Iğdır Üniversitesi Fen Bilimleri Enstitüsü Dergisi, 12(3):1550 - 1557, 2022
Journal of the Institute of Science and Technology, 12(3): 1550 - 1557, 2022

ISSN: 2146-0574, eISSN: 2536-4618

Food Engineering **DOI: 10.21597/jist.1099409**

Research Article

Received: 06.04.2022 **Accepted: 06.06.2022**

To Cite: Oguz Akin S, Yesil Celiktas O, Sevimli Gur C, 2022. Anticancer, Antioxidant and Antimicrobial Activities of Some Mediterranean Plants Extracts. Journal of the Institute of Science and Technology, 12(3): 1550 - 1557.

Anticancer, Antioxidant and Antimicrobial Activities of Some Mediterranean Plants Extracts

Sevgi OĞUZ AKIN[1], Özlem YEŞİL ÇELİKTAŞ[2], Canan SEVİMLİ GÜR[3*]

ABSTRACT: This research was carried out to determine the anti-tumorigenic, antioxidant and antimicrobial activities of extracts obtained from *Juniperus oxycedrus L. oxycedrus* (Cupressaceae) and *Smilax aspera L.* (Smilacaceae) fruits. The cytotoxic effects of ethanol extracts of *Juniperus oxycedrus L. oxycedrus* and *Smilax aspera L.* fruits were determined with six different tumorogenic cell lines including breast adenocarcinoma, small cell lung carcinoma, osteosarcoma, neuroblastoma and healthy kidney epithelial cells. Among the tested ethanol extracts of *Juniperus oxycedrus L. oxycedrus* and *Smilax aspera L.* fruits the ethanol extract obtained from *Juniperus oxycedrus* fruits was determined to have the highest anti-tumorigenic effect against small cell lung carcinoma with an IC_{50} value of 7.2 µg ml^{-1}. At the end of cytotoxicity studies, ethanol extracts of *Juniperus oxycedrus L. oxycedrus* fruits proved to be good candidates for small cell lung carcinoma (A569). Antimicrobial effects were analyzed by the MIC test. MIC values of ethanol extracts of *Juniperus oxycedrus L. oxycedrus* and *Smilax aspera L.* fruits against *Escherichia coli an*d *Candida albicans* were found to be 31.25 µg ml^{-1}. Moreover, the radical scavenging capacity of *Juniperus oxycedrus L. oxycedrus* and *Smilax aspera L.* fruit extracts was elucidated. *S. aspera L.* (61%) and *J. oxycedrus L. oxycedrus* (47.3%) were found to have good free radical scavenging capacity.

Keywords: Antitumorogenic, antioxidant, antimicrobial, *Juniperus oxycedrus*, *Smilax aspera*

[1] Sevgi OGUZ AKIN (**Orcid ID:** 0000-0001-6349-4536) Kocaeli University, Science Faculty, Biology Department, Kocaeli, Turkey

[2] Ozlem YESIL CELIKTAS (**Orcid ID:** 0000-0003-4509-2212), Ege University, Engineering Faculty, Bioengineering Department, Izmir, Turkey

[3] Canan SEVIMLI GUR (**Orcid ID:** 0000-0002-2210-5925), Izmir Katip Celebi University, Pharmacy Faculty, Basic Pharmaceutical Science Department, Izmir, Turkey

***Corresponding Author:** Canan SEVIMLI GUR, e-mail: cananSevimli@yahoo.com

This study was produced from Sevgi OĞUZ AKIN's Master's thesis.

The article was presented as an oral presentation at the "ICONTES" congress held in Antalya on October 1-15, 2018.

INTRODUCTION

A wide variety of plant species have been scientifically researched for bioactive compounds and contributed to the development of new drugs. Some potential antitumor compounds isolated from natural products have led researchers to determine the antitumor activity of different parts of plants. Also, it is the development of multi-drug resistance in human pathogenic microorganisms due to the random and misuse of commercial antimicrobial drugs that we have witnessed much more in recent years. This has prompted the detection of new antimicrobial agents from medicinal plants, which are good source of new antimicrobial chemotherapeutic agents (Karaman et al., 2003). Many conifer species have been reported to have cytotoxic activities on some tumor cell lines (Sadeghi-Aliabadi et al., 2013, Subramanyan et al., 2022). The most common species of the genus *Juniperus,* one of the conifers of the Cupressaceae family, is *Juniperus oxycedrus L. oxycedrus.* Although it can be seen in Thrace and many parts of Anatolia, it is essentially a Mediterranean plant (Koyuncu et al., 2007). In Turkey, it is generally used as a traditional medicine to heal wounds, treatments of stomachache and stomach ailments, gynecological diseases, hemorrhoids, colds, cough, bronchitis, arthritis, fungal infections, diabetes, kidney inflammation and kidney stones (Tuzlaci and Erol, 1993).

Saponins have attracted a growing interest in many different biological activities including antidiabetic, antitumor, cough suppressant and anti-demantia and platelet aggregation inhibitors (Sparg et al., 2004). *Smilax aspera* L., known as Saparna, which is the subject of many studies conducted in recent years due to the saponins it contains, is a typical Mediterranean Region plant of evergreen, extremely hard bushes belonging to the family Liliaceae. It has been reported that *Smilax* has many pharmacological properties used to treat diseases such as cancer, diabetes, wounds, inflammations, boils and skin diseases including ulcers (Damayanthi et al., 2011; Wang et al. 2022). It is also used as an antioxidant source in the treatment of diseases such as fever, gout, diuretic, ophthalmia, infertility. In this study, the ethanolic extracts of *J. oxycedrus* and *S. aspera* were screened for anticancer, antioxidant and antimicrobial activities.

MATERIALS AND METHODS

Collection and Storage of Plant Material

Fruits of *Juniperus oxycedrus L. oxycedrus* and *Smilax aspera L.* species were collected in October 2009 from Mordogan location, İzmir. After washing with tap water, they were dried at room temperature (+24^{o}C) protected from moisture and light. then ground in a conventional grinder and stored at +4^{o}C in the dark until extraction.

Extraction of Samples with Soxhlet

Extraction of ground and stored fruits of *Juniperus oxycedrus L. oxycedrus* and *Smilax aspera* L. species was done with 1000 ml ethanol (99,5%) for five cycles (approximately 10 hours) using a soxhlet (500 ml) apparatus. Then, the extracts were concentrated to dryness by using Laborato 4001, Heidolph rotary evaporator in vacuum at 70 ºC and finally lyophilized and stored (-20^{o}C) (Yesil-Celiktas O. et al. 2009).

Cell Lines

MCF7 (human, breast, adenocarcinoma), A549 (human, small cell lung carcinoma), MDA-MB-231 (human, breast, adenocarcinoma), Saos2 (human, osteosarcoma), Neuro2A (*Mus musculus,* neuroblastoma), NA2B (human, neuroblastoma) cancer cell lines and Vero (African green monkey kidney epithelium) healthy cell line purchased from the American Cell Culture Collection (ATCC Manuassas, VA) using medium in DMEM F-12 supplemented with 10% fetal bovine serum. Then,

they were grown at 37ºC in a 5%CO_2 humid atmosphere. They were also stored in liquid nitrogen (-196ºC) until MTT test (Sevimli-Gur et al., 2013).

Measuring the Cytotoxic Effect of Extracts

The cytotoxic activities of the obtained herbal extracts were analyzed on different cancer cells lines and healthy cell line by using the MTT test for determination of anticancer effects. Doxorubicin (10 µg ml^{-1}) was used as positive control in cytotoxic activity assays. Cells were distributed at 6000 cells/well in 96-well plates. Then, the ethanol-solublextracts were added into the wells containing 100 µl of medium cells each. Cells were treated with the extracts for 72 hours. The negative control was treated with 0.1% ethanol. Formazan crystal formation was measured spectrophotometrically (Versamax, Tunable Microplate Reader, USA) at 570-690 nm. Final data was found from the mean values of the dependent and independent triplicate analyses. Cytotoxicity was made sense by normalizing to the percentage of cell viability (Sevimli-Gur et al., 2013).

Determination of Free Radical Scavenging Effects of the Extracts

While determining the free radical scavenging activities of fruit extracts *of Juniperus oxycedrus L. oxycedrus and Smilax aspera L.* species, firstly, extracts at a concentration of 25 mg ml^{-1} were dissolved in 4 ml of methanol. 0.5 ml of 1 mM 2, 2-diphenyl-1-picrylhydrazil hydrate (DPPH) was added to the methanolic solution. The resulting solution was stirred for 15 seconds and then left in the dark for 30 minutes at room temperature. The absorbance of the solution was read spectrophotometrically against methanol at 517 nm using a Shimadzu UV-2401 spectrophotometer. The absorbance readings were calculated as the % inhibition of DPPH radical (% I) using the antioxidant activity Equation (1).

$$\% \text{ Inhibition (I)} = [(A_{DPPH} - A_{Ext})/A_{DPPH}) \times 100] \quad (1)$$

A_{DPPH}: absorbance of control DPPH solution at 0 min

A_{Ext}: absorbance in the presence of the sample of the extract after 30 min

Determination of Antimicrobial Activity of the Extracts (MIC Test)

Minimum inhibitory concentrations (MIC) of the extracts were established by liquid microdilution method in 96-well culture dishes. The test bacteria MRSA (*Staphylococcus aureus*, ATCC 43300) and *Escherichia coli*, O157: H7 RSKK 234 CLSI) (Weinstei, 2018) inoculum regulated to the 0.5 McFarland standard after preparation of active cultures for 18 hours or overnight at 37 °C in cation-adjusted MHB (Mueller-Hinton Broth) according to the M07-A9 standard. 100 µl of MHB was dispensed into each well. Then, serial dilutions were made by adding 1250 ug ml^{-1} -0.225 µg ml^{-1} concentrations of the extracts arranged in stock solutions in DMSO to the wells. For bacteria, the final inoculum concentration was added to 100 µl wells at a concentration of 5 x 10^5 CFU ml $^{-1}$. After inoculation, the plates were incubated at 37 °C for 24 hours and the results were evaluated. MIC testing for *Candida albicans* was performed using RPMI-1640 medium according to CLSI, M27-A2 standard, and extracts were dispensed into wells by serial dilutions as mentioned above. After the 24-hour active culture of the yeast was prepared, a final concentration of $0.5x10^3$-$2.5x10^3$ CFU ml $^{-1}$ was included to the wells and incubated at 35°C for 24-48 hours. The test was done in triplicate. For sterility control, a separate well containing only the extract and medium was used. For the control of growth, a well containing only bacteria and medium was used as a separate test group. Ampicillin was used as positive control for bacteria and cycloheximide for the yeast.

Statistical Evaluation of Data

Data analyzes were done statistically using the student-t test. In this study, the probability value of $p \leq 0.01$ indicates the strength of significance. A $p \leq 0.05$ indicates a statistically significant difference. In this study, data are given as mean values ± standard error of mean (S.E.M.).

RESULTS AND DISCUSSION

Anticancer Activity

A growing number of studies in literature suggests that *Juniperus* and *Smilax* species may be the sources of bioactive compounds with potential anticancer activity (El-Sayed et al., 2022; Begum et al., 2022). In this study, ethanol extracts of *Juniperus oxycedrus L. oxycedrus* and *Smilax aspera* strains were tested on six cancer cell lines including breast adenocarcinoma, small cell lung carcinoma, osteosarcoma neuroblastoma cells and kidney epiteliel healthy cells. Besides, IC_{50} values were determined, and cytotoxicity tests were carried out with MTT at concentrations of 6.25-100 µg ml^{-1}. Taviano et al. (2013) found that *J. oxycedrus* had no cytotoxic effect on HepG2 (human hepatocellular liver carcinoma) tumor cell line at a concentration of 10 µg ml^{-1} with 100% cell viability at concentrations of 0-10 µg ml^{-1}. In this study, *J. oxycedrus* showed a cytotoxic effect on NA2B (human neuroblastoma) tumor cell line at a concentration of 6.25 µg ml^{-1} with a 73% cell viability.

As a result of cytotoxicity tests, growth inhibition of A549 and NA2B tumor cells at 100 µg ml^{-1} concentration of crude ethanol extract of *J. oxycedrus* was found to be 37% and 24.5%, respectively. At a concentration of 6.25 µg ml^{-1}, it was calculated as 50.5% and 73%, respectively. The inhibition of doxorubicin, the positive control, on these cell lines was 35% and 30%, respectively; *J. oxycedrus* was found to have a better cytotoxic effect than doxorubicin with a cell viability of 24.5%, especially in the NA2B cell line at a concentration of 100 µg ml^{-1}. In addition, the cell viability of doxorubicin in Vero normal cell line was 62%, while the cell viability of *J. oxycedrus* at a concentration of 100 µg ml^{-1} was 110.5%. This suggests that this cytotoxic effect of *J. oxycedrus*, in particular on the NA2B cell line, may prove promising for its use in the treatment of neuroblastoma (Table 1).

Table 1. The effect of different concentrations of *Juniperus oxycedrus* crude ethanol extracts at different concentrations on the cell viability of various tumor cell lines

Cell lines	**Positive control**	**Concentrations (µg/ml ± SD)**				
	Doxorubicine (**µg ml^{-1} ± SD**)	**100**	**50**	**25**	**12.5**	**6.25**
A-549	35.0 (±1.4)	37.0 (±2.4)	42.0 (±4.4)	44.5 (±2.4)	46.5 (±6.4)	50.5 (±3.4)
MDA-MB-231	65.0 (±4.6)	47.5 (±5.6)	59.0 (±7.6)	64.0 (±5.6)	65.5 (±9.6)	69.0 (±6.6)
Saos-2	44.0 (±5.4)	60.5 (±6.4)	62.5 (±8.4)	64.5 (±6.4)	66.5 (±10.4)	68.5 (±7.4)
MCF-7	69.0 (±7.5)	63.5 (±8.5)	68.0 (±10.5)	75.5 (±8.5)	86.0 (±12.5)	99.0 (±9.5)
NA2B	30.0 (±1.7)	24.5 (±2.7)	47.5 (±4.7)	62.0 (±2.7)	68.5 (±6.7)	73.0 (±3.7)
Neuro2A	120.0 (±5.9)	43.5 (±6.9)	69.5 (±8.9)	70.0 (±6.9)	78.5 (±10.9)	82.5 (±7.9)
Vero	62.0 (±0.3)	110.5 (±1.3)	115.0 (±3.3)	122.5 (±1.3)	133.0 (±5.3)	146.0 (±2.3)

Ivanova et al. (2011) using *S. aspera* as a result of cytotoxic tests performed on FL (normal amniotic human cell) normal cell line and A549 (human lung carcinoma), they found that some saponins isolated from *S. aspera* could be potential therapeutic agents with an IC_{50} value of 62.94 µg ml^{-1} for the A549 cell line. In our study, the IC_{50} value for the A549 cell line was determined to be 138.3 µg ml^{-1}.

Growth inhibition on A549 and NA2B tumor cells at a concentration of 100 µg ml^{-1} of crude ethanol extract of S. aspera was found to be 78% and 53%, respectively (Table 2). It was found that *S. aspera* was not cytotoxic with a cell viability of 225% on the Vero normal cell line.

Table 2. The effect of different concentrations of *Smilax aspera* crude ethanol extracts at different concentrations on the cell viability of various tumor cell lines

Cell lines	Positive control (µgml $^{-1}$± SD)	Concentrations (µg ml $^{-1}$ ± SD)				
		100	50	25	12.5	6.25
A-549	35.0 (±1.4)	78.0 (±2.7)	88.0 (±4.7)	93.0 (±2.7)	97,0 (±6.7)	105.0 (±3.7)
MDA-MB-231	65.0 (±4.6)	99.0 (±5.9)	122.0 (±7.9)	132.0 (±5.9)	135.0 (±9.9)	142.0 (±6.9)
Saos-2	44.0 (±5.4)	125.0 (±6.7)	129.0 (±8.7)	133.0 (±6.7)	137.0 (±10.7)	141.0 (±7.7)
MCF-7	69.0 (±7.5)	53.0 (±3.0)	99.0 (±5.0)	128.0 (±3.0)	141.0 (±7.0)	150.0 (±4.0)
NA2B	30.0 (±1.7)	24.5 (±2.7)	47.5 (±4.7)	62.0 (±2.7)	68.5 (±6.7)	73.0 (±3.7)
Neuro2A	120.0 (±5.9)	91.0 (±7.2)	143.0 (±9.2)	144.0 (±7.2)	161.0 (±11.2)	169.0 (±8.2)
Vero	62.0 (±0.3)	225.0 (±1.6)	234.0 (±3.6)	249.0 (±1.6)	270.0 (±5.6)	296.0 (±2.6)

The IC_{50} values showed the lowest value of *Juniperus oxycedrus* extract with a value of 7.2 µg ml against the A549 cancer cell line. This was followed by NA2B with a value of 47.5 µg ml^{-1} MDA-MB-231 with 62.4 µg ml^{-1} and Neuro2A cell lines with 87.6 µg ml^{-1} (**Table 3**). According to these results it may be appropriate to concentrate on *Juniperus oxycedrus L. oxycedrus* which is common in our country.

Table 3. IC_{50} values (µg ml^{-1}) of *J. oxycedrus L. oxycedrus and S. aspera* extracts

Samples	IC_{50} values (µg/ml ± SD)						
	A549	MDA-MB-231	Saos2	MCF-7	NA2B	Neuro2A	Vero
J. oxycedrus	7.2 (±0.21)	62.4 (±0.52)	112.4 (±2.21)	124.7 (±2.38)	47.5 (±0.29)	87.6 (±1.69)	280.2 (±3.36)
S. aspera	138.3 (±3.29)	197.4 (±5.11)	243.2 (±7.16)	267.4 (±8.21)	104.6 (±1.61)	186.8 (±1.76)	372.7 (±8.18)

Antioxidant Activity

Crude ethanol extracts of *J. oxycerdrus L. oxycedrus* and *S. aspera* were evaluated by DPPH test for antioxidant activity. Extracts from *J. chinensis* showed a strong antioxidant activity in terms of DPPH radical scavenging activity due to the presence of various phenolic compounds (quercetin, naringenin, taxifolin, aromadendrine and isocerrin) which were identified and argued to be responsible for the activity (Lim et al., 2002). Orhan et al. (2011) reported DPPH radical scavenging activity of ethanol extracts obtained from *J. oxycedrus* as 55%. This is in agreement with our study, where DPPH radical scavenging activity of *J. oxycedrus* extract was determined as 47.3%. As for the antioxidant activities of methanolic extracts of *S. zeylanica* root and rhizome, DPPH radical scavenging activity was 7.6 µg ml^{-1} and also reported that the extract exhibited a strong antioxidant activity in different *in vitro* environments (Murali et al., 2011). In this study, DPPH radical scavenging activity of *S. aspera* crude ethanol extract was found to be 61%. In another study, *S. macrophylla* leaves were shown to exhibit high total phenolic (2.2 - 6. 2 mg gallic acid equivalent /g extract) and total flavonoid contents (1.2 - 4.5 mg catechin equivalent /g extract). Leaf extract and fractions were found to possess relatively high levels of antioxidant potential as measured by the DPPH radical scavenging test [(Inhibitor concentration was 50% = 33.4-72.3 µg ml^{-1}) (Zubair et. al. 2017)].

Antimicrobial Activity

After the detection of the first methicillin-resistant *S. aureus* (MRSA) strain in 1961, the treatment of the diseases caused by these strains has been tackled as an important problem among the hospital infectious agents worldwide. Therefore, it is particularly important to screen the activity of various extracts against MRSA to elicit potential compounds. In this study, *J. oxycedrus* extract was determined to have a MIC value of 31.25 µg ml^{-1} against MRSA (Table 4).

Cavaleiro et al. (2006) and Ninich et. al. (2022) found the MIC value of Candida albicans as 1.25-2.5 µg ml-1 in an antifungal activity study with the essential oils obtained from J. oxycedrus. In this study, the MIC values of ethanol extracts of Juniperus oxycedrus L. oxycedrus and Smilax aspera L. fruits against E. coli and C. albicans were found to be 31.25 µg ml-1. In a study, it was found that J.

macrocarpa essential oils exhibited significant antimicrobial activity against Gram-positive bacteria, especially Clostridium perfringens with MIC values ranging between 2-8 µg ml-1 (Lesjaka et al., 2014). In the thesis study of Memis (2011), J. oxycedrus ssp. extracts obtained from fruits were tested against S. aureus Koog (+), E. coli (ATCC 35218), C. albicans (ATCC 16231) and reported not to display antimicrobial activity at concentrations of 0.3 g ml-1 and 0.6g ml-1, respectively (Memis Y., 2011). Based on the results of antimicrobial activity test, the MIC value of J. oxycedrus extract against MRSA is about 40% lower than the value of ampicillin, which indicates the high potential of the extract to be utilized as a biological agent.

Table 4. Minimum inhibition concentration (MIC) values of extracts along with ampicillin as a positive control for bacteria and cyloheximide as a positive control for yeast

Test organisms	*J. oxycedrus* extract (µg ml^{-1})	*S. aspera* extract (µg ml^{-1})	Ampicillin (µg ml^{-1})	Cyloheximide (µg ml^{-1})
MRSA (*S. aureus* ATCC 43300)	31.25	62.50	80	-
***E. coli* O157:H7 RSKK 234**	31.25	31.25	5	-
C. albicans	31.25	31.25	-	12.05

'-': Not used or not tested

Anticancer, antioxidant and antimicrobial activity studies conducted with *J. oxycedrus* and *S. aspera* crude ethanol extracts showed that the most active extract of the anticancer activity against the A549 cancer cell line was *J. oxycedrus* with 7.2 µg/ml IC_{50} value. When antimicrobial activity was examined, it was found that the most active species among these species was *J. oxycedrus* with MIC of 31.25 µg ml^{-1} against MRSA.

CONCLUSION

Based on the IC_{50} values of the study of *J. oxycedrus, S. aspera* fruit extracts on both tumor cells and normal cell lines, it can be suggested that it would be appropriate to focus on *Juniperus oxycedrus* species, which is common in Turkey. In this study, the extract with the highest DPPH radical scavenging activity belonged to *S. aspera* with 61%. Considering all these results, it can be said that *S. aspera* is a potential antioxidant and it is predicted that it can act as a potential natural antioxidant that can be used in the health care.

According to the antimicrobial activity test results the extracts have a significant activity in terms of antimicrobial and antifungal effects. Especially with its MIC value of 31.25 µg ml^{-1}, the antimicrobial effect of *J. oxycedrus* against MRSA is promising as a biological agent.

In the future, in the light of the data obtained from this study, new active compounds responsible for biological activity can be identified using bioactivity-guided isolation studies, especially focusing on *Juniperus oxycedrus L oxycedruss*.

ACKNOWLEDGEMENTS

Thank you for supporting this project by Kocaeli University Fund Accounting Office (KOU/BAP/2017/113).

Conflict of Interest

The article authors declare that there is no conflict of interest between them.

Author's Contributions

The authors declare that they have contributed equally to the article.

REFERENCES

Begum, S. J., Pratibha, S., Rawat, J. M., Venugopal, D., Sahu, P., Gowda, A., Jaremko, M. 2022. Recent Advances in Green Synthesis, Characterization, and Applications of Bioactive Metallic Nanoparticles. Pharmaceuticals, 15 (4), 455.

Cavaleiro C, Pinto E, Goncalves MJ and Salgueiro M, 2006. Antifungal activity of *Juniperus* essential oils against dermatophyte *Aspergillus* and *Candida* strains, Journal of Applied Microbiology, 100 (6), 1333-1338.

Damayanthi D, Mab A, Ahk A, Hamid A, Khh N, 2011. Effects of *Smilax myosotiflora* on testicular 11 a-hydroxysteroid dehydro-genase oxidative activity and plasma hormone levels in rats, Biomed. Res., 22, 188–193.

El-Sayed, A. S., Hassan, W. H., Sweilam, S. H., Alqarni, M. H. S., El Sayed, Z. I., Abdel-Aal, M. M., Abdelaziz, S. 2022. Production, Bioprocessing and Anti-Proliferative Activity of Camptothecin from Penicillium chrysogenum,"An Endozoic of Marine Sponge, Cliona sp.", as a Metabolically Stable Camptothecin Producing Isolate. Molecules, 27 (9), 3033.

Ivanova A, Mikhova B, Batsalova T, Dzhambazov B, Kostova I, 2011. New furostanol saponins from *Smilax aspera* L. and their in vitro cytotoxicity, Fitoterapia, 82, 282–287.

Karaman I, Sahin F, Gulluce M, Ogutcu H, Sengul M, Adiguzel A., 2003. Antimicrobial activity of aqueous and methanol extracts of *Juniperus oxycedrus* L., Journal of Ethnopharmacology, 85, 231–235.

Koyuncu M, Coskun M, Tanker N, 2007. Farmasotik Botanik, 3. Baski**,** Ankara Universitesi, Eczacilik Fakultesi, Ankara.

Lesjaka MM, Beara IN, Orcic DZ, Petar KN, Simin N-D, Emilija S-D, Mimica-Dukic NM, 2014. Phytochemical composition and antioxidant, anti-inflammatory and antimicrobial activities of *Juniperus macrocarpa* Sibth. et Sm., Journal of Functional Foods, 7, 57– 268.

Lim JP, Song YC, Kim JW, Ku CH, Eun JS, Leem KH, Kim DK, 2002. Free radical scavengers from the heartwood of *Juniperus chinensis*, Arch. Pharm. Res., 25, 449–452.

Memis Y, 2011. Bazi *Juniperus* turlerinden ucucu yaglarin ekstraksiyonu ve bunlarin biyolojik aktivitelerinin incelenmesi, Nigde Omer Halisdemir Universitesi, Fen Bilimleri Enstitusu, Yuksek Lisans Tezi (Basılmıs).

Murali A, Ashok P, Madhavan V, 2011. In vitro antioxidant activity and HPTLC studies on the roots and rhizomes of *Smilax zeylenica* L. (*Smilacaceae*), International Journal of Pharmacy and Pharmaceutical Sciences, 3, 192-195.

Orhan N, Orhan IE, Ergun F, 2011. Insights into cholinesterase inhibitory and antioxidant activities of five *Juniperus* species, Food and Chemical Toxicology, 49, 2305–2312.

Sadeghi-aliabadi H, Emami A, Saidi M, Sadeghi B, Jafarian A, 2009. Evaluation of in vitro cytotoxic effects of *Juniperus foetidissima* and *Juniperus sabina* extracts against a panel of cancer cells,Iranian Journal of Pharmaceutical Research, 8 (4), 281-286.

Sevimli-Gur C, Cetin B, Akay S, Gulce-Iz S, Yesil-Celiktas O, 2013. Extracts from black carrot tissue culture as potent anticancer agents. Plant Foods for Human Nutrition, 68, 293- 298.

Sparg SG, Light ME, Van Staden J, 2004. Biological activities and distribution of plant saponins, Journal of Ethnopharm, 94, 219–243.

Subramanyan, S., Karunakaran, V., Deepika, S., Gracy, A. J., Sheeba, V., Joseph, K., Radhakrishnan, K. V. 2022. Libocedroquinone: A Promising Anticancer Lead against Lung Cancer from Calocedrus Decurrens. Planta Medica International Open, 9 (01), 54-59.

Taviano MF, Marino A, Trovato A, Bellinghieri V, Melchini A, Dugo P, Cacciola F, Donato P, Mondello L, Guvenc A, De Pasquale R, Miceli N., *Juniperus oxycedrus* L. subsp. *oxycedrus* and *Juniperus oxycedrus* L. subsp. *macrocarpa* (Sibth. & Sm.) Ball. ''berries'' from Turkey: Comparative evaluation of phenolic profile, antioxidant, cytotoxic and antimicrobial activities, Food and Chemical Toxicology, 2013, 58, 22–29.

Tuzlaci, E, Erol MK, 1993. Turkish folk medicinal plants, Part II, Egirdir (Isparta) *Fitoterapia*, 70, 593–610.

Wang, T., Ye, Y., Ji, J., Zhang, S., Yang, X., Xu, J., Sun, X. 2022. Astilbin from Smilax glabra Roxb. alleviates high-fat diet-induced metabolic dysfunction. Food & Function, open access.

Weinstein M. P., 2018. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically, 11th Edition, ISBN Number: 1-56238-836-3.

Yesil-Celiktas O, Otto F, Gruener S, Parlar H 2009. Determination of extractability of pine bark using supercritical CO2 extraction and different solvents - optimization and prediction. Journal of Agricultural and Food Chemistry, 57, 341-347.

Yildiz-Ozturk E, Tag O, Yesil-Celiktas O, 2014. Subcritical water extraction of steviol glycosides from *Stevia rebaudiana* leaves and characterization of the raffinate phase. Journal of Supercritical Fluids, 95, 422-430.

Zubair M, Rizwan K, Rashid U, Saeed R, Saeed AA, Rasool N, Riaz M, 2017. GC/MS profiling, in vitro antioxidant, antimicrobial and haemolytic activities of *Smilax macrophylla* leaves, Arabian Journal of Chemistry, 10, 1460–1468.