J. Int. Environmental Application & Science, Vol. 14(3): 110-115 (2019)

Research Paper

Antibacterial, Antioxidant and DNA Interaction Properties of *Cistus creticus* L. Extracts

Dudu Duygu Kilic[1], Belgin Siriken[2], Omer Erturk[3], Güler Tanrikulu[4], Melek Gül[4], Ceren Başkan[5,*]

[1]Amasya University, Faculty of Arts and Sciences, Department of Biology, Amasya, Turkey; [2]*Ondokuz Mayis University, Faculty of Veterinary Medicine, Aquatic Animal Diseases, Samsun, Turkey;* [3]*Ordu University, Faculty of Arts and Sciences, Department of Biology, Ordu Turkey;* [4]*Amasya University, Faculty of Arts and Sciences, Department of Chemistry, Amasya, Turkey;* [5]*Amasya University, Sabuncuoğlu Şerefeddin Health Services Vocational School, Amasya, Turkey*

Received June 08, 2019; Accepted September 03, 2019

Abstract: Medicinal plants are a natural source that possesses significant biological functions. *Cistus* (*C.*) *creticus* which are medical plant are of antibacterial, antioxidant and cytotoxic activities. This study was performed to investigate the antibacterial, antioxidant and DNA interaction properties of *C. creticus* extracts. Samples of *C. creticus* L. were obtained in May 2018 in the region of Amasya. The different extracts of *C. creticus* leaves were obtained by using soxhlet apparatus for 4 hours. Antibacterial activity of extracts was tested against four Gram positive and four Gram negative by the disc diffusion method. Antioxidant activities of extracts were determined with DPPH radical scavenging, ferric reducing and metal chelating methods. The ability to repair the plasmid DNA breaks created by hydroxyl radicals was also determine using pUC18 plasmid DNA. As a result, *C. creticus*. extracts have strong inhibitory activity against all tested bacteria. The chloroform extract was also very effective against Gram positive bacteria especially *Bacillus cereus*. Among the Gram-negative bacteria, the most susceptible bacterium was identified as *Pseudomonas aeruginosa*. Moreover, ethanol extract had repair effects on plasmid DNA in H_2O_2 condition. Among the different extracts of *C. creticus* chloroform extract showed the highest in vitro antioxidant activity. In this study, *C. creticus* collected from Amasya has shown significant antibacterial, antioxidant and DNA interaction activity and could be therefore a useful source in the discovery of new antibacterial and antioxidant compounds. However, the use of extracts as a complement to the treatment requires further research to thoroughly understand the activities and interactions with recommended medicines.

Keywords: *Cistus creticus L. Antioxidant, Antimicrobial, DNA interaction.*

Introduction

Cistus species are found in the Cistaceae family, which is a large family. They are in the form of perrenial shrubs with pink or white flowers (Stępień et al., 2018; Menor et al., 2013). *Cistus* species are colloquially known as "rock roses" (Hocking, 1997; Bouamamaa *et al*., 2006). There are 20 *Cistus* species in the world, and they are mostly distributed in the Mediterranean region, Middle East Europe, West Africa and Asian countries. There are five different types of *Cistus* in Turkey, which are *C. creticus* L., *C. laurifolius* L., *C. monspeliensis* L., *C. parviflorus* Lam, *C. salviifolius* L. (Szeremeta et al., 2018; Catoni et al., 2012; Comandini et al., 2006; Güvenç et al., 2005).

Some plants are used in the parfum industry, while others have been used in traditional folk medicine since ancient times. Chemical studies on different genera have basically shown that their antioxidant properties, which are considered to have therapeutic potential, originate from profenolic compounds and terpenoids (Kupeli & Yesilada, 2007; Menor *et al*., 2013; Stępień, 2017). Oxygen reactive forms such as peroxide, superoxide and hydroxyl radicals cause many oxidative stress-related diseases, including diabetes and Alzheimer's disease. The ability of antioxidants to sweep toxic oxygen radicals is very important in this sense.

Thanks to its rich chemical compounds, it has anti-inflammatory, antibacterial, antifungal, antiviral, anti-allergic effects and is used as a therapeutic agent against various diseases, strengthening the body resistance. In addition, various ethnobotanical studies have shown that *Cistus* species are a good remedy for infections (Bassole & Juliani, 2012; Salin *et al*., 2011). Furthermore, the studies have shown that

*Corresponding: E-Mail: ceren.yavuz@amasya.edu.tr; Tel: 00903582400016

extracts obtained from *Cistus* species have antiulcer, wound healing, vasodilator and cytotoxic effects (Güvenç *et al*., 2005; Stępień, 2017). However, no study has been conducted on DNA interaction of creticus.

The aim of this work is to determine the antibacterial activity of extracts obtained from the leaves of *C. creticus* L. plant found in Amasya province by using disk diffusion test, and to determine the antioxidant activities with the methods of radical scavenging activity (DPPH), metal chelating activity and ferric reducing antioxidant power assays. Additionally, the protective role of *C. creticus* L. extracts on hydroxyl radical-induced DNA damage has been investigated through plasmid DNA.

Materials And Methods

Sample extraction

C. creticus L. was collected from natural population which are located in Amasya in May 2018 (Baytop, 1999). The leaves of *C. creticus* L. were then dried at room temperature. The plants (25 g) were extracted with the soxhlet tool (Isolab, Turkey) for 4 hours in ethanol, dichloromethane (DCM) and n-hexane (HXN) (prepared by using solvents of different polarity). The extracts were filterd by Whatman No. 1 paper. The solvents were removed under a rotary vacuum until dry (Heidolph Collegiate, LV28798826, New Jersey, USA). Then the residue dissolved in Tetrahydrofuran (THF, Sigma) for antimicrobial activity (20 mg/mL). The extracts samples were stored at 4 °C in dark bottle for investigation (Bouyahya et al., 2016).

Test organisms and culture condition for antibacterial analysis

Extracts of *C. creticus* L. were tested against Gram positive (*Staphylococcus aureus* ATCC 25923, *Staphylococcus aureus* ATCC 25953, *Bacillus cereus* ATCC 7064 and *Bacillus subtilis* ATCC 6633) and Gram-negative bacteria (*Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Pseudomonas aeruginosa* ATCC 9027 and *Salmonella enteritidis* ATCC 13076). Before use, Bacterial strains were subcultured overnight at 37°C for 18 to 24 h in Tryptic Soy Broth (TSB, Oxoid, Hampshire, UK).

Antibacterial activity

Antibacterial activity of the *C. creticus* L. extracts were investigated by the disc diffusion method (CLSI 2010). *S. aureus* ATCC 25923, *S. aureus* ATCC 25953, *E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853, *P. aeruginosa* ATCC 9027, *B. cereus* ATCC 7064, *B. subtilus* ATCC 6633, and *S. enteritidis* ATCC 13076 were used in bacterial strains. The concentrations of the microorganisms were adjusted using turbidity measurements (0.5 McFarland) using serum physiologic solution. The concentration of bacterial suspensions was adjusted to 10^8 cells/mL. Then, extracts (20 mg/mL) prepared in THF loaded 6 mm diameter sterile blank discs (Oxoid). Inhibition zones were determined after incubation at 37 °C for 24 h. As a positive control for bacteria, gentamicin (10 µg) and ceftriaxone (30 µg) were placed in Petri dishes. All tests were carried out in three times.

Antioxidant activity

Free radical scavenging activity

The free radical scavenging activity was determined with 1,1-diphenyl-2-picryl-hydrazyl (DPPH•) using methods of Brand-Williams (Brand-Williams et al., 1995). Different concentrations of plant material were prepared, and 0.75 mL of this extract was added the 1.5 mL of 20 mg/L DPPH• solution in methanol. This solution was added to, butylated hydroxytoluene (BHT), and trolox (25-400 µg/mL). The mixture was shaken vigorously, and the decrease in absorbance at 517 nm was measured for 30 min at room temperature. Water (0.75 mL) in place of the sample was used as control. IC50 value was calculated to use the linear regression as the concentration required for 50% reduction of the DPPH radical. The percent inhibition activity was calculated using the following equation: free radical scavenging effect % = $[(A0 - A1)/A0]\cdot 100$. ($A0$ = the control absorbance and $A1$ = the sample solution absorbance).

Metal chelating activity

The chelating activity of extract on ferrous ions (Fe^{2+}) was measured according to the method of Decker and Welch (Decker and Welch, 1990). Aliquots of 1 mL of different concentrations (100–500 µg/mL)

of extracts were mixed with 3.7 mL of deionized water. The mixture was incubated with $FeCl_2$ (2 mM, 0.1 mL) for 30 min. After incubation, the reaction was initiated by addition of ferrozine (5 mM and 0.2 mL) for 10 min at room temperature, and then the absorbance was measured at 562 nm. A lower absorbance indicates a higher chelating power. The chelating activity of the extract on Fe^{2+} was compared with that of EDTA at the same concentrations. Metal chelating activity (%) = $[(A0 - A1)/A0]\cdot 100$.

Estimation of total phenolic content

According to the methods of Slinkard and Singleton (Slinkard and Singleton, 1977), using Folin–Ciocalteu reagent were determined depending on phenolic standard gallic acid. 1 mL of the plant extract was introduced into test tubes followed by 1 mL Folin–Ciocalteu's reagent. The solution was kept in the dark for 5 min and then 3 mL of sodium carbonate (2%) was added. The tubes were covered with parafilm and kept again in the dark for 1 h and were measured absorption at 765 nm with a spectrophotometer and compared to a gallic acid calibration (GAE) curve. The results were expressed as mg gallic acid/g dried sample. Each assay was carried out in triplicate.

Estimation of total flavonoid content

Total flavonoid content was determined with quercetin standard solution using Park method (Park et al., 2008). The plant extract in 0.3 mL of was introduced into test tubes followed by 3,4 mL 30% methanol, 0.15 mL of 0,5 M $NaNO_2$ and 0,3 M $AlCl_3$ reagent. After 5 min 1 mL of 1 M NaOH was added and measured absorption at 506 nm with a spectrophotometer and compared to a quercetin calibration curve. Each assay was analyzed in three times. The total flavonoids were described as mg of quercetin equivalents (QTE) per g of dried fraction.

DNA interaction assay

To explore the beneficial effect of the *C. creticus* L. extracts on hydroxyl radical-mediated DNA damage plasmid DNA pUC18 (Thermo Scientific) was used. Firstly, the *C. creticus* L. extracts were dissolved Dimethyl sulfoxide (DMSO, concentration range from 12.5 to 100 mg/ml). A reaction mixture (20 µl final volume) containing 0.25 µg/µl plasmid DNA pUC18, 1 µl of 3% H_2O_2, 0.1 g/ml *C. creticus* L. extracts in Tris-EDTA (TE) buffer was prepared. H_2O_2 and 0.1% tetrahydrofuran treated plasmid DNAs were used as control groups. Secondly, the prepared mixture for each *C. creticus* L. extracts was incubated for 24 h at 37°C. 2 µl loading dye (bromophenol blue [0.025%] and sucrose [4%] in dH_2O) was added into the mixture (10 µl total volume) and loaded on to the 1% agarose gel. Electrophoresis process was for 90 minutes at 80 V in TBE running buffer (pH 8). The Gel was imaged under UV light (Attaguile et al., 2000; Ayvaz et al., 2018).

Results and Discussion

In this work, results indicate that ethanol, DCM and HXN extracts showed antibacterial activity with specific differences according to the test microbial strain. Gram negative bacteria are more sensitive than Gram positive bacteria. Infact, the disc diffusion tests ranged from 9-18 mm for the *C. creticus* L. ethanol extract, 8-11 mm for the *C. creticus* L. DCM extract and 8-14 mm for the *C. creticus* L. HXN extract. Extracts revealed an improved antibacterial activity against *Staphylococcus aureus* compared with that of antibiotics ceftriaxone and gentamicin. The highest diameter of inhibition is found with ethanolic extract against *Pseudomonas aeruginosa* ATCC 27853 and *P. aeruginosa* ATCC 9027 strains. Thus, *P. aeruginosa* shows extreme sensitivity, and the six other bacteria espond very positively to its antibacterial activity. Furthermore, the extracts of *C. creticus* L. were effective against *E. coli* except for the DCM extracts. Especially ethanol of *C. creticus* L. were found to be active against all bacteria. The results are showed in Table 1.

Table 1. Antibacterial effect of the extract against wild type microorganisms (mm)

	Microorgansims							
	B.s	**B.c**	**P.a 9027**	**P.a 27853**	**S.a 25953**	**S.a 25923**	**S.e**	**E.c**
Ethanol	11	10	14	18	10	10	9	10
Dichloromethane	9	14	10	9	8	8	8	-
n-Hexane	10	11	14	9	9	8	10	8
CRO 30 µg	27	13	14	30	24	32	14	30
CN 10 µg	20	21	21	21	21	20	23	21

Microorganisms: B.s: *Bacillus subtilis* ATCC 6633; B.c: *Bacillus cereus* ATCC 7064; P.a. *Pseudomonas aeruginosa* ATCC 27853 and *Pseudomonas aeruginosa* ATCC 9027; S.a: *Staphylococcus aureus* ATCC 25953 and *Staphylococcus aureus* ATCC 25923; S.e: *Salmonella enteritidis* ATCC 13076; E.c: *Escherichia coli* ATCC 25922. CRO: Ceftriaxone, CN: Gentamicin.

Overall, Gram-negative bacteria exhibited higher sensitivty to the extract antimicrobial properties than the Gram-positive ones. These results are consistent with those found in literature. These results could be partially related to the phenolic composition of the extract, Additionally, the antioxidant capacities of those extracts showing high polyphenolic content were determined via a panel of antioxidant measurements. The DPPH assay of the extracts showed potent antioxidant activity, which was comparable to that of the synthetic antioxidant BHT and Trolox. High DPPH activity was found at the ethanol extracts. Also, calculated IC50 value of the ethanol, DCM, HXN; 165.10, 189.71, 397.29 respectively. One recent study reported that *C. creticus* L. subsp. eriocephalus plant possess highest activity (Bullitta et al., 2013). Antioxidant properties of *C. creticus* using metal chelating assay are shown in Figure 1.

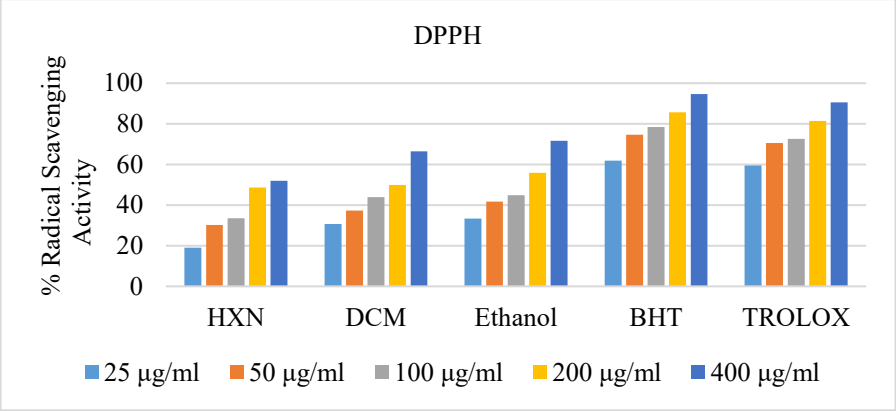

Figure 1. DPPH radical scavenging of extracts

Among extracts, antioxidant activity in terms of metal chelating activity, ranged between 43 and 32%, whereas it ranged between 95 and 75 % in the EDTA standard.

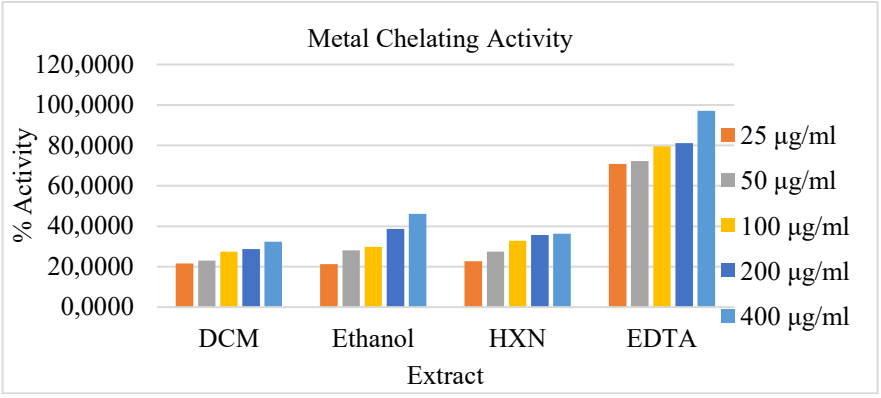

Figure 2. The results for metal chelating activities of extracts

Table 2 shows the total phenolic and total flavonoids content in the different solvent extracts of *C. creticus* in Amasya. Among the extracts, the highest phenolic content was found in ethanol (130.32 mg GAE/g dry wt.) followed by DCM and HXN. The flavonoid content was the highest in ethanol extracts. The differences in flavonoid content in extracts DCM and HXN were observed to be the insignificant however total phenolic content amount were very different in extracts.

Table 2. Total phenolic and flavonoid content

	Total Phenolic Content (mg GAE/g)	**Total Flavonoid Content (mgQTE/g)**
n-Hexane	68.3163	60.71
Dicholoromethane	112.1473	63.98
Ethanol	130.3205	83.94

Reactive oxygen species, from both endogenous and exogenous sources, may be involved in the etiology of diverse human diseases, such as coronary heart disease, infammation, neuro-degenerative diseases. According to gel electrophoresis, extracts were dissolved in DMSO and 0.25 µg/µl pUC18 plasmid DNA was treated with 12.5, 25, 50 and 100 mg/mL extracts respectively (Figure 1).

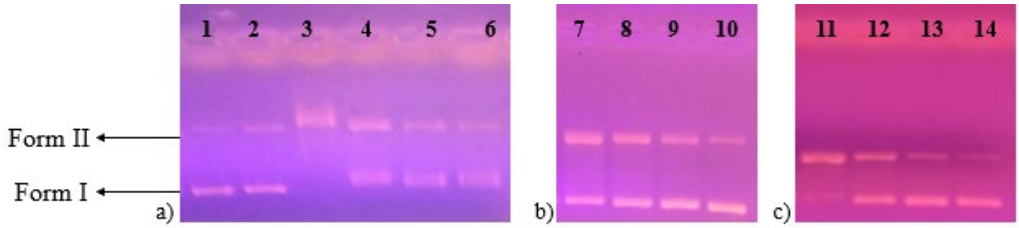

Figure 3. Gel image of extracts of *C. creticus* L. a) Lane 1: pUC18 plasmid DNA control (blank); Lane 2: DMSO control; Lane 3-6: H_2O_2, pUC18 plasmid DNA and different concentration of ethanolic extracts (12,5-100 mg/mL). b) Lane 7-10: H2O2, pUC18 plasmid DNA and different concentration of dichloromethan extracts (12,5-100 mg/mL). c) Lane 11-14: H_2O_2, pUC18 plasmid DNA and different concentration of n-Hexanolic extracts (12,5-100 mg/mL)

Figure 3 shows the gel imagene of DNA after UV-photolysis of H_2O_2 in the absence and presence of different extracts of leaves of *C. creticus*. DNA derived from pUC18 plasmid showed two bands on agarose gel electrophoresis (lane 1), the faster-moving band corresponding to the native form of supercoiled circular DNA and the slower-moving band being the open circular form (Attaguile et al., 2000). Increasing doses of *C. creticus* extracts had a protective effect on hydroxyl radical-mediated plasmid DNA damage, but a low concentration of these extracts had no protective effect on plasmid DNA in H_2O_2 conditions. It appears that extracts, ethanol, DCM and HXN, exhibit relatively similar effects against plasmid DNA.

Conclusion

The findings highlighted that *C. creticus* extracts were able to inhibit the growth of a wide spectrum of bacterial strains, known for their implications in infections. Moreover, the results of this study suggest that their antioxidant and DNA interaction activities. Hence, this extracts of *C. creticus* can be a promising agent to control microbial growth, even if more detailed reports on its toxicity and mechanisms of action are requested to overcome the impediment of its application in several industries.

References

Attaguile G, Russo A, Campisi A, Savoca F, Acquaviva R, Ragusa N, Vanella A, (2000) Antioxidant activity and protective effect on DNA cleavage of extracts from *Cistus incanus* L. and *Cistus monspeliensis* L. *Cell Bio. & Toxico.*, **16**, 83-90.

Ayvaz MÇ, Ömür B, Ertürk Ö, Kabakçı D, (2018) Phenolic profiles, antioxidant, antimicrobial, and DNA damage inhibitory activities of chestnut honeys from Black Sea Region of Turkey. *Journal Food Biochemistry*, **42,** 1-10.

Bassole IHN, Juliani HR, (2012) Essential oils in combination and their antimicrobial properties. *Molecules*, **17**, 3989-4006.

Baytop T, (1999) *Therapy with Medicinal Plants in Turkey (Past and Present)*, Nobel Medical Press, Istanbul.

Bouamamaa H, Noel T, Villard J, Benharref A, Jana M, (2006) Antimicrobial activities of the leaf extracts of two Moroccan *Cistus* L. species. *Journal of Ethnopharmacology*, **104**, 104-107.

Bouyahya A, Moussaoui NE, Abrini J, Bakri Y, Dakka N, (2016) Determination of Phenolic Contents, Antioxidant and Antibacterial Activities of Strawberry Tree (*Arbutus unedo* L.) Leaf Extracts. *British Biotech. J.*, **14** (3), 1-10.

Brand-Williams W, Cuvelier ME, Berset C, (1995) Use of a Free Radical Method to Evaluate Antioxidant Activity. *LWT-Food Sci. Techno.*, **28**, 25–30.

Bullitta, S., Piluzza, G., Manunta, M.D.I. (2013). Cell-Based and Chemical Assays of The Ability to Modulate the Production of Intracellular Reactive Oxygen Species of Eleven Mediterranean Plant Species Related to Ethnobotanic Traditions. *Genet Resour Crop Evol*. **60**, 403–412.

Catoni R, Gratani L, Varone, L, (2012) Physiological, morphological and anatomical trait variations between winter and summer leaves of Cistus species. *Flora –Morphology, Distribution, Functional Ecology of Plants*, **207**, 442-449.

Clinical and Laboratory Standards Institute (CLSI) (2010) Performance Standards for Antimicrobial Susceptibility Testing; Twentieth Informational Supplement. M100-S20, Vol:**30** No: 1.

Comandini O, Contu M, Rinaldi AC, (2006) An overview of *Cistus* ectomycorrhizal fungi. *Mycorrhiza*, **16**, 381-395.

Decker EA, Welch B, (1990) Role of Ferritin as a Lipid Oxidation Catalyst in Muscle. *Food J. Agric. Food Chem.*, **38**, 674–677.

Güvenç A, Yıldız S, Özkan AM, Erdurak CS, Coşkun M, Yılmaz G, Okuyama T, Okada Y, (2005) Antimicrobiological studies on Turkish *Cistus* species. Pharmaceutical Biology, 43 (2), 178-183.

Hocking GMD, (1997). *A Dictionary of Natural Products*, 2nd Ed., Plexus Publishing, Medford, N.J. 190-191.

Kupeli E, Yesilada E, (2007) Flavonoids with anti-inflammatory and antinociceptive activity from *Cistus laurifolius* L. leaves through bioassay-guided procedures. *J. Ethnoph.*, **112**, 524-530.

Menor TL, Soto MA, Catalan BE, Segura RC, Carretero SA, Micol V, (2013) Correlation between the antibacterial activity and the composition of extracts derived from various Spanish *Cistus* species. *Food & Chem. Toxico*., **55**, 313-322.

Oyaizu M, (1986) Studies on Product of Browning Reaction Prepared from Glucose Amine. *The Jap. J. Nutrition & Dietetics*, **44**, 307–315.

Park YS, Jung ST, Kang SG, Heo BKH, Arancibia-Avila P, Toledo F, Drzewiecki J, Namiesnik J, Gorinstein S, (2008) Antioxidants and Proteins in Ethylenetreated Kiwifruits. *Food Chem*., **107**, 640–648.

Salin OP, Pohjala LL, Saikku P, Vuorela HJ, Leinonen M, Vuorela PM, (2011) Effects of coadministration of natural polyphenols with doxycycline or calcium modulators on acute Chlamydia pneumoniae infection in vitro. *J Antibiotics*, **64**, 747-752.

Slinkard K, Singleton VL, (1977) Total Phenol Analyses: Automation and Comparison with Manual Methods. *Am. J. Enology & Viticulture*, **28**, 49–55.

Stępień AE, (2017) Cytotoxic and anti-cancer activity of the *Cistus* species of herbal plants.

Stępień A, Aebisher D, Aebisher BD, (2018) Biological properties of *Cistus* species. *Eu. J .Clini. & Experi. Med.*, 16 (2), 127-132.

Szeremeta D, Knaś M, Długosz E, Krzykała K, Mrozek-Wilczkiewicz A, Musioł R, Kowalska T, Ott PG, Sajewicz M, Móricz AM, (2018) Investigation of antibacterial and cytotoxic potential of phenolics derived from *Cistus incanus* L. by means of thin-layer chromatography-direct bioautography and cytotoxicity assay. *Journal of Liquid Chromatography & Related Technologies,* **41** (6), 349-357.