Journal of Medicinal Plants Research Vol. 4(16), pp. 1637-1642, 18 August, 2010
Available online at http://www.academicjournals.org/JMP
DOI: 10.5897/JMPR10.087
ISSN 1996-0875 ©2010 Academic Journals

Full Length Research Paper

Antimicrobial and antioxidant activities of medicinal plant species *Ornithogalum alpigenum* stapf. from Turkey

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Accepted 31 March, 2010

In this study, examination of the antioxidant and antimicrobial properties inherent in leaves and bulbs of geophytic, endemic species, *Ornithogalum alpigenum* Stapf. were carried out. Of all the extracts derived from the leaves and bulbs of this plant with various solvents, the acetone bulb extract was most inhibitory to the growth of with inhibition zone diameter of 12 ± 2 mm *Candida albicans* ATCC 10239. It was determined that the extracts of *Ornithogalum* Leaf-ethanol (OLE) (11 ± 2 mm diameter) and OTA (8 ± 2 mm diameter) were effective on *Bacillus subtilis* ATCC 6633, OTB (9 ± 1 mm diameter) and OTA (10 ± 2 mm diameter) was effective on *Bacillus cereus* RSKK 86. We studied that the total antioxidant and free radical scavenging activities of the extractions obtained from *O. alpigenum* Stapf. Total antioxidant activity of the extract from *O. alpigenum* Stapf. was determined using β–carotene-linoleic acid model system and was found the highest antioxidant activity of 88.12 ± 0.9% in methanol bulb extracts. Free radical scavenging activity of the extracts using the free radical 1,1-diphenyl-2-picrylhydrazyl (DPPH) was also determined. The overall results showed that some solutions have values (90.9% > 90.5% > 90.4% > 88.4%) very close to those of Butylated Hydroxytoluene (BHT) (90.0%). The leaf extracts were more potent with respect to free radical scavenging activity.

Key words: *Ornithogalum alpigenum*, antimicrobial activity, antioxidant activity, Butylated Hydroxytoluene, 1,1-diphenyl-2-picrylhydrazyl, geophytes, organic extracts, radical scavenging.

INTRODUCTION

Reactive Oxygen Species (ROS), such as hydroxyl radical, hydrogen peroxide, and superoxide anions, are produced as by-products in aerobic organisms and have been implicated in the pathology of a vast variety of human diseases including cancer, atherosclerosis, diabetic mellitus, hypertension, AIDS and aging. Therefore, antioxidant activity is important in view of the free radical theory of aging and associated diseases (Halliwell and Gutteridge, 1984; Wallace, 1999; Lee et al., 2000). Researchers have reported antimicrobial activity of several mushrooms (Lee et al., 1999; Kim and Fung, 2004). The chloroform and ethyl acetate extracts of the dried mushroom have antibacterial activity against *Streptococcus mutans* and *Prevotella intermedia* (Hirasawa et al., 1999). Both fruiting body and the mycelium contain compounds with wide-ranging antimicrobial activity. Researchers reported antimicrobial activity of several mushrooms (Gezer et al., 2006; Mercan et al., 2006; Turkoglu et al., 2007). It is studied antimicrobial activity *Cyclamen mirabile* Hildebr and *Cyclamen trochopteranthum* O. Schwarz. For studying antimicrobial activity *C. mirabile* Hildebr and *C. trochopteranthum* O. Schwarz tests with microorganisms are used; a gram-negative (*Escherichia coli* ATCC 25922), gram-positive (*Bacillus subtilis* ATCC 6633) and fungus (*Candida albicans* ATCC 1023). As appears from

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the present data, *C. mirabile* Hildebr and *C. trochopteranthum* O. Schwarz have not antimicrobial activity, and only it is appreciable antifungal activity (diameter of a zone, a sterile disk, 11 and 12 mm) (Gundogan et al., 2005).

The ability of *Sternbergia candida* Mathew. extract to suppress attack by *Postia placenta,* (a brown-rot) and *Trametes versicolor*, (a white-rot) was investigated. The extract was dissolved from *Sterinbergia candidum*'s bulb and leaves in 96% ethyl alcohol. Poisonous extract impregnated into wood blocks of Turkish oriental beech (*Fagus orientalis* L.) and Scottish pine (*Pinus sylvestris* L.). Extract dosages were 0.25, 0.75, 1.50 and 3.00%. Treated blocks were exposed to *P. placenta* and *T. versicolor* attack for 12 weeks by the soil block method. Only extract dosages of 0.25 and 0.75% were found efficacious in suppressing attack (Goktas et al., 2007). The chloroform and ethyl acetate extracts of the dried mushroom have antibacterial activity against *S. mutans* and *P. intermedia* (Hirasawa et al., 1999). In recent years, multiple drug resistance in human of pathogenic microorganisms has developed due to indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious diseases. This situation forced scientists to search for new antimicrobial substances from various sources which are the good sources of novel antimicrobial chemotherapeutic agents (Karaman et al., 2003). Infusion of *Lepista nuda* is used to prevent beriberi disease. In addition, the decoction is used for the treatment of abscesses and wounds (Dulger et al., 2002).

Almost all organisms are well protected against free radical damage by enzymes, such as superoxide dismutase and catalase, or compounds such as ascorbic acid, tocopherols and glutathione (Mau et al., 2002). Although, almost all organisms possess antioxidant defense and repair systems that have evolved to protect them against oxidative damage, these systems are insufficient to prevent the damage entirely (Simic, 1988). However, antioxidant supplements or foods containing antioxidants may be used to help the human body reduce oxidative damage (Yang et al., 2001).

The *in vitro* antioxidant activity of the methanol extract of *Plantago bellardii* All. aerial parts evaluated using two different tests: scavenging of 1,1-diphenyl-2-picrylhydrazil (DPPH) radical, and inhibition of lipid peroxidation on liposomes prepared from bovine brain extract (Galvez et al., 2005). In another study carried out with *Ornithogalum longibracteatum* species, a new homoisoflavonoid, which is a 3-benzil, 4-chromanoid derivation, was identified and isolated (Dulcie et al., 2004). Kuroda et al. (1999) suggested that six unknown cholestan glikozides related to six membered hemiacetal ring system were isolated from *Ornithogalum saundersiae* and called Saundersioside C-H (1-6). These glikozides are observed to have high cytostatic effects on the HL-60 cells in humans. In another study six *O. saundersiae* type cholestan ramnozides were isolated. Another study on this type isolated three new acetyl cholestan glikozides from its bulbs (Kubo et al., 1992). Kuroda et al. (2006) reported that isolated four new steroidal gikozides from *Ornithogalum thyrsoides* and named them Ornithosaponin A-D.

In 1993, a new 22-homo-23 norcholestan trisaccharide having an inhibitive effect on cyclic AMP phosphordiesterase was isolated from the bulbs of *O. saundersiae* (Kuroda et al., 1993). A compound derivative of saponin isolated from *O. saundersiae* species, icogenin was tested for antitumor activity, and positive results were gained (Hou et al., 2006). Another study researched on the nitrogen reductase activity of *Ornithogalum nutans* and identified a rather high activity (Arslan and Guleryuz, 2005). *Ornithogalum alpigenum* Stapf. is an endemic species to Turkey that belongs to the family Hyacinthaceae (Manning et al., 2009). The extracts of this plant traditionally using on alopecia for microbial reasons (Baytop, 1999). The bulbs of *Ornithogalum* L. have rich contents cholestan glikozides and saponin (Kubo et al., 1992). In this study, we aimed the antimicrobial and antioxidant activities of traditionally using species *O. alpigenum* Stapf. from turkey.

MATERIALS AND METHODS

Plant materials and chemicals

Different parts (leaves and bulbs) of wild *O. alpigenum* Stapf. were collected from the natural environment of Muğla city in Turkey and cleaned to remove any residual compost. The air-dried leaves and bulbs were ground to fine powder and then stored in an air-tight container until further use. Tween-20, methanol, ethanol, benzene, acetone, β-carotene, chloroform, linoleic acid were obtained from E. Merck (Darmstadt, Germany). DPPH, Butylated Hydroxytoluene (BHT) were obtained from Sigma Chemical Co. (St. Lois, MO). Disk of ampicillin (10 µg, Merck), penicillin (10 U, Sigma) were used as positive controls. All other chemicals and solvents were analytical grade.

Extraction and plant extract preparations

After the plant was collected when it had flowered, its bulbs and leaves were dried, chopped up with a blender, and prepared for the experiment. In this study 10 g of the plant and 100 ml of solvent (Merck) were used for every sample (Darwish et al., 2002). These extractions were prepared using different solvents (methanol, ethanol, acetone and benzene). The mixture was extracted after being heated in a vibrating water bath at 55°C. Having been acquired as a result of extraction, the mixture filtered through filter paper (Whatman No: 1), and the solvents were evaporated in a rotary evaporator at 48 - 49°C. The water in each extract was frozen in freeze-drying machine and then drawn out.

Plant extracts

Ornithogalum (O), Bulb-methanol (OBM), Bulb-ethanol (OBE), Bulb-acetone (OBA), Bulb-benzene (OBB), Leaf-methanol (OLM), Leaf-ethanol (OLE), Leaf-acetone (OLA) and Leaf-benzene (OLB).

Determination methods of antimicrobial activity

In the tests carried out, the bacteria *E. coli* and *B. subtilis* and the

yeast *C. albicans* were used. The diffusion method was implemented (Collins et al., 2004). The bacterial strains were cultivated in Nutrient Broth at 37°C for 24 h, and the yeast was cultivated in Sabouraud Dextrose Broth at 28°C for 48 h. On the preparation of bacteria, a 1 mg/ml of concentration was prepared from the extracts obtained from the bulbs and leaves of the *O. alpigenum* Stapf. plant using methanol, ethanol, acetone, and benzene. The extracts were saturated into paper discs 6 mm in diameter. For the control, discs that only had solvents were prepared. These discs were placed should have bacteria on the agar plate. The petri dishes that had bacteria were incubated at 37°C for 24 h and the yeast at 28°C for 48 h.

Determination of total antioxidant activity

The antioxidant activity of the extracts was determined using β-carotene-linoleic acid model system (Amin and Tan, 2002). The β-carotene solution was prepared by dissolving 2 mg of β-carotene in 10 ml of chloroform. 40 mg of linoleic acid and 400 mg of Tween 20 were added to each millilitre of the solution, which was mixed with 100 ml of distilled water after the chloroform was evaporated in the rotary evaporator. 4.8 ml of this emulsion was placed into test tubes which had 0.2 mg of the sample and 0.2 of the extract in them. For control, 0.2 ml of solvent (methanol, ethanol, acetone or benzene) was placed the extract. As soon as the emulsion was added into the test tubes, initial absorbances were measured with a spectrophotometer (Shimadzu UV–1601, Japanese) at 470 nm. The tubes were incubated at 50°C until β-carotene discolored (120 min) (Amin et al., 2004; Amin et al., 2006). The measurements were made using the equation below:

$$AA = \left[1 - \frac{A_0 - A_t}{A_0^{o} - A_t^{o}}\right] x\,100$$

Where AA is the total antioxidant activity, A_0 is the initial absorbance of the sample, A_t is the initial absorbance of the control, A_0^{o} is the sample's absorbance after 120 min, and A_t^{o} is the control's absorbance after 120 min.

Determination of free radical scavenging activity

Free radical scavenging activity of the extracts was determined using the free radical DPPH (Cuendet et al., 1997; Kirby and Schmidt, 1997; Wu et al., 2006). 4 ml of the DPPH's 0.004% metanolic solution was mixed with 1 ml (0.2 - 1.0 mg) of the extracts, and their absorbances were measured to be at 517 nm after incubation for 30 min at room temperature the absorbance value of the samples were evaluated against empty control group (where all determinants except the test compound were present). Every test was treated three times and the averages as determined. Free radical scavenging activity was measured using the equation below:

$$\text{Scavenging effect \%} = 1 - \left[\frac{A_t\ 517nm}{A_0\ 517nm}\right] x\,100$$

Where; A_0 is the control's absorbance and A_1 is that of the sample.

Of the obtained inhibition values, the extract concentrations that provided 50% inhibition were measured in IC_{50} value.

RESULTS AND DISCUSSION

Antimicrobial activity results

Recently, there has been considerable interest in extracts from plants with antimicrobial activities for controlling pathogens and/or toxin producing microorganisms in foods (Alzoreky and Nakahara, 2003; Valero and Salmeron, 2003; Li et al., 2005). The antimicrobial activities of different *O. alpigenum* Stapf. extracts tested by agar dilution method were shown in Table 1. There are some values of inhibitions zones which are determined in experiment with studying the antibiotics of ampicillin and penicillin effects on microorganisms: 31 mm (the highest penicillin effect on *Staphylococcus aureus* ATCC 25923), 22 mm (penicillin effect on *Bacillus cereus* RSKK 863), 18 mm (ampicillin on *E. coli* ATCC 35218) and 12 mm (penicilin effect on *B. subtilis* ATCC 6633).

Extracts, which is to get by various solvents (methanol, ethanol, acetone and benzen) of the bulbs and leaves of *O. alpigenum* Stapf. are studied antimicrobial activity and results are shown in Table 1. The result of research is *O. alpigenum* Stapf. plant extract is more effective (12 ± 2 mm diameter) on *C. albicans* ATCC 10239 is determined. Partially low values are determined the extracts of OBB (6 ± 1 mm diameter) and OLB (8 ± 0 mm diameter) effect on *C. albicans* ATCC 10239. It is determined that the extracts of OLE (11 ± 2 mm diameter) and OBA (8 ± 2 mm diameter) is effective on *B. subtilis* ATCC 6633, OBB (9 ± 1 mm diameter), OBA (10 ± 2 mm diameter), OLB (8 ± 1 mm diameter) and OLE (7 ± 1 mm diameter) are effective on *B. cereus* RSKK 86. The highest value (10 ± 1 mm diameter) is observed in experimental which is done with *Pseudomonas aeruginosa* NRRLB-23 with OBA extracts. The extracts of OBB and OLB are more effective on *E. coli* ATCC 35218 bacteria. OLB (8 ± 0 mm diameter), OLE (7 ± 0 mm diameter) and OBB (6 ± 0 mm diameter) extracts are effective on *S. aureus* ATCC 25923. In some extracts there is no any antimicrobial activity (Table 1).

Total antioxidant activity

The total antioxidant activities of all the extracts from *O. alpigenum* Stapf. were determined by β-carotene-linoleic acid model system. This system depends on the principle that β-carotene discolors rapidly when no antioxidant is present as a result of the process in which free radicals produce hydroperoxides from linoelic acid. The addition of antioxidant containing extracts to the system enables peroxide products formed from linoleic acid to be neutralized by these antioxidants, and thus β-carotene preserves its characteristic orange as yellow color. Therefore, the higher absorbance of the samples proves a higher antioxidant activity. Researchers have studied the antioxidant activity of many plants. Tepe et al. (2005)

Table 1. Antimicrobial activities of the ampicillin, penicillin and various organic extracts of *O. alpigenum* Stapf. (Inhibition zone diameter, mm).

Test bacteria	Antibiotic and plant extracts									
	A	P	OBM	OBE	OBA	OBB	OLM	OLE	OLA	OLB
E. coli ATCC 35218	18	nt	–	7 ± 2	–	9 ± 1	5 ± 1	–	–	8 ± 1
S. aureus ATCC 25923	nt	31	–	–	4 ± 1	6 ± 0	–	7 ± 0	–	8 ± 0
P. aeruginosa NRRL B-23	nt	nt	4 ± 1	–	10 ± 1	4 ± 1	5 ± 0	–	3 ± 1	7 ± 2
B. subtilis ATCC 6633	nt	12	–	–	8 ± 2	5 ± 1	–	11 ± 2	–	–
B. cereus RSKK 863	nt	22	3 ± 1	–	9 ± 1	10 ± 2	–	7 ± 1	3 ± 1	8 ± 1
C. albicans ATCC 10239	nt	nt	–	8 ± 0	12 ± 2	6 ± 1	–	9 ± 2	6 ± 1	8 ± 0

A, Ampicillin (10 µg); P, Penicillin (10 U); nt, Not tested; –, No inhibition; O, *Ornithogalum*; OBM, Bulb-methanol; OBE, Bulb-ethanol; OBA, Bulb-acetone; OBB, Bulb-benzene; OLM, Leaf-methanol; OLE, Leaf-ethanol; OLA, Leaf-acetone; OLB, Leaf-benzene.

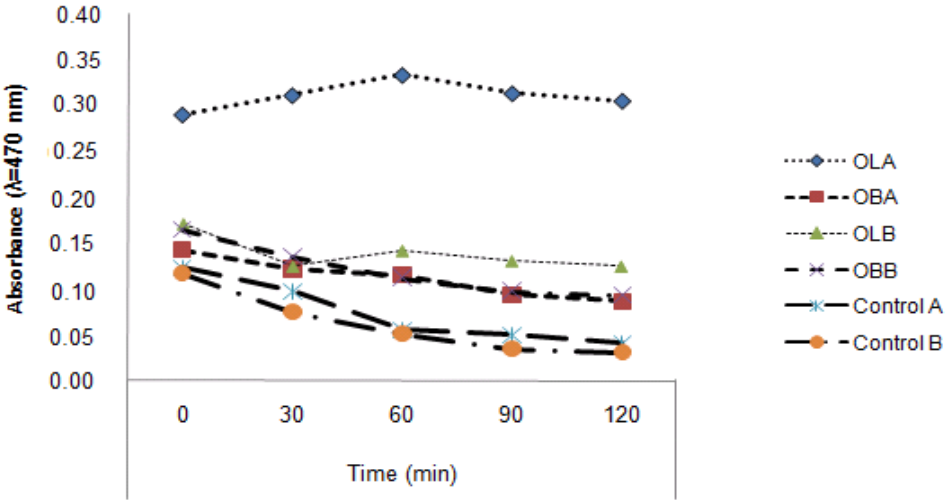

Figure 1. Absorbance values of aseton (Control A) and benzene (Control B) extracts determined with β-carotene-linoleic acid model system ($p < 0.05$). OLA, *Ornithogalum* Leaf-acetone; OBA, *Ornithogalum* bulb-acetone; OLB, *Ornithogalum* Leaf-benzene; OBB, *Ornithogalum* bulb-benzene.

The determination of antioxidant activity of *O. alpigenum* plant extracts as the control event methanol, ethanol, benzene and acetone solvents were used. Absorbance values of control substances were very low according to absorbance values of the extract samples. Highest absorbance value was observed Control A (0.041) in the control group and the highest absorbance value extracts was observed OLA solution (0.306) within 120 min (Figure 1).

However, the lowest absorbance value in the Control M (0.028) and OBE solution (0.069) have been determined (Figure 2). The highest antioxidant activity in the extracts of *O. alpigenum* Stapf. (88.12 ± 0.9%) was found in the extract obtained with methanol solvent from its bulbs. Also, the lowest antioxidant activity (24.20 ± 0.4%) was found in the extract obtained with benzene solvent from the bulbs (Figure 3). The reason why the extracts obtained from the same plant with different solvents have very different antioxidant activity can be seen in the polarities of the solvents.

Free radical scavenging activity

The free radical scavenging activity of the extracts depends on the ability of the antioxidant compounds to give away their hydrogen atoms and the structural conformation of the compound (Duha et al., 1992; Singleton et al., 1999; Mammadov, 2009). DPPH has maximum absorption spectrum of at 517 nm and is used as free radical in the determination of antioxidant activity in certain natural compounds. As a result of the reaction below, the DPPH free radical can easily get an electron or hydrogen radical from antioxidant molecules in order to be a stable molecule. The antioxidant activity of *Ruellia*

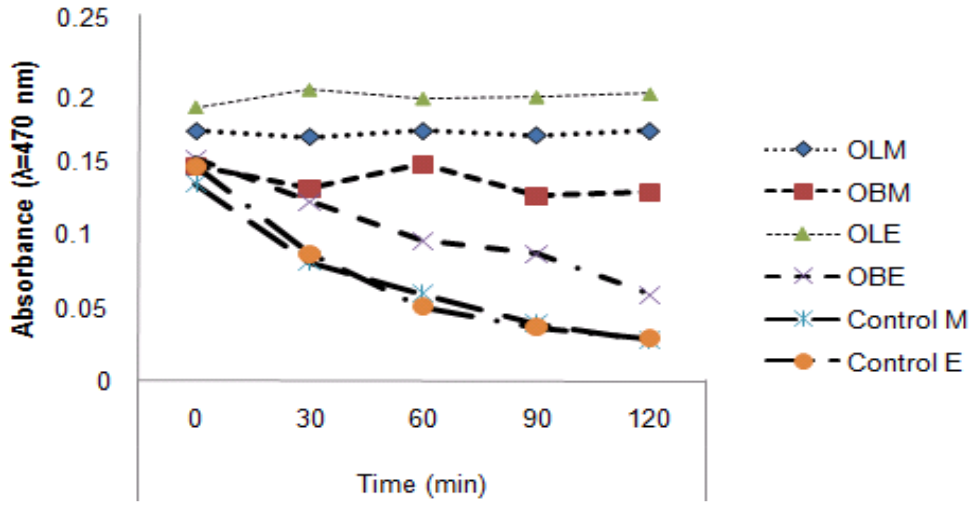

Figure 2. Absorbance values of methanol (Control M) and ethanol (Control E) extracts determined with β-carotene-linoleic acid model system ($p < 0.05$). OLM, *Ornithogalum* Leaf-methanol; OBM, *Ornithogalum* bulb-methanol; OLE, *Ornithogalum* Leaf-ethanol; OBE, *Ornithogalum* bulb-ethanol.

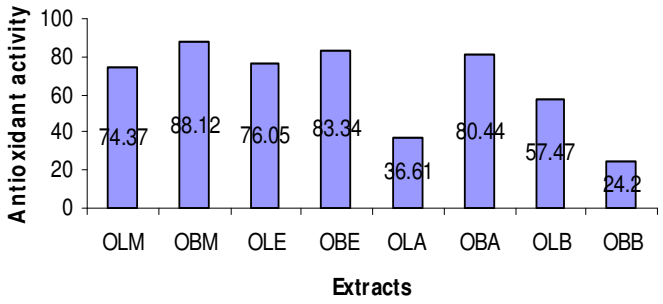

Figure 3. The antioxidant activities in the methanol, ethanol, acetone and benzene extracts ($p < 0.05$).OLM, *Ornithogalum* Leaf-methanol; OBM, *Ornithogalum* Bulb-methanol; OLE, *Ornithogalum* Leaf-ethanol; OBE, *Ornithogalum* Bulb-ethanol; OLA, *Ornithogalum* Leaf-acetone; OBA, *Ornithogalum* bulb-acetone; OLB, *Ornithogalum* Leaf-benzene; OBB, *Ornithogalum* bulb-benzene.

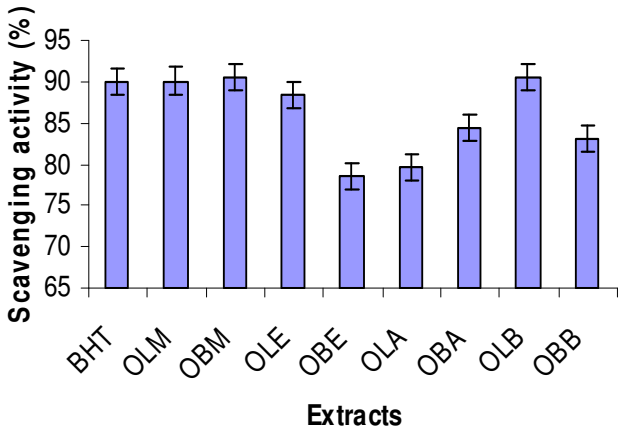

Figure 4. The free radical scavenging capacity of the extracts with methanol, ethanol, acetone and benzene through DPPH method. BHT, Butylated hydroxytoluene; OLM, *Ornithogalum* Leaf-methanol; OBM, *Ornithogalum* bulb-methanol; OLE, *Ornithogalum* Leaf-ethanol; OBE, *Ornithogalum* bulb-ethanol; OLA, *Ornithogalum* Leaf-acetone; OBA, *Ornithogalum* bulb-acetone; OLB, *Ornithogalum* Leaf-benzene; OBB, *Ornithogalum* bulb-benzene.

tuberosa L. (Acanthaceae) was investigated by the DPPH freeradical-scavenging assay and the hydrogen peroxide-induced luminol chemiluminescence assay. The Methanolic Extract (ME) and its four fractions of water (WtF), ethyl acetate (EaF), chloroform (CfF), and n-hexane (HxF) were prepared and then subjected to antioxidant evaluation (Chen et al., 2006). The free radical scavenging capacity of this plant is between 10 – 40%. In this study chemicals such as water, methanol, hexane and chloroform were used as solvents. The scavenging activity changed according to the type of the solvent. A study into the free radical scavenging capacity of peanut found the result to be 25% (Yen et al., 2005).

The extracts and the free radical scavenging effects of BHT were tested on DPPH, a stable free radical. The results of the free radical scavenging effects were calculated to be a concentration the 50% of which was scavenged by DPPH (IC_{50}). The low IC_{50} value shows the high antioxidant activity. For this reason, the amount of unused DPPH in the system was determined, and the percentage of the amount used later was measured. The overall results show that the values certain solvents have are very close to those of the BHT (90.04%) (OLM-90.80% > OLB-90.46% > OBM-90.38% > OLE-88.42%). Leaf extracts are generally more potent than the free radical scavenging activity (Figure 4). All these result

from the high amount of flavonoid derivatives in the structure of extracts.

ACKNOWLEDGEMENTS

The authors would like to thank Assistant Prof. Dr. Gulsen Ulukoy from University of Mugla, Faculty of Fisheries, for her excellent help to this study.

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