I., Satana, M. E.,Yürüker, A., Kelican, P., Demirdamar, R., Alacam, R., Tanker, N., Rüegger H., and Sticher, O. 1997. Triterpene saponins from *Cyclamen mirabile* and their biological activities. Journal of Natural Products, 60, 315-318.

[42] Varposhti, M., Ahya, A. A., Mohammadi, P., Saboora, A. 2013. Effects of Extracts and an Essential Oil from Some Medicinal Plants against Biofilm Formation of *Pseudomonas aeruginosa*. Journal of Medical Microbiology and Infectious Diseases, 1(1), 36-40.

[43] Okmen, G., Erdal, P., Isik, D., Bayrak, D. 2014. The antibacterial activities against mastitis pathogens of *Cyclamen mirabile* Hildebr. tubers and its non-enzymatic antioxidant activities. Pelagia Research Library European Journal of Experimental Biology, 4(2), 370-374.