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Likenized Askomiset *Tornabea scutellifera*'nın Antioksidan ve Antimikrobiyal Özelliklerinin Araştırılması

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Anahtar Kelimeler
Tornabea scutellifera, likenler, antimikrobiyal aktivite, antioksidan aktivite, fenolik madde

Özet: Bu çalışmada Türkiye'nin Doğu Akdeniz Bölgesi'nden toplanan *Tornabea scutellifera*'dan elde edilen metanollü ekstrenin antioksidan ve antimikrobiyal aktivitesinin değerlendirilmesi amaçlanmıştır. Antioksidan aktivitenin belirlenmesi için fosfomolibdenyum, β-karoten/linoleik asit ve 2,2-difenil-1-pikrilhidrazil (DPPH) serbest radikal süpürme yöntemleri kullanılmıştır. β-karoten/linoleik asit sisteminde *T. scutellifera* metanollü ekstresi linoleik asit oksidasyonuna karşı %72.0 inhibisyon göstermiştir. DPPH yönteminde metanollü ekstrenin IC_{50} değeri 147.06 µg/mL olarak belirlenmiştir. Metanollü ekstredeki toplam fenolik madde miktarı Folin- Ciocalteu yöntemi ile spektrofotometrik olarak belirlenmiştir. Ekstrenin antimikrobiyal aktivitesi 13 bakteri ve 2 adet mayaya karşı agar difüzyon yöntemi ile belirlenmiş, *Aeromonas hydrophila, Klebsiella pneumonia*, *Pseudomonas aeruginosa*, *Bacillus brevis*, *Bacillus cereus* ve *Staphylococcus aureus*'a karşı etkili olduğu belirlenmiştir. Mayalara karşı (*Candida albicans* ve *Saccharomyces cerevisiae*) antimikrobiyal aktiviteye sahip olmadığı belirlenmiştir. Bu çalışmadan elde edilen sonuçlar *T. scutellifera*'nın doğal antioksidan ve antimikrobiyal ajanların potansiyel kaynağı olabileceğini göstermektedir.

Screening the Antioxidant and Antimicrobial Properties of the Lichenized Ascomycete *Tornabea scutellifera*

Keywords
Tornabea scutellifera, lichens, antimicrobial activity, antioxidant activity, penolic content

Abstract: The study was aimed at evaluating the antioxidant and antimicrobial activity of the methanol extract of *Tornabea scutellifera* collected from Eastern Mediterranean Region of Turkey. Antioxidant activity was employed by three complementary test systems namely phosphomolybdenum, β-carotene/linoleic acid and 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging systems. In β -carotene-linoleic acid system, *T. scutellifera* methanol extract exhibited 72.0% inhibition against linoleic acid oxidation. In DPPH assay, IC_{50} value of the methanol extract was determined as 147.06 µg/mL. Total phenolic content in the methanol extract was determined spectrometrically applying the Folin- Ciocalteu assay. The extract was tested against thirteen bacteria and two yeasts by agar diffusion assay and found active against *Aeromonas hydrophila, Klebsiella pneumonia*, *Pseudomonas aeruginosa*, *Bacillus brevis*, *Bacillus cereus* and *Staphylococcus aureus*. No antimicrobial activity against the yeasts (*Candida albicans* and *Saccharomyces cerevisiae*) was detected. The results obtained in the present study indicate that *T. scutellifera* may be a potential source of natural antioxidant and antimicrobial agents.

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1. Introduction

Food borne illness resulting from consumption of food contaminated with pathogenic bacteria has been of vital concern to public health. To reduce health hazards and economic losses due to food borne microorganisms, the use of natural products as antibacterial compounds seem to be an interesting way to control the presence of pathogenic bacteria and to extend the shelf life of processed food [1].

Lipid oxidation is a complex free radical chain process involving a variety of radicals. There is a growing interest in the problem of lipid oxidation because of its significance for food deterioration. The use of antioxidants in lipid containing foods is one method to minimize rancidity, retard the formation of toxic oxidation products, maintain nutritional quality and increase the shelf life of food products [2]. However, the commonly used synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxy toluene (BHT), are restricted by legislative rules because they are suspected to have some toxic effects and as possible carcinogens [3]. Thus, there is an increased interest in identifying alternative natural and safe sources of antioxidant and antimicrobial agents.

It is known that lichens have been used for medical purposes since ancient times and are known to produce unique secondary metabolites, a number of which have considerable biological activities such as antimicrobial, antiherbivore and antibiotic [4-6]. All of the secondary substances which are so characteristic of lichens are of fungal origin. Consequently it seems rather suprising that with more than 630 secondary metabolites known from lichens, most are unique to these organisms and only a small minority (c. 50-60) occur in other fungi or higher plants [4]. Huneck [7,8] provided the lists of the antibacterial and antifungal activities of lichen compounds and lichens.

Tornabea scutellifera (With.) J.R.Laundon (Ascomycota, Physciaceae) is a fruticose epiphytic lichen which has a broad, but disjunctive distribution in semi-arid areas with frequent period of air humidity, including Europe (with a Mediterranean-Atlantic range), North Africa, southwestern Asia, California, Mexico, western Chile and Peru [9]. Previously; no lichen substances were reported from *T. scutellifera* [10]. However, Řezanka et al. [11] isolated recently tornabeatins A, B, C and D as new natural products from this species and reported to show antibiotic and antitumor activity.

Although antioxidant and antimicrobial activities of different extracts and substances obtained from many lichen species have been investigated by many researchers, there is no reports on biological activity of *T. scutellifera* except report of Řezanka et al. [11]. Therefore, the methanol extract of *T. scutellifera* were tested for their antimicrobial and antioxidant activities and the results are presented and discussed here.

2. Material and Method

2.1. Chemicals

Folin-Ciocalteau reagent, 2,2-diphenyl-1-picrylhydrazyl (DPPH), sodium carbonate, gallic acid, ascorbic acid, Mueller–Hinton agar, Mueller–Hinton broth, Malt extract agar and Malt extract broth were purchased from Merck. The other chemicals and solvents used in this experiment were of analytical grade, purchased from Merck.

2.2. Collection and identification of lichen samples

The lichenized ascomycete *T. scutellifera* which is densely found on the bark of *Pinus brutia* was collected from İçel Province which is a shore city in the eastern Mediterranean part of Turkey (Turkey, İçel, South of Güzelyayla Village, *Pinus brutia* forest, alt. 840 m, 37º 02’ 40" N, 34º 29’ 45" E, 07 August 2009). The lichen specimens were authenticated by Dr. M.G. Halıcı & M. Candan and stored in the lichen herbarium of Erciyes University, Science Faculty, Department of Biology, Kayseri, Turkey (Voucher no. MGH 0.5781).

2.3. Preparation of lichen extractions

Dried parts of the *T. scutellifera* at room temperature were ground to a fine powder with a grinder. Then the powdered material (10 g) was extracted using a Soxhlet type extractor with 100 mL methanol (MeOH) at 60 ^{0}C for 6 h. Thereafter, the extract was filtered and evaporated to dryness under vacuum at 40 ^{0}C with a rotary evaporator. After determining the yield, the extract was dissolved in methanol for further study.

2.4. Determination of total phenolic content

The total phenolic content of the extract was determined using the Folin-Ciocalteau reagent [12]. The reduction of the Folin-Ciocalteau reagent by phenolic compounds under alkaline conditions, which resulted in the development of a blue colour, was recorded at an absorbance of 765 nm. Briefly, 40 µl of the methanol solution

of the extract (1 mg/mL) was mixed with 2.4 mL of distilled water. 200 µl of Folin-Ciocalteau reagent was added and the contents of the flask mixed thoroughly. After 1 min, 600 µL of sodium carbonate (20% Na_2CO_3) was added and the volume was made up to 4.0 mL with distilled water. After 2 h incubation at room temperature, the absorbance was measured at 765 nm with spectrophotometer (Shimadzu) and compared to a gallic acid calibration curve. The data are presented as the average of triplicate analyses. Results were expressed as mg of gallic acid (GAE) equivalents/g extract.

2.5. Determination of antioxidant activity

2.5.1. Phosphomolybdenum assay

The antioxidant activity was determined by the phosphomolybdenum method of Prieto, Pineda, and Aguilar [13]. 0.4 mL of the methanol extract (1 mg/mL) was mixed with 4 mL of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes were capped and incubated in water bath at 95 °C for 90 min. After the samples had cooled to room temperature, the absorbance of the green phosphomolybdenum complex was measured at 695 nm. In the case of the blank, 0.4 mL of methanol was used in place of sample. The antioxidant activity was determined using a standard curve with ascorbic acid solutions as the standard. The mean of three readings was used and the reducing capacity of the extract was expressed as mg of ascorbic acid equivalents
(AAE)/g extract.

2.5.2. β-Carotene bleaching assay

The ability of the methanol extract to inhibit the bleaching of the β-carotene–linoleic acid emulsion was determined [14]. β-carotene (10 mg) was dissolved in 10 mL of chloroform ($CHCl_3$). An aliquot (0.2 mL) of this solution was added into a boiling flask containing 20 mg of linoleic acid and 200 mg of Tween 40. The chloroform was removed using a rotary evaporator at 40 °C for 5 min. Distilled water (50 mL) was slowly added to the residue with vigorous agitation, to form an emulsion. The emulsion (5 mL) was added to a tube containing 0.2 mL of the extract solution (2 mg/mL). The test emulsion was incubated in a water bath at 50 °C for 2h, when the absorbance was measured at 470 nm. In the negative control, the extract was substituted with an equal volume of ethanol. BHT (Butylated hydroxytoluene) was used as the positive control.

2.5.3. DPPH radical scavenging activity

The capacity of methanol extract to scavenge the lipid-soluble DPPH (2,2-diphenyl-1-picrylhydrazyl) radical, which results in the bleaching of the purple colour exhibited by the stable DPPH radical, is monitored at an absorbance of 517 nm. The ability of the extracts to scavenge DPPH radical was assessed spectrophotometrically [15]. 50 µL aliquots of the proper methanolic extract dilution at a concentration range of 0.1–5 mg/mL were mixed with 1 mL of the methanolic DPPH solution (0.1 mM). Methanol was used as a control instead of extract. The mixtures were left for 30 min at room temperature in the dark and the absorbance at 517 nm measured using methanol as blank. IC_{50} (concentration causing 50% inhibition) values of methanolic extracts were determined graphically. The same procedure was repeated with BHT as a positive control. The measurements were performed in triplicate and the results were averaged. Radical scavenging activity was expressed as percentage inhibition of DPPH radical and was calculated by following equation:

Inhibition% = $(A_{blank} - A_{sample} / A_{blank})$ x 100
where A_{blank} is the absorbance of the control reaction (containing all reagents except the test compound) and A_{sample} is the absorbance of the test compound.

2.5.4. Determination of antimicrobial activity

The microorganism strains used in this study were *Aeromonas hydrophila* ATCC 7965, *Bacillus brevis* FMC 3, *Bacillus cereus* RSKK 863, *Bacillus subtilis* ATCC 6633, *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* FMC 5, *Listeria monocytogenes* 1/2B, *Morganella morganii*, *Proteus mirabilis* BC 3624, *Pseudomonas aeruginosa* ATCC 27853, *Salmonella typhimurium* NRRLE 4463, *Staphylococcus aureus* ATCC 29213, *Yersinia enterocolitica* ATCC 1501, *Candida albicans* ATCC 1223 and *Saccharomyces cerevisiae* BC 5461. The agar-well diffusion method was employed for the determination of antimicrobial activities of extracts [16]. Each microorganism was suspended in sterile nutrient broth. Test yeasts (*C. albicans*, *S. cerevisiae*) were suspended in malt extract broth and each microorganism was diluted at ca. 10^6–10^7 colony forming units (cfu)/mL. 250 µL of each microorganism was added into a flask containing 25 mL sterile Mueller–Hinton agar or malt extract agar at 45 °C and poured into Petri dishes (9 cm in diameter). Then the agars were allowed to solidify at 4 °C for 1 h. The wells (4 mm in diameter) were cut from the agar. The extracts were prepared at 1%, 2.5%, 5% and 10% concentrations in absolute methanol and 40 µl of extract solutions were applied to the wells. Absolute methanol without herb extract was used as a control. *Y. enterocolitica, C. albicans* and *S. cerevisiae* was incubated at 25 °C for 24–48 h in the inverted position. The other microorganisms were incubated at 37 °C for 18–24 h. At the end of the period, all plates were examined for any zones of growth

inhibition and the diameters of these zones were measured in millimetres. Ampicillin (AMP-10 µg/disc). Chloramphenicol (C-30 µg/disc), Erythromycin (E-15 µg/disc), Gentamycin (CN-10 µg/disc), Oxacillin (OX-1 µg/disc) (Oxoid) standard antibiotics were used as positive control.

3. Results

The yield of extract obtained by using methanol and their contents of total phenols are shown in Table 1. The yield of the methanolic extract of *T. scutellifera* was calculated based on a dry weight basis and was found to be 8.67 ± 0.9% (Table 1). The total phenolic content of *T. scutellifera* methanol extract was estimated to be 7.03 ± 0.3 mg gallic acid equivalents/g of plant extract from triplicate measurements (Table 1).

T. scutellifera methanol extract was subjected to screening for the possible antioxidant activity by three complementary test systems namely phosphomolybdenum, β-carotene/linoleic acid and DPPH free radical scavenging systems. The phosphomolybdenum method is based on the reduction of Mo(VI) to Mo(V) by the antioxidant compounds and the formation of green Mo(V) complexes with a maximal absorption at 695 nm [13]. Total antioxidant capacity of *T. scutellifera* methanol extract was determined by the phosphomolybdenum method and expressed as mg of ascorbic acid equivalents (AAE)/g extract. The total antioxidant activity of the methanol extract was found as 80.61 ± 03 mg AAE/g dry extract (Table 1).

The mechanism of bleaching of β-carotene is a free radical mediated phenomenon resulting from the hydroperoxides formed from linoleic acid. In this model system, β-carotene undergoes rapid discoloration in the absence of an antioxidant. The linoleic acid free radical, formed upon the abstraction of a hydrogen atom from one of its diallylic methylene groups, attacks the highly unsaturated β-carotene molecules. As β-carotene molecules lose their double bonds by oxidation, the compound loses its chromophore and characteristic orange color, which can be monitored spectrophotometrically. The presence of different extracts can hinder the extent of β-carotene-bleaching by neutralizing the linoleate-free radical and other free radicals formed in the system [17]. In β-carotene/linoleic acid system, *T. scutellifera* methanol extract exhibited 72.0% inhibition against linoleic acid oxidation (Table 1). *T. scutellifera* methanol extract showed lower activity on the inhibition of linoleic acid oxidation than BHT (98.68%) at 2 mg/mL concentration.

DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule. The method is based on the reduction of alcoholic DPPH solution in the presence of a hydrogen-donating antioxidant due to the formation of the non-radical form DPPH-H by the reaction. Free radical scavenging capacities of the methanol extract measured by DPPH assay are shown in Figure. *T. scutellifera* methanol extract showed free radical scavenging capacity at all the concentrations studied (0.1-5 mg/mL). The scavenging activity increased with increasing concentration of the methanol extract. The percentage inhibition of DPPH radical by 6.66, 8.3, 16.6, 33.3 and 66.6 µg/ mL was found to be 31.89%, 34.03%, 37.69%, 41.10% and 42.79% respectively (Fig.)When compared to BHT, *T. scutellifera* methanol extract has been found less effective than that synthetic antioxidant agent. The percentage inhibition of 66.6 µg/mL concentration of BHT was found to be 92.15%. The IC_{50} value for methanol extract was calculated and defined as the concentration of extract causing 50% inhibition of absorbance. As can be seen from the Table 1, IC_{50} value of the *T. scutellifera* methanol extract was determined as 147.06 µg/mL. Antioxidant and the scavenging activity might be related to its phenolic substances due to the active hydrogen donating ability of hydroxyl substitutions.

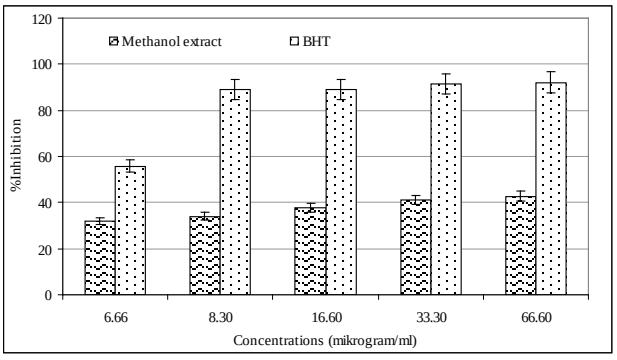

Figure. %Inhibition values of *T. scutellifera* methanolic extracts tested by DPPH assay

The antimicrobial activities of *T. scutellifera* methanol extract against microorganisms examined at 1%, 2.5%, 5% and 10% concentrations and their potency were assessed by the presence or absence of inhibition zones and zone diameter. Pure methanol (control) used as solvent had no inhibitory effects on the tested fifteen

microorganism. The results are given in Tables 2. The results showed that *T. scutellifera* methanol extract had antibacterial activities against only 6 out of 13 bacteria tested. No activity was found against yeasts tested (*C. albicans* and *S. cerevisiae*). While the methanol extract had inhibitory effect only against *A. hydrophila, K. pneumonia*, *P. aeruginosa* among the Gram (-) bacteria tested, it was only effective against *B. brevis*, *B. cereus* and *S. aureus* among Gram (+) bacteria tested. *S. aureus* were the most sensitive bacteria to *T. scutellifera* methanol extract. The antimicrobial activities of extracts were compared with standard antibiotics, in this study (Table 2).

4. Discussion and Conclusion

As far as we know could ascertain, there were only three previous reports concerning the antioxidant properties of lichens in the literature [18-20]. The antioxidant activity, reducing power, superoxide anion radical scavenging and free radical scavenging activities of aqueous extract of *Cetraria islandica* were determined by Gülçin et al. [18] and their antioxidant activity was found to be higher than that of α-tocopherol. It has been reported that the methanol extracts of *Cladonia foliacea*, *Everinia divaricata*, *Evernia prunastri*, and *Neofuscella pulla* did not exhibit any activity in DPPH assay [19]. *Dermatocarpon miniatum* showed DPPH radical scavenging activity with an IC_{50} of 396.1 µg/mL which is higher than that of the *T. scutellifera* methanol extract [19]. In a previous study, Gulluce et al. [20] showed that the methanol extracts of *Parmelia saxatilis*, *Platismatia glauca*, *Ramalina pollinaria* and *Ramalina polymorpha* did not exert any activity in DPPH assay, whereas those of *Umbilicaria nylanderiana* provided 50% inhibition at 400.2 µg/mL, which is higher than that of the *T. scutellifera* methanol extract. Lower IC_{50} value reflects better protective action of the extract. Based on this information, total phenolic content, total antioxidant activity, free-radical scavenging and inhibition of linoleic acid oxidation of *T. scutellifera* methanol extract were determined for the first time in this study.

Antimicrobial screening of extracts from a number of lichen species have been carry out by different researchers. However, this is the first study reporting antimicrobial activity of methanol extract of *T. scutellifera*. In only one study, antibacterial activities of tornabeatins A-D, isolated as new substances from *T. scutellifera* were investigated previously [11]. These researchers reported that tornabeatins A-D had antibacterial activity against *S. aureus* and *B. subtilis*, but they had no activity against the *E. coli* [11]. The acetone, diethyl ether and ethanol extracts of the lichen *Cetraria aculeata* were found active against *Escherichia coli*, *Staphylococcus aureus*, *Aeromonas hydrophila*, *Proteus vulgaris*, *Streptococcus faecalis*, *Bacillus cereus*, *Bacillus subtilis, Pseudomonas aeruginosa*, *Listeria monocytogenes* [21]. Aslan et al. [19] stated that the methanol extracts of *C. foliacea*, *D. miniatum E. divaricata*, *E. prunastri*, and *N. pulla* possess antimicrobial activity against some of the bacteria and fungi tested, but no activity was observed against the yeasts, which is agrees with our results. The methanol extracts of *P. saxatilis*, *P. glauca*, *R. pollinaria*, *R. polymorpha* and *U. nylanderiana* were also found to possess antimicrobial activity against some test bacteria, fungi and yeast in a previous study [20]. The antimicrobial activity of some lichens from south Spain against Gram (+) bacteria has been reported [22]. It was determined that the diethyl ether, acetone, chloroform, petroleum ether, and ethanol extracts of the lichen *Xanthoparmelia pokornyi* showed antimicrobial activity against *Aeromonas hydrophila*, *Bacillus cereus*, *Bacillus subtilis*, *Listeria monocytogenes*, *Proteus vulgaris*, *Staphylococcus aureus*, *Streptococcus faecalis*, *Yersinia enterocolitica*, *Candida albicans* and *Candida glabrata* [23]. The different activities of the extracts can be ascribed to their different phenolic compositions. Polyphenols are among the most widespread class of metabolites in nature, and their distribution is almost ubiquitous. Phenolic compounds, including depsides, depsidones, dibenzofurans and pulvinic acid derivatives are secondary metabolites of lichen samples [24]. Polyphenols have received considerable attention because of their physiological functions, including free radical scavenging, antioxidant [25] and antimicrobial activity [26]. The antioxidant activity of phenolics is mainly due to their redox properties which make them act as reducing agents, hydrogen donors, and singlet oxygen quenchers. They also may have a metal chelating potential [27].

As a conclusion, *T. scutellifera* possess antioxidant, free radical scavenging and antimicrobial activities. The results of the present work indicate the presence of compounds possessing high antioxidant and antimicrobial activity in *T. scutellifera* methanol extract. However, further investigation of individual phenolic compounds, their *in vivo* antioxidant activity and the different antioxidant and antimicrobial mechanisms are warranted.

References

[1] Oussalah, M., Caillet, S., Saucier, L., Lacroix, M. 2007. Inhibitory effects of selected plant essential oils on the growth of four pathogenic bacteria: *E. coli* O157:H7, *Salmonella typhimurium*, *Staphylococcus aureus* and *Listeria monocytogenes*, Food Control, 18, 414-420.

[2] Maisuthisakul, P., Suttajit, M., Pongsawatmanit, R. 2007. Assessment of phenolic content and free radical-scavenging capacity of some Thai indigenous plants. Food Chemistry, 100, 1409-1418.

[3] Abdille, Md. H., Singh, R. P., Jayaprakasha, G. K., Jena, B. S. 2005. Antioxidant activity of the extracts from *Dillenia indica* fruits. Food Chemistry, 90, 891-896.

[4] Elix, J. A. 1996. Biochemistry and secondary metabolites. In: Lichen Biology (Nash III T. H., ed.). Cambridge University Press, Cambridge, pp., 154-181.

[5] Lawrey, J. D. 1989. Lichen secondary compounds: evidence for a correspondence between antiherbivore and antimicrobial function. Bryologist, 92, 326-328.

[6] Richardson, D. H. S. 1988. Medicinal and other economic aspects of lichens. In: CRC Handbook of Lichenology, Vol. 3 (Galun M., ed.). CRC Press, Boca Raton, FL, pp., 93-108.

[7] Huneck, S. 1999. The significance of lichens and their metabolites, Naturwissenschaften, 86, 559-570.

[8] Huneck, S. 2001. New results on the chemistry of lichen substances. In: Progress in the Chemistry of Organic Natural Products, 81 (Herz W., Falk H., Kirby G. W., and Moore R. E., eds.). Springer, Wien, New York, pp., 224-230.

[9] Nimis, P. L., Tretiach, M. 1997. A Revision of *Tornabea*, a Genus of Fruticose Lichens New to North America. Bryologist, 100, 217-225.

[10] Culberson, C. F. 1979. Chemical and Botanical Guide to Lichen Products *Reprint*, Otto Koeltz, Science Publishers, Koenigstein.

[11] Řezanka, T., Temina, M., Hanuš, L., Dembitsky, V. M. 2004. The torna beatins, four tetrahydro-2-furanone derivates from the lichenized ascomycete *Tornabea scutellifera* (With.) J.R.Laundon. Phytochemistry, 65, 2605-2612.

[12] Singleton, V. L., Rossi, J. A. Jr. 1965. Colorimetry of total phenolics with phosphomolybdic–phosphotungstic acid reagents. American Journal of Enology and Viticulture, 16, 144-158.

[13] Prieto, P., Pineda, M., Aguilar, M. 1999. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: Specific application to the determination of vitamin E. Analytical Biochemistry, 269, 337-341.

[14] Cao, L., Si, J. Y., Liu, Y., Sun, H., Jin, W., Li, Z., Zhao, X. H., Pan, R. L. 2009. Essential oil composition, antimicrobial and antioxidant properties of *Mosla chinensis* Maxim. Food Chemistry, 115, 801-805.

[15] Lee, S. K., Mbwambo, Z. H., Chung, H. S., Luyengi, L., Games, E. J. C., Metha, R. G., Kinghorn, A. D., Pezzuto, J. M. 1998. Evaluation of the antioxidant potential of natural products. Combinatorial Chemistry & High Throughput Screening, 1, 35-46.

[16] Albayrak, S., Aksoy, A., Sagdic, O., Hamzaoglu, E. 2010. Compositions, Antioxidant and Antimicrobial Activities of *Helichrysum* (Asteraceae) Species Collected from Turkey. Food Chemistry, 119, 114-122.

[17] Jayaprakasha, G. K., Singh, R. P., Sakariah, K. K. 2001. Antioxidant activity of grape seed (*Vitis vinifera*) extracts on peroxidation models in vitro. Food Chemistry, 73, 285-290.

[18] Gülçin, I., Oktay, M., Küfrevioğlu, O. I., Aslan, A. 2002. Determination of antioxidant activity of lichen *Cetraria islandica* (L) Ach. Journal of Ethnopharmacology, 79, 325-329.

[19] Aslan, A., Güllüce, M., Sökmen, M., Adigüzel, A., Sahin, F., Özkan, H. 2006. Antioxidant and Antimicrobial Properties of the Lichens *Cladonia foliacea*, *Dermatocarpon miniatum*, *Everinia divaricata*, *Evernia prunastri*, and *Neofuscella pulla.* Pharmaceutical Biology, 44, 247-252.

[20] Gulluce, M., Aslan, A., Sokmen, M., Sahin, F., Adiguzel, A., Agar, G., Somken, A. 2006. Screening the antioxidant and antimicrobial properties of the lichens *Parmelia saxatilis*, *Platismatia glauca*, *Ramalina pollin*aria, *Ramalina polymorpha* and *Umbilicaria nylanderiana*. Phytomedicine, 13, 515-521.

[21] Türk, A. Ö., Yılmaz, M., Kıvanç, M., Türk, H. 2003. The Antimicrobial Activity of Extracts of the Lichen *Cetraria aculeata* and Its Protolichesterinic Acid Constituent. Zeitschrift für Naturforschung, 58c, 850-854.

[22] Saenz, M. T., Garcia, M. D., Rowe, J. G. 2006. Antimicrobial activity and phytochemical studies of some lichens from south of Spain. Fitoterapia, 77, 156-159.

[23] Candan, M., Yılmaz, M., Tay, T., Kıvanc, M., Türk, H. 2006. Antimicrobial activity of extracts of the lichen *Xanthoparmelia pokomyi* and its gyrophoric and stenosporic acid constituents. Zeitschrift für Naturforschung., 61c, 319-323.

[24] Odabasoglu, F., Aslan, A., Cakir, A., Suleyman, H., Karagoz, Y., Halici, M., Bayir, Y. 2004. Comparison of Antioxidant Activity and Phenolic Content of Three Lichen Species. Phytotherapy Research, 18, 938-941.

[25] Suhaj M. 2006. Spice antioxidants isolation and their antiradical activity: a review. Journal of Food Composition and Analysis, 19, 531-537.

[26] Cushnie, T. P. T., Lamb, A. J. 2005. Antimicrobial activity of flavonoids. International Journal of Antimicrobial Agents, 26, 343-356.

[27] Pietta, P. G. 2000. Flavonoids as antioxidants. Journal of Natural Products, 63, 1035-1042.

Table List

Table 1. The yields, total phenolic content, total antioxidant activities, IC_{50} values and the effects on β-carotene bleaching of *T. scutellifera*

	Methanol Extract
Yield (%)	8.67 ± 0.9
Total phenolic content (mg GAE/g extract)	7.03 ± 0.3
Total antioxidant activity (mg AAE/g extract)	80.61 ± 0.3
β-carotene bleaching (I%)	72.00
IC_{50} (µg/mL)	147.06

Total phenolic content expressed as gallic acid equivalent (GAE), total antioxidant activity expressed as ascorbic acid equivalent (AAE).

Table 2. Antimicrobial activities of *T. scutellifera* methanol extract (inhibition zones, mm).

Microorganisms	Methanol extract (%)				Antibiotics (µg)				
	10	5	2.5	1	AMP	C	CN	E	OX
Gram (-)									
A. hydrophila	8.0*	8.0	7.0	7.0	27.0 [a]	18.0	8.5	20.0	15.0
E. coli	-	-	-	-	6.5	17.0	9.0	-	-
M. morganii	-	-	-	-	-	11.0	-	-	-
K. pneumoniae	17.0	15.0	7.0	-	14.0	13.0	6.5	11.0	-
P. mirabilis	-	-	-	-	26.0	19.0	8.0	-	-
P. aeruginosa	8.0	7.0	7.0	7.0	25.0	15.0	12.0	-	-
S. typhimurium	-	-	-	-	24.0	22.0	8.0	-	-
Y. enterocolitica	-	-	-	-	8.0	17.0	9.0	7.0	-
Gram (+)									
B. brevis	12.0	11.0	9.0	7.0	8.0	20.0	16.0	22.0	-
B. cereus	13.0	12.0	8.0	-	31.0	21.0	11.0	18.0	20.0
B. subtilis	-	-	-	-	24.0	25.0	12.0	20.0	19.0
L. monocytogenes	-	-	-	-	28.0	25.0	13.0	23.0	-
S. aureus	20.0	15.0	-	-	16.0	15.0	7.0	12.0	-
Yeasts									
C. albicans	-	-	-	-	-	-	-	-	-
S. cerevisiae	-	-	-	-	-	-	-	-	-

*: Inhibition zones include diameter of hole (5 mm). Sample amount 50 µL.

[a]: inhibition zones include diameter of disc (6 mm).

Ampicillin (AMP-10 µg/disc). Chloramphenicol (C-30 µg/disc). Erythromycin (E-15 µg/disc). Gentamycin (CN-10 µg/disc). Oxacillin (OX-1 µg/disc).

-: Not active

Figure List

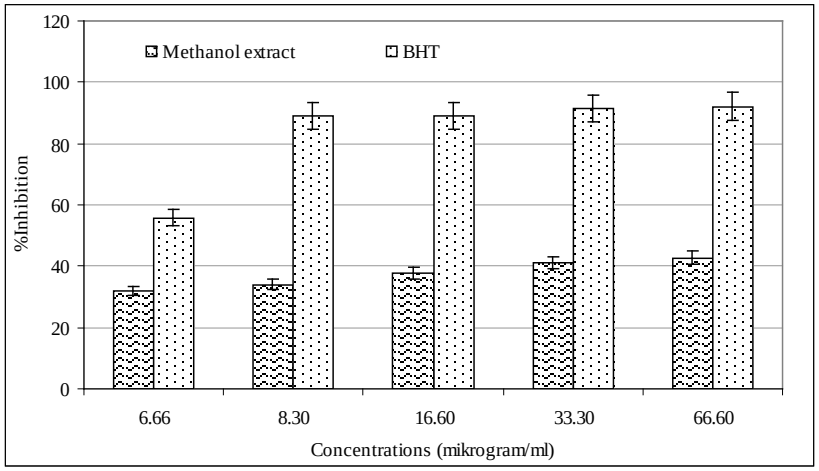

Figure. %Inhibition values of *T. scutellifera* methanolic extracts tested by DPPH assay