



Phenolic constituents, antioxidant and antimicrobial activities of methanolic extracts of some female cones of gymnosperm plant

Alican Bahadır Semerci*, Dilek İnceçayır, Tuğba Konca, Hatice Tunca & Kenan Tunç
Department of Biology, Sakarya University, Sakarya-54300, Turkey

Received 05 September 2019; revised 12 March 2020

In the present study, the total antioxidant activity, the total phenolic content, and the antimicrobial activity of methanol extracts obtained from the female cones of *Pinus brutia*, *Pinus nigra*, *Cupressus sempervirens* L., *Thuja orientalis* L., and *Cedrus libani* were evaluated. The *in vitro* total phenolic content, the antioxidant, and the antimicrobial activities were determined using the Folin-Ciocalteu reaction, the DPPH radical scavenging assay and the disc diffusion method, respectively. The results of the present study showed that the extracts of the female cones prepared in methanol had the antimicrobial activity against the test microorganisms and the highest antimicrobial effect was observed against *Staphylococcus epidermidis*, *Staphylococcus aureus*, and *Bacillus subtilis*. The total phenolic constituents of the extracts were determined to be in the ranges from 69 to 220 mg GA/100 g. All extracts exhibited antioxidant activity with concentrations necessary to inhibit the activity of DPPH radical by 50% (IC₅₀) ranging from 0.35 to 17.21 µg/mL. The results indicate that the extracts of the cones exhibit acceptable antioxidant and antimicrobial activities and suggest that these cones' extracts may serve as a potential source of natural antioxidants and antimicrobials for food or medical purposes.

Keywords: Antioxidant, Antimicrobial effect, Cupressaceae, Pinaceae

Plant extracts obtained from aromatic and medicinal plants have been known to possess biological activity, most notably antibacterial, antifungal and antioxidant properties for long times. With growing interest in the use of essential oils in both the food and the pharmaceutical industries, the systematic and the potential usefulness of plant extracts studies have become increasingly important¹. Therefore, the antimicrobial and antioxidant activities of medicinal plants can be explored to maintain alternative drugs in the pharmaceutical industry or to protect food quality in the food industry²⁻⁴.

On the other hand, increasing the incidence of multi-resistant microbial strains represents the major issue in medical microbiology, which raises the need for new, efficient, and safe antimicrobial agents. Therefore, there are many studies focused on finding new drugs with antimicrobial properties, which are, in many cases, inspired by the traditional utilization of medicinal plants^{5,6}.

Also, gymnosperm species have antioxidant activities and thus they have great potential as sources for natural health products⁷.

The buds, leaves, young needles, and female cones of the families of Cupressaceae and Pinaceae and

their essential oils and resins are used in various diseases in the traditional treatments^{8,9}. Also, these species have been used for anti-inflammatory, antioxidant, antiseptic, antipyretic, anthelmintic, astringent, anti-rheumatic, anti-hemorrhoidal, anti-diarrheal and vasoconstrictive properties¹⁰.

The aim of this study is to determine the total phenolic constituents, antioxidant and antimicrobial activities of the methanolic extracts of the female cones of *Pinus brutia*, *Pinus nigra*, *Cupressus sempervirens* L., *Thuja orientalis* L., and *Cedrus libani*.

Materials and Methods

Chemicals and reagents

All the chemicals and reagents (Folin-Ciocalteu, Gallic Acid, 2,2-diphenyl-1-picrylhydrazyl (DPPH), Methanol, Mueller Hinton Agar (MH agar), Tryptic Soy Broth, Saboraud Dextrose Agar, Sodium Carbonate, Ascorbic Acid) used in this study were of analytical grade and obtained from Merck Company, Germany.

Plant materials

The female cones of *Pinus brutia*, *Cupressus sempervirens* L., *Thuja orientalis* L., and *Cedrus libani* were randomly collected from natural populations in Esentepe campus of Sakarya University, Sakarya, Turkey; *Pinus nigra* were randomly collected in National Park of Camlık, Yozgat, Turkey in June and July 2018.

*Correspondence:
E-mail: alicannn5434@gmail.com

Preparation of methanolic extracts

Ten grams of powdered cones were placed in the soxhlet device, with 200 mL of solvent (methanol), and subjected to 12 h of extraction. The solvents in the extracts were evaporated by using a rotary evaporator (Heidolph) under vacuum at 45°C for 15 min and the dried extracts were used for all investigations. The extract concentrations were adjusted by adding own solvent (methanol) to each extract at the doses of 6400 µg/mL for the antimicrobial activity tests and 1000 µg/mL for the antioxidant activity and the total phenolics analyses.

Disc diffusion method

All strains used throughout this study have been obtained from the Microbiology Research Laboratory of Sakarya University. For the determination of the antimicrobial activity, the disc diffusion method was used according to the National Committee for Clinical Laboratory Standards¹¹. Briefly, microbial suspension with adjusted density (0.5 McFarland) was spread on the MH solid media plate by using sterile swabs. Filter paper discs (6 mm in diameter, Himedia) were impregnated with 10 µL of the methanol extracts. After keeping at room temperature for 2 h, the discs impregnated with extracts were slightly pressed on the inoculated plates under aseptic conditions. Then, they were incubated 37°C for 24 h. Methanol-impregnated discs were used as negative controls and the commercial antibiotic discs (Gentamicin and Amphotericin B) were used as positive ones. At the end of the incubation, the diameters of the inhibition zone were measured by using an electronic digital caliper.

Antioxidant activity (DPPH assay)

The modified Blois method was used for antioxidant activity determination¹². Briefly, 1 mL of 0.004% solution of DPPH radical in methanol was mixed with 1 mL of extract solution in methanol (containing different concentrations of dried extract). These solutions were kept in dark for 30 min and the optical density was measured at 517 nm using spectrophotometer and methanol was used for the blank. The following equation was employed to determine the % DPPH radical scavenging activity.

$$\% \text{ DPPH radical scavenging} = \frac{(\text{control absorbance} - \text{extract absorbance})}{(\text{control absorbance})} \times 100$$

Total phenolic content (TPC)

The total phenolic content was determined according to Singleton and Rossi method with a slight

modification¹³. For the total phenolic content determination, the plant extracts were prepared at 100 µg/mL concentrations. 100 µL of the sample was mixed with 200 µL of 50% Folin and Ciocalteu's phenol reagent (Sigma). After 3 min, 1 mL of 2% Na₂CO₃ (~35%) (Riedel-de Haën) was added to the mixture. The reaction was kept in dark for 60 min, after which its absorbance was read at 760 nm. A calibration curve was constructed with different concentrations of gallic acid (Sigma) (0.01-0.1 mM) as a standard.

Statistical Analysis

All measurements were performed in triplicate and the results were represented as mean ± SD. Statistical analyses were realized with the SPSS 20.0 statistics program. Data statistical analyses were achieved by using One-way ANOVA and Duncan-test. The level of significance was set at $P < 0.05$.

Results

Antimicrobial activity

In the present study, the antimicrobial activity of the female cones of *Pinus brutia*, *Pinus nigra*, *Cupressus sempervirens* L., *Thuja orientalis* L., and *Cedrus libani* against *Bacillus subtilis* ATCC 6633, *Escherichia coli* ATCC 8739, *Enterococcus faecalis* ATCC 29212, *Staphylococcus aureus* ATCC 29213, *Staphylococcus epidermidis* ATCC 12228, *Salmonella typhimurium* ATCC 14028, *Pseudomonas aeruginosa* ATCC 9027, *Candida albicans* ATCC 1029 were determined.

The results of the antimicrobial screening of five conifer species against the seven bacteria and one yeast have been summarized in (Table 1) (the diameters of the inhibition zone against the test microorganisms).

All the tested extracts revealed the antimicrobial activity showing different selectivity for each microorganism. The methanolic extracts of cones showed that the greatest inhibitory effects were determined to be 12-15 mm, 11.5-15.2 mm, 10.5-13 mm against *B. subtilis*, *S. epidermidis*, and *S. aureus*, respectively. Additionally, no inhibitory effect against *C. albicans*, and *S. typhimurium* was observed for all extracts. Activities of the cones towards the test microorganisms were found to be lower than those of the known antibiotics. Only the methanolic extract of *Cedrus libani* showed antibacterial activity against *E. coli* and *E. faecalis*. The results of the present study demonstrated that *Cedrus libani* cones had a broad spectrum of activity on various microbial infections.

Scavenging effects on DPPH radical

Antioxidant activity is most commonly evaluated by the DPPH scavenging activity test¹⁴. The IC₅₀ of a

Table 1 — Inhibition zone diameters of extracts

Test Microorganisms	Inhibition Zones (mm) (\pm SD)					Antibiotics	
	Methanolic Extracts (6400 μ g/mL)					GC	AMB
	<i>P. brutia</i>	<i>P. nigra</i>	<i>C. sempervirens</i>	<i>T. orientalis</i>	<i>C. libani</i>		
<i>B. subtilis</i>	15.0 \pm 0.5	13.0 \pm 0.02	12.0 \pm 0.0	12.5 \pm 0.0	13.0 \pm 0.05	26.0	-
<i>C. albicans</i>	0	0	0	0	0	-	17.0
<i>E. coli</i>	0	0	0	0	11.0 \pm 0.0	28.0	-
<i>E. faecalis</i>	0	0	0	0	12.0 \pm 0.02	23.0	-
<i>P. aeruginosa</i>	0	15.0 \pm 0.0	0	0	13.0 \pm 0.05	22.0	-
<i>S. aureus</i>	13.0 \pm 0.5	12.0 \pm 0.0	13.0 \pm 0.5	12.0 \pm 0.5	10.5 \pm 0.1	28.0	-
<i>S. epidermidis</i>	15.2 \pm 0.1	14.0 \pm 0.5	13.0 \pm 0.5	14.0 \pm 0.0	11.5 \pm 0.1	21.0	-
<i>S. typhimurium</i>	0	0	0	0	0	25.0	-

GC: Gentamicin, AMB: Amphotericin B

compound is related to its antioxidant capacity inversely, as it expresses the amount of antioxidant required to decrease the DPPH concentration by 50%, which is obtained by interpolation from a linear regression analysis. A lower IC₅₀ indicates a higher antioxidant activity of a compound¹⁵.

Table 2 shows the IC₅₀ values in the DPPH radical scavenging activity assay of the extracts. The DPPH radical-scavenging activities of plant extracts display a concentration-dependent manner. The IC₅₀ values were found to range from 0.35 to 17.20 μ g/mL. The highest antioxidant activity was determined in the *Cedrus libani* cone and the lowest antioxidant activity was determined in the *Thuja orientalis* L. cone. The methanolic extracts, obtained from the cones of *Cedrus libani*, *Pinus brutia*, and *Pinus nigra*, showed higher antioxidant activity than ascorbic acid.

The antioxidant activities of the cones belonging to the conifer species used in the present study exhibited higher activity than the fruits and roots belonging to the same species in the literature¹⁶⁻¹⁸.

Total phenolic contents (TPCs)

Antioxidant compounds are usually in the phenolic form. Phenols, which are the most abundant structures in plants, are compounds that have the ability to destroy radicals because they contain hydroxyl groups; hence, they play an important role in the antioxidant activity¹⁹. Therefore, determination of the quantity of the phenolic compounds is of importance in order to regulate the antioxidant capacity of plant extract²⁰.

The total content of phenolics in the extracts has been evaluated according to the Folin-Ciocalteu method using gallic acid as the standard (Table 2).

TPCs of the extracts range from 69 to 220 mgGA/100 g, with *C. libani* showing the highest value of 220 mgGA/100 g, followed by *P. nigra*, *P. brutia*,

Table 2 — Antioxidant activities and total phenolic contents of the extracts

Species	Antioxidant Activity		Total Phenolics	
	IC ₅₀ (μ g/mL) \pm SD	R ²	mg GA/ 100 g \pm SD	R ²
<i>Cedrus libani</i>	0.58 \pm 0.09 ^a	0.88	220.0 \pm 0.08 ^d	0.98
<i>Cupressus sempervirens</i> L.	9.83 \pm 0.72 ^b	0.82	69.0 \pm 0.05 ^a	0.98
<i>Pinus brutia</i>	2.13 \pm 0.13 ^a	0.94	91.0 \pm 0.04 ^b	0.98
<i>Pinus nigra</i>	0.35 \pm 0.01 ^a	0.98	135.0 \pm 0.22 ^c	0.98
<i>Thuja orientalis</i> L.	17.20 \pm 0.39 ^b	0.93	80.0 \pm 0.04 ^{a,b}	0.98
Ascorbic acid	3.20 \pm 0.01 ^a	0.96	-	-

Data were represented as mean \pm SD of three measurements. Different letters symbolized significant differences ($P < 0.05$) by mean of the ANOVA Duncan-test

T. orientalis, and *C. sempervirens* with TPC values of 135, 91, 80, and 69 mgGA/100 g, respectively (Table 2). Total phenolic compounds and IC₅₀ values show an inverse relation.

Xie *et al.* determined that the TPCs of the essential oil extracts obtained from needles of six *Pinus* species ranged from 86.60 to 138.34 mg GA/100 g²¹. In the present study, it was determined that the total phenolic values of *Pinus* cones were within this range. In the literature, it has been reported that the total phenolics content was significantly correlated with the antioxidant activities as in the present study^{15,22}.

Discussion

Different parts of *Thuja* plants have been found to contain secondary metabolites such as flavonoids^{23,24}, phenolics, and terpenes²⁵⁻²⁷. It is known that the essential oils of *Thuja orientalis* L. have antimicrobial properties²⁸. Akers *et al.* reported that *Thuja orientalis* L. leaf extract exhibited antimicrobial activity for

Salmonella typhimurium c and d strains and concluded that the antimicrobial activity arise from α , β , γ thujaplicin in the extract²⁹. Bissa *et al.* and Jasuja *et al.* reported that the leaves of *Thuja orientalis* L. had more antimicrobial activity than the stem against selective bacterial species^{30,31}. In the research of Jasuja *et al.*, 70% methanol extract of the leaves of *Thuja orientalis* L. plant contained more phenolic compounds than the methanol extract of female cones with respect to our study³¹. This may be the reason why the female cone has less antimicrobial activity.

Xu *et al.* specified the compounds obtained from the fruit extracts such as catechin, quercitrin, hypolaetin 7-O- β -xylopyranoside, isoquercitrin, myricitrin shown antioxidant activity due to a catechol group or a pyrogallol group in ring Dubey and Batra (found that the IC₅₀ values of the extract obtained from dried branches were 202.45 μ g/mL^{32,33}). The IC₅₀ value in our study was lower than Dubey and Batra's study³³. Due to this fact that this could be related to the female cones containing more metabolites than branches.

Cupressus sempervirens L. is a plant that is used for medical purposes. Cupresses have antioxidant properties exhibiting phenolic and flavonoid compounds in different parameters³⁴. In various studies, the extracts and essential oils of *Cupressus sempervirens* L. have been found to have high antioxidant activity and it was reported that these extracts had α -pinene and 3-carene in abundance³⁵⁻³⁷. However, in some studies, the extracts of this plant have a moderate antioxidant activity³⁸. Zouaghi *et al.* compared the antioxidant activity of *C. sempervirens* stem, leaf and cone extract and concluded that the inhibition features of essential oils obtained from stem were similar to those of BHA and BHT activities but the extract of cones has shown less DPPH radical scavenging activity and they suggested that this could be caused by diterpenoids³⁹. In the present study, DPPH scavenging activity of *C. sempervirens* cone extracts has lower positive control value as in the study of Zouaghi *et al.*³⁹.

In various studies, *E. faecalis* and *P. aeruginosa* have been shown to have less sensitivity than *S. aureus* against the essential oils of leaves and the aerial parts of *Cupressus sempervirens* L.^{2,40}. Selim *et al.* determined that the *Cupressus sempervirens* L. essential oil had no antimicrobial activity against *E. coli* and *C. albicans*⁴⁰. The results of these studies are similar to the results of *Cupressus sempervirens* L. female cones in the present study.

Pinus brutia contains lipophilic compounds such as fatty acids, resin acids, resin alcohols, sterols α -pinene and β -pinene are the main compounds in the essential oil of *P. brutia*^{18,41}. The cones include terpene compounds such as monoterpene hydrocarbons, monoterpene alcohol, sesquiterpene hydrocarbons, sesquiterpene alcohols⁴². Ulukanlı *et al.* found that the total phenolic compound content of *P. brutia* was 17.02 mg gallic acid equivalent/g essential oil¹⁸. This result is higher than the value we found, due to the different extraction methods. The results of the total phenolic compound content contribute significantly to the antioxidant activity of *P. brutia*. Ustun *et al.* reported that the extract of *Pinus* species exhibits moderate or less DPPH scavenging activity⁴³. Yesil-Celiktas *et al.* observed that the bark extracts of *P. brutia* showed high radical scavenging effect⁴⁴. Guri *et al.* reported that the dry leaf and bark extracts of *P. brutia* had antioxidant properties. Guri *et al.* and Yesil-Celiktas *et al.* supported that the antioxidant scavenging effect of *P. brutia* was higher than the antioxidant effect of ascorbic acid in our study^{44,45}.

In the study of Ulukanlı *et al.*, the antimicrobial activity of essential oils obtained from resin of *Pinus brutia* was determined against *B. subtilis*, *E. coli*, *E. faecalis*, *S. aureus*, and *C. albicans* (inhibition zone values in the range of 8-16 mm); no effect against *P. aeruginosa*¹⁸.

The major constituents in essential oils of *C. libani* leaves were Germacrene D and β -caryophene^{46,47}. *C. libani* cones and the leaves have antimicrobial activity⁴⁸. There is no study in the literature about the antioxidant characteristics of *Cedrus libani*. In our study, the DPPH radical scavenger effect of *Cedrus libani* was higher than ascorbic acid.

Kızıl *et al.* reported that the ethanol extract of resins obtained from the stems and roots of *Cedrus libani* have shown antimicrobial activity (inhibition zone values in the range of 10-14 mm) against *E. coli*, *S. aureus*, *P. aeruginosa*, and *C. albicans*⁴⁹.

The cone extracts of *Pinus nigra* contain lipophilics such as fatty acids, resin acids, resin alcohols, resin hydrocarbons, sterols, sterols, and triglycerides⁴¹. The main compound found in essential oils of *P. nigra* is α -Pinene⁵⁰. It contains also isopimaric acid⁴¹. Ustun *et al.* observed that *Pinus nigra* extracts displayed a weak scavenging effect compared to gallic acid⁴³. Yesil-Celiktas *et al.* reported that the extracts of *Pinus nigra* showed a high scavenging

effect and they found that the antioxidant activity of *P. nigra* was bigger than *P. brutia*⁴⁴. The study of Yesil-Celiktas *et al.* is coherent with our study. In the present study, *Pinus nigra* has a more scavenging effect than the ascorbic acid and its antioxidant activity is due to its constituents⁴⁴. The DPPH radical scavenging activity of *Pinus nigra* is the highest of all the extracts.

Šarac *et al.* reported that *S. aureus* showed sensitivity against essential oils of *Pinus nigra* needle⁵¹. The result of that study is consistent with the current one. The results of the present study have shown that the cones used in the study have antimicrobial activity and that the cones of forest trees, which are one of the major sources of nature, can be evaluated.

Conclusion

It was determined that all the coniferous extracts obtained in the study had antimicrobial effects on *B. subtilis*, *S. aureus* and *S. epidermidis* bacteria, while there was no antimicrobial effect on *S. typhimurium* and *C. albicans*. All extracts showed high antioxidant activity compared to ascorbic acid used as standard.

Antioxidant evaluation of the extracts has shown that the antioxidant potency correlated well with the total phenolic content (TPC) and revealed that the cone's extracts exhibit acceptable antioxidant activities. Therefore, it is possible that the cones may take an active role in the prevention of many diseases that are likely to occur as a result of the radical-mediated damage. The results of the antimicrobial and antioxidant activity of the cone's components obtained in pure form will be even more informative.

Conflict of interest

All authors declare no conflict of interest.

Acknowledgment

The authors would like to thank Dr. Mehmet Sağıroğlu and Dr. İbrahim Okur for their invaluable support.

References

- Kilic O & Kocak A, Essential oil composition of six *Pinus* L. Taxa (Pinaceae) from Canada and their chemotaxonomy. *J Agric Sci*, 4 (2014) 67.
- Mazari K, Bendimerad N, Bekhechi C & Fernandez X, Chemical composition and antimicrobial activity of essential oils isolated from Algerian *Juniperus phoenicea* L. and *Cupressus sempervirens* L. *J Med Plants Res*, 4 (2010) 959.
- Dziria S, Hassenb I, Fatnassia S, Mrabeta Y, Casabiancac H, Hanchid B & Hosnia K, Phenolic constituents, antioxidant and antimicrobial activities of rosy garlic (*Allium roseum* var. *odoratissimum*). *J Funct Anal*, 4 (2012) 423.
- Namasivayam SKR, Shivaramakrishnan K & Bharani RSA, Potential antioxidative protein-pigment complex *Spirulina platensis* mediated food grade phycocyanin C -Extraction, purification, antioxidative activity and biocompatibility. *Indian J Biochem Biophys*, 56 (2019) 230.
- Govindarajan M, Rajeswary M & Benelli G, Chemical composition, toxicity and non-target effects of *Pinus kesiyana* essential oil: An eco-friendly and novel larvicide against malaria, dengue and lymphatic filariasis mosquito vectors. *Ecotox Environ Safe*, 129 (2016) 85.
- Mitić ZC, Jovanović B, Jovanović CZ, Krsteva T, Radić ZZ, Cvetkovića VJ, Mitrović TL, Marin PD, Zlatković BK & Stojanović GS, Comparative study of the essential oils of four *Pinus* species: Chemical composition, antimicrobial and insect larvicidal activity. *Ind Crop Prod*, 111 (2018) 55.
- Priyanka C, Kadam Da, Kadam AS, Ghule YA & Aparadh VT, Free radical scavenging (DPPH) and Ferric reducing ability (FRAP) of some gymnosperm species. *Int J Res Bot*, 3 (2013) 36.
- Menkovic N, Savikin K, Tasic S, Zunic G, Stesevic D, Milosavljevic S & Vincek D, Ethnobotanical study on traditional uses of wild medicinal plants in Prokletije Mountains (Montenegro). *J Ethnopharmacol*, 133 (2010) 97.
- Ari S, Kargioglu M, Temel M & Konuk M, Traditional tar production from the Anatolian black pine [*Pinus nigra* Arn. sub sp. *Pallasiana* (Lamb.) Holmboe var. *pallasiana*] and its usages in Afyonkarahisar, central western Turkey. *J Ethnobiol Ethnomed*, 10 (2014) 29.
- Eryılmaz M, Tosun A & Tumen I, Antimicrobial activity of some species from Pinaceae and Cupressaceae. *Turk J Pharm Sci*, 13 (2016) 35.
- Kiehlbauch JA1, Hannett GE, Salfinger M, Archinal W, Monserrat C & Carlyn C, Use of the national committee for clinical laboratory standards guidelines for disk diffusion susceptibility testing in New York state laboratories. *J Clin Microbiol*, 38 (2000) 3341.
- Blois MS, Antioxidant determinations by the use of a stable free radical. *Nature*, 181 (1958) 1199.
- Singleton VL & Rossi JA, Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *Am J Enol Viticult*, 16 (2017) 144.
- Tohidi B, Rahimmalek M, Arzani M, Essential oil composition, total phenolic, flavonoid contents, and antioxidant activity of *Thymus* species collected from different regions of Iran. *Food Chem*, 220 (2017) 153.
- Do QD, Angkawijaya AE, Tran-Nguyen PL, Huynh LH, Soetaredjo FE, Ismadji S & Ju Y, Effect of extraction solvent on total phenol content, total flavonoid content, and antioxidant activity of *Limnophila aromatic*. *J Food Drug Anal*, 22 (2014) 296.
- Emami SA, Asili J, Mohagheghi Z & Hassanzadeh MK, Antioxidant activity of leaves and fruits of Iranian conifers. *Evid Based Complement Altern Med*, 4 (2007) 313.
- Saharan P, Duhan JS & Gahlawat SK, Antioxidant potential of various extracts of stem of *Thuja orientalis* L.: *in vitro* study. *Int J Appl Biol Pharm*, 3 (2012) 264.
- Ulukanlı Z, Karaborklu S, Bozok F, Ates B, Erdogan S, Cenet M & Karaaslan MG, Chemical composition, antimicrobial, insecticidal, phytotoxic and antioxidant

- activities of mediterranean *Pinus brutia* and *Pinus pinea* resin essential oils. *Chin J Nat Med*, 12 (2014) 901.
- 19 Shrivastava A, Aggarwal LM, Mishra SP, Khanna HD, Shahil UP & Pradhan S, Free radicals and antioxidants in normal vs cancerous cells —An overview. *Indian J Biochem Biophys*, 56 (2019) 7.
 - 20 Aksoy L, Kolay E, Aglonu Y, Aslan Z & Kargioglu M, Free radical scavenging activity, total phenolic content, total antioxidant status, and total oxidant status of endemic *Thermopsis turcica*. *Saudi J Biol Sci*, 20 (2003) 235.
 - 21 Xie Q, Liu Z, Li Z, Chemical Composition and Antioxidant Activity of essential oil of six *pinus* taxa native to China, *Molecules*, 20 (2015)9380.
 - 22 Stankovic MS, Petrovic M, Godjevac D, Stevanovic ZD, Screening inland halophytes from the central Balkan for their antioxidant activity in relation to total phenolic compounds and flavonoids: Are there any prospective medicinal plants, *J Arid Environ*, 120 (2015) 26.
 - 23 Pelter A, Warren R, Hameed N, Khan NU, Ilyas M & Rahman W, Biflavonyl pigments from *Thuja orientalis* L. (Cupressaceae). *Phytochemistry*, 9 (1970) 1897.
 - 24 Natarajan S, Murti VVS & Seshadri TR, Biflavones of some Cupressaceae plants. *Phytochemistry*, 9 (1970) 575.
 - 25 Tomita B, Hirose Y & Nakatsuk T, Terpenoids. XVI new constituents of *Biota orientalis*. *Tetrahedron Lett*, 9 (1968) 843.
 - 26 Inoue M, Hasegawa S & Hirose Y, Terpenoids from the seed of *Platyclusus orientalis*. *Phytochemistry*, 24 (1985) 1602.
 - 27 Sung SH, Koo KA, Lim HK, Lee HS, Cho JH, Kim HS & Kim YC, Diterpenes of *Biota orientalis* leaves. *Kor J Pharmacog*, 29 (2012)347.
 - 28 Bagcı E & Dıgırak M, Antibacterial activities of essential oils from Turkish spices and citrus. *FlavourFragr J*, 11 (1996) 251.
 - 29 Akers HA, Abrego VA & Garland E, Thujaplicins from *Thujaplicata* as iron transport agents for *Salmonella typhimurium*. *J Bacteriol*, 141 (1980) 164.
 - 30 Bissa S, Bohra A & Bohra A, Antibacterial potential of three naked- seeded (Gymnosperm) plants. *Nat Prod Res*, 7 (2008) 420.
 - 31 Jasuja ND, Sharma SK, Saxena R, Choudhary J, Sharma R & Joshi SC, Antibacterial, antioxidant and phytochemical investigation of *Thuja orientalis* L. leaves. *J Med Plants Res*, 7 (2013) 1886.
 - 32 Xu GH, Ryoo IJ, Kim YH, Choo SJ & Yoo ID, Free radical scavenging and antielastase activities of flavonoids from the fruits of *Thuja orientalis*L. *Arch Pharm Res*, 32 (2019) 275.
 - 33 Dubey SK & Batra A, Antioxidant activities of *Thuja occidentalis* Linn. *Asian J Pharm Clin Res*, 2 (2009) 73.
 - 34 Al-Snafi, AE, Medical importance of *Cupressus sempervirens* L.-A review. *IOSR J Pharm*, 6 (2016) 66.
 - 35 Ibrahim NA, El-Seedi HR & Mohammed MMD, Constituents and biological activity of the chloroform extract and essential oil of *Cupressus sempervirens* L. *Chem Nat Compd*, 45 (2009) 309.
 - 36 Boukhris M, Regane G, Yangui T, Sayadi S & Bouaziz M, Chemical composition and biological potential of essential oil from Tunisian *Cupressus sempervirens* L. *J Arid Land*, 22 (2012) 329.
 - 37 Nouri AB, Dhifi W, Belili S, Ghazghazi H, Aouadhi C, Chérif A & Mnif W, Chemical composition, antioxidant potential, and antibacterial activity of essential oil cones of Tunisian *Cupressus sempervirens* L. *J Chem-NY*, (2015) 1.
 - 38 Sacchetti G, Maietti S & Muzzoli M, Comparative evaluation of 11 essential oils of different origin as functional antioxidants, antiradicals and antimicrobials in foods. *Food Chem*, 91 (2015) 621.
 - 39 Zouaghi N, Bellel C, Cavaleiro C, Nadjemi B & Youfı M, Identification of volatile compounds, antimicrobial properties and antioxidant activity from leaves, cones and stems of *Cupressus sempervirens* L. from Algeria. *Afr J Microbiol Res*, 9 (2015) 83.
 - 40 Selim S, Adam M, Hassan S & Albalawi AR, Chemical composition, antimicrobial and antibiofilm activity of the essential oil and methanol extract of the Mediterranean cypress (*Cupressus sempervirens* L.). *BMC Adv Exp Med Biol*, 14 (2014) 179.
 - 41 Kılıc A, Hafızoglu H, Donmez IE, Tumen I, Sivrikaya H, Reunanen M & Hemming J, Extractives in the cones of *Pinus* species. *Eur J Wood Wood Prod*, 69 (2011) 37.
 - 42 Tumen I, Hafızoglu H, Kılıc A, Donmez IE, Sivrikaya H & Reunanen M, Yields and constituents of essential oil from cones of *Pinaceae* spp. natively grown in Turkey. *Molecules*, 15 (2010) 5797.
 - 43 Ustun O, Senol FS, Kurkcuoglu M, Orhan IE, Kartal M & Baser KHC, Investigation on chemical composition, anticholinesterase and antioxidant activities of extracts and essential oils of Turkish *Pinus* species and pycnogenol. *Ind Crop Prod*, 38 (2012) 115.
 - 44 Yesil-Celiktas O, Ganzera M, Akgun I, Sevimli C, Korkmaz KS & Bedir E, Determination of polyphenolic constituents and biological activities of bark extracts from different *Pinus* species. *J Sci Food Agric*, 89 (2009) 1339.
 - 45 Guri A, Kefalas P & Roussis V, Antioxidant potential of six pine species. *Phytother Res*, 20 (2006) 263.
 - 46 Saab AM, Harb F & Koenig W, Essential oil components in the leaves of *Cedrus libani* and *Cedrus deodara* from Lebanon. *Minerva Biotechnol*, 21 (2009) 201.
 - 47 Derwich E, Benziane Z & Boukir A, Chemical composition and *in vitro* antibacterial activity of the essential oil of *Cedrus atlantica*. *Int J Agric Biol*, 12 (2010) 381.
 - 48 Metin D, Ahmet I & Hakkı A, Antimicrobial activities of several parts of *Pinus brutia*, *Juniperus oxycedrus*, *Abies cilicia*, *Cedrus libani* and *Pinus nigra*. *Phytother Res*, 13 (1999) 584.
 - 49 Kızıl M, Kızıl G, Yavuz M & Aytekin C, Antimicrobial Activity of Resins Obtained from the Roots and Stems of *Cedrus libani* and *Abies Cilicia*. *Prikl Biokhim Mikrobiol*, 38 (2002) 166.
 - 50 Tumen I, Hafızoglu H, Kılıc A, Donmez IE, Sivrikaya H & Reunanen M, Yields and constituents of essential oil from cones of *Pinaceae* spp. natively grown in Turkey. *Molecules*, 15 (2010) 5797.
 - 51 Šarac Z, Matejić JS, Radić ZZ, Veselinović JB, Džamić AM, Bojović S & Marin PD, Biological activity of *Pinus nigra* terpenes-Evaluation of FtsZ inhibition by selected compounds as contribution to their antimicrobial activity. *Comput Biol Med*, 54 (2014)72.