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DETERMINATION OF CHEMICAL CONTENT OF *LEMNA MINOR* L. BY GC-MS AND INVESTIGATION OF ANTIOXIDANT ACTIVITY

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ABSTRACT. *Lemna minor* L. has been traditionally used for a long time for its analgesic, antipyretic, vitamin C supplement, astringent, antipruritic effects. Although there are many heavy metal removals using *L. minor*, unfortunately, biological activity studies are very limited. In this study, the chemical content and total phenol content, DPPH removal, metal chelation (Fe^{2+}) and β-Carotene-lycopene methods of the *L. minor* macrophyte we obtained from Turkey were determined by GC-MS. The results of the study showed that our plant contains 25 different essential oils and has a high phenol content. In addition, 72% DPPH removal of *L.minor* was determined when it had 71% iron chelating ability. As a result of our study, it has been revealed that the *L. minor* we use is an effective antioxidant. It is thought that its usability in the fields of food and medicine can be investigated with further studies.

1. INTRODUCTION

Free radicals are high-energy, unstable molecules that carry one or more unpaired electrons in their final orbitals. These unpaired electrons give free radicals great reactivity, causing them to damage many biological materials such as proteins, lipids, DNA and coenzymes. Under normal conditions, there is a balance between free radicals and antioxidant defense system in our body. However, an increase in reactive oxygen species and/or a deficiency in the defense systems cause the antioxidant balance in the body to deteriorate and "oxidative stress" conditions occur. Recent studies have shown that this oxidative damage caused by free radicals can be the cause

Keywords. Lemna minor, antioxidants, DPPH, total phenol, GC-MS

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of many important diseases such as diabetes [1], cancer [2], atherosclerosis [3], immune system [4] and cardiovascular diseases [3-4-5]. Antioxidants are natural or synthetic substances that play an important role in preventing cancer formation by neutralizing free radicals, which are toxic by-products of normal cell metabolism [6-7-8].

Plants have a high antioxidant activity due to the secondary metabolites they contain, and due to these properties, they have been used both in folk medicine and in the pharmaceutical industry for years. Flavonoids, cinnamic acid derivatives, cumarins, tocopherols and phenolic acids are the most common herbal antioxidants. Studies have shown that plants increase antioxidant enzyme activity and reduce lipid oxidation thanks to these phenolics they have [6-9-10].

Lemna minor (Duckweed) belongs to the Lemnaceae family and is a perennial, simple, small plant that can be found floating or submerged in fresh waters. Although there are limited scientific studies, *L. minor* is a herb that has been used frequently in traditional medicine and homeopathy for many years. It is known that it is used externally as an antipyretic, diuretic, anti-inflammatory in upper respiratory tract and chronic rheumatic diseases, as well as in eczema, acne, wound healing and insect bites [11]. There are many commercial drugs prepared with *L. minor* extract for use in allergic asthma, rhinitis and nasal congestion problems. The fact that Lemna species contain phenolic compounds such as gallic acid, tannins, flavonoids, anthocyanins, quercetin, and compounds such as thiol and terpene known as steroids suggest that they may have antimicrobial, antioxidant and even anticarcinogenic properties. Studies have shown that they have antimicrobial activity against the pathogens *Bacillus subtilis, B. cereus, Staphylococus aureus, S. saprophyticus, S. warneri, Proteus vulgaris, Citrobacter freundii, C. koseri, Neisseria lactamica, Micrococus luteus* and *Streptococus pneumoniae* [12-13]. Gülçin et al. showed that the *Lemna minor* has antibacterial, anticandidal and antioxidant effects in their study with ethanol and water extracts [13]. In the studies by Popov et al., it has been shown that lemnan isolated from *Lemna minor* macrophyte has safety and tolerability in cellular and humoral immunity, as well as ease of formulation and can be used as an adjuvant in vaccines developed for various infections [14].

Within the scope of our study, the chemical content, total phenol amount, DPPH scavenging activity, iron chelating activity and β-carotene/lycopene content of *L. minor* was determined and its usability in medicine, pharmacy and food fields was investigated.

2. Materials and Methods

2.1. Extraction of *L. minor*

L. minor used in the study was developed in the aquarium of Hydrobiology Laboratory from Ankara University. The collected plants were washed twice with distilled water and left to dry at room temperature. The dried plants were crushed to powder and stored in room conditions, out of the sun until the study was carried out.

Microwave assisted extraction method was used for extraction. Microwave extraction method is a frequently used method for obtaining plant extract in recent years. It has advantages such as less solvent consumption, shorter time and no evaporation phase compared to the classical soxhlet method. The dried and powdered plant samples were prepared in proportion to 1g/20ml volume and extracted in 60% ethanol using microwave at 850 Watt, 90 seconds conditions. The obtained extracts were centrifuged at 2500 rpm for 20 minutes, the supernatant was passed through filter paper and dried in a lyophilizer (Christ, Alpha 1-2 LD). The samples were stored at +4˚C until the study was carried out [15].

2.2. Determination of Chemical Contents by GC-MS

Gas Chromatography/Mass Spectrometer (GC-MS) method was used to identify the volatile components obtained by microdistillation, and Gas Chromatography method was used to determine their relative percentages. System was used Agilent 7890B GC 5977B Mass Selective System that consisted of Agilent HP-Innowax. The sample taken into the hexane phase was injected into the system as 1 microliter with a 10:1 split ratio. definitions were made with the help of the Wiley-9 Nist 11 Mass Spectral database (Anadolu University Plant, Medicine and Scientific Research Application and Research Center, AUBIBAM).

2.3. Determination of Total Phenolic Content

The total amount of phenolic compounds was determined spectrophotometrically by adapting the Folin-Ciocalteu method of Barros et al. to 96-well plates. 0.02 ml (concentration 1 mg / ml) was taken from the extracts, mixed with 40 µl of Folin-Ciocalteus reagent:water (50:50) and 0.2 ml of 2% sodium carbonate, and incubated for 90 minutes at room temperature. After incubation, absorbances were read at 760 nm wavelength (Epoch, BioTek) and calculations were made according to the Gallic acid standard [16].

2.4. Investigation of DPPH Scavenging

The scavenging capacity of 1,1-diphenyl-2-picrylhydrazil (DPPH) free radical was determined by spectrophotometric method. 0.004% DPPH solution was added to the determined concentrations (25-400 µg/ml) of the extracts and incubated for 30 minutes in the dark. At the end of the incubation, the free radical scavenging effect was determined according to the following formula (2.1) by measuring spectrophotometrically (Epoch microplate reader, BioTek) at a wavelength of 517 nm [17].

$$\% \ Scavenging = [(A_{Control} - A_{Sample}) / A_{Control}] \times 100 \quad (2.1)$$

2.5. Investigation of Iron (Fe^{+2}) Ion Chelating Activity

Iron ion chelating activity was determined by making some changes in Decker and Welch's method. By adding 0.05 ml 2 mM FeCl2 and 0.1 ml 5 mM ferrosine to the concentrations of the extracts (25-400 µg/ml), the samples were left for incubation at room temperature for 15 minutes After incubation, the absorbance was measured at 562 nm wavelength and the calculation was made according to the following equation (2.2)[18].

$$\% \ Chelating = [(A_{Control} - A_{Sample}) / A_{Control}] \times 100 \quad (2.2)$$

2.6. Determination of β-Carotene and Lycopene Contents

100 mg of dry extract was completely dissolved in 10 ml of acetone:hexane (6:4) mixture and passed through a 0.45 µm filter. The absorbances at 453, 505, 645 and 663 nm wavelengths were measured and calculated according to the following formulas (2.3) [19].

$$Lycopene \ (mg/100 \ ml) = -0.0458 A_{663} + 0.204 A_{645} + 0.372 A_{505} - 0.0806 A_{453}$$
$$\beta\text{-}Carotene \ (mg/100 \ ml) = 0.216 A_{663} - 1.22 A_{645} - 0.304 A_{505} + 0.452 A_{453} \quad (2.3)$$

3. RESULTS

3.1. Determination of Chemical Contents of *L. minor*

It was determined that the *L. minor* methanol extract we used in our study contained 25 different volatile compounds, and the compound with the highest rate was phytol (19,8 %) (Table 1). Phytol is an acyclic diterpene alcohol that can be used as a precursor in the production of synthetic forms of vitamin E and vitamin K. Many studies with Phytol have shown that this molecule has antimicrobial, antidiabetic, antidiuretic, anti-inflammatory and anticarcinogenic properties [20]. Moraes et al. reported that phytol, which is widely used as a food additive and in medical fields, is effective against parasites (antiscistosomal) in vitro and in vivo studies with mice [21]. Other compounds in *L. minor* were found to be hexanal (5.6 %), 2,4-Di-tert-butylphenol (2.2 %), 5-Tetradecene (5.0 %), cetene (4.5 %). 2,4-Di-tert-butylphenol is known to have antifungal and antioxidant properties. The 2,4 DTBP compound obtained from the bacterium (*Lactococcus* sp.) can be an antifungal and antimicrobial food additive that can improve food safety and also contribute positively to health [22]. Hexanal, also called hexanaldehyde or caproaldehyde, is used as a food additive due to its shelf life extension and flavoring properties. Tacheva et al., in their similar study, they stated that *L. minor* extract has 12 different antioxidant compounds (phytol, campesterol, loliolide, dihydroactinidiolide, ascorbic acid, vanillic acid, 2,3-dihydroxybenzoic acid, caffeic acid, chlorogenic acid, esculetin, esculin and fraxetin) [23]. Their content analysis is similar to our study. When the chemical contents of 0.5% relative ratio are analyzed using GC-MS, it is seen that *L. minor* contains antimicrobial and antioxidant compounds.

3.2. Determination of Total Phenolic Content

It is known that phenolic content and antioxidant capacity are parallel. Therefore, the *L. minor* total phenol content was calculated in the next step. Plants with high phenolic content show higher antioxidant and anticarcinogenic activity. As a result of our study, it was determined that the *L.minor* extract we studied contained 20.44 mg GAE/100g total phenol. There are very few antioxidant activity studies with *L. minor*. However, the total phenol results found in the study of Gülcin et al. are similar to our study. They were reported that water (WELM) and ethanol (EELM) extracts had phenolic substances between 22.0 ± 0.8 and 16.7 ± 0.0 μg GAE.

TABLE 1. Volatile compound content of *Lemna minor*.

No	Compound	%
1	Pentanal $$ n-Pentanal $$ n-Valeraldehyde $$ Valeral	5.0
2	Hexanal $$ n-Hexanal $$ Hexaldehyde $$ Caproaldehyde $$ Capronaldehyde	5.6
3	2-Pentylfuranm (2-Amylfuran)	3.8
4	1-Dodecene	2.2
5	*p*-Cymene	4.4
6	Isopropyl pentyl ketone (2-Methyl-3-octanone)	1.8
7	*Spektrum-1*	1.0
8	6-Methyl-5-hepten-2-one	1.5
9	Nonanal (CAS) $$ n-Nonanal $$ n-Nonylaldehyde $$ Nonaldehyde $$ n-Nonaldehyde	1.8
10	(*E*)-5-Tetradecene	5.0
11	Cetene (1-Hexadecene)	4.5
12	Heptadecane	4.4
13	1-Octadecene	2.0
14	*Spektrum-2* ((*E*)-Geranylacetone)	2.3
15	Neophytadiene	2.1
16	(*E*)-beta.-Ionone	2.6
17	*Spektrum-3* Neophytadiene isomer I: II	7.1
18	Pentadecanal- $$ 1-Pentadecanal $$ n-Pentadecanal	5.3
19	Hexahydrofarnesyl acetone	1.6
20	*Spektrum-4*	4.3
21	Thymol	1.3
22	*Spektrum-5*	5.8
23	2,4-Di-tert-butylphenol	2.2
24	Farnesyl acetone	1.6
25	Phytol	19.8
	Toplam	**99.0**

* ≥ % 0.5

Gülçin et al., in their study with Lemna minor, reported that water (WELM) and ethanol (EELM) extracts had phenolic substances between 22.0 ± 0.8 and 16.7 ± 0.0 µg GAE [13].

3.3. DPPH Scavenging Activity

It was observed that the DPPH scavenging effect increased in a dose-dependent manner. Although *L. minor* methanol extract had activity (18-72 %) at all concentrations studied, it showed the highest efficiency at 400 µg /ml with 72% removal (Figure 1).

L. minor is a fast growing plant with high protein content and is an important food source for aquatic animals. It is also known for its antipyretic and analgesic properties. Based on these properties, Kim et al., in their study, claiming that the substances in the environment will affect the metabolite content, the total phenol amount and indirectly the antioxidant activity, and when they added 3% sucrose and 0.5 mM proline, they found the DPPH scavenging effect to be 69.1% [24]. In our study, *L. minor* showed a similar effect and swept the DPPH radical in the environment by 62%. Antioxidant studies are among the subjects of much research today, and now more comprehensive and advanced methods are tried to increase the effectiveness of plants whose effectiveness is known. Saying that *L. minor* macrophyte contains high protein and is a good source of bioactive peptides, Tran et al. investigated the antioxidant activity of hydrolyzed proteins obtained from lean *L. minor*. It was observed that *L. minor* samples hydrolyzed with flavourzyme and alcalase removed 28.91-54.15% DPPH. The results showed that protein recovery, hydrolysis degree values and antioxidant activities were found increased with increasing enzyme concentration and hydrolysis time. They said that under the same enzymatic hydrolysis condition, samples hydrolyzed with flavourzyme had a higher inhibitory effect on ABTS and DPPH radical scavenging than samples hydrolyzed by alcalase and alkaline treatment [25]. Saritha and Saraswathi, on the other hand, studied another popular topic, nanoparticles, and looked at the DPPH scavenging effect of gold nanoparticles they synthesized using *L. minor*. As a result of their study, it was determined that the *L. minor* extract had a DPPH scavenging effect, however, they found that the DPPH scavenging activity of the gold nanoparticles synthesized with *L. minor* was higher. *L. minor* is an important food source for living creatures in the aquatic environment. In addition to its rich nutritional content and known pharmacological properties, its rapid vegetation period and easy development have attracted the attention of researchers [26]. *L. minor*, which is considered to have antioxidant properties because it contains high amounts of vitamin E, carotenoids and flavonoids, is also used as a feed and feed additive due to its high nutritional content. Iskandar et al. found the DPPH scavenging ability of *L. minor* extract, which they plan to use as a fish feed additive, as IC_{50}; 54.517 ppm. In the next stage of the study, they looked at the effect of antioxidant effect on growth and immunity in Nile tilapia fish, which they fed with ready-to-feed + 25,50,75% and 100 IC_{50} extract. As a result of the study, it was found that the group fed with 25% IC_{50} *L. minor* extract increased the daily growth rate, vitality and immune system [27].

When all these studies are examined, we can say that the *L. minor* macrophyte we use has high DPPH removal, thus it has the potential to be used as an antioxidant in many fields such as food, cosmetics, feed additives, and the pharmaceutical industry.

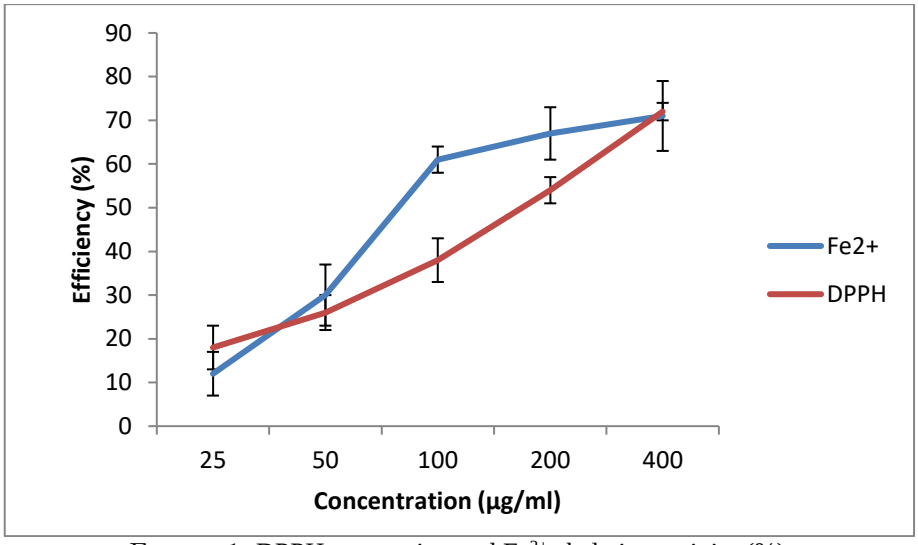

FIGURE 1. DPPH scavenging and Fe^{2+} chelating activity (%).

3.4. Determination of Metal chelating activity on ferrous ions (Fe^{2+})

Among the transition metals, iron is known as the most important pro-oxidant in lipid oxidation due to its high reactivity. At the same time, iron ions play a role in the occurrence of Fenton reactions and cause the formation of hydroxyl radicals. It is known that the hydroxyl radical, the amount of which increases in the environment, damages DNA and causes genetic mutations and cancer in advanced processes. Secondary antioxidants that come into play in this process can prevent this process, which progresses to cancer formation, by forming chelate with metal ions and inhibiting the Fenton reactions as in the equation $Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + {}^*OH + OH–$. Our study results showed that the *L. minor* we analyzed was effective at every concentration studied. The chelating efficiency increased in a dose-dependent manner (Figure 1). Our macrophyte, which has a chelating effect of 12-71%, showed the highest efficiency at a concentration of 400 µg /ml. There are a limited number of studies showing that *L. minor* have antioxidant effects and iron chelating activity. Gülçin et al., in their study with *L. minor*, stated that ethanol extract (EELM) was 61%, water extract (WELM) 63%, and the chelating effect was BHA > WELM > EELM > BHT > trolox > αtocopherol, respectively [13]. When we compare these results, we can say that *L. minor* in our study has higher activity.

3.3. Determination of β-Carotene and Lycopene Contents

β-Carotene and Lycopene are the main hydrocarbon carotenoids with apolar properties. Carotenoids are secondary plant pigments that give yellow, orange and red colors and can be synthesized by plants and some bacteria, algae and fungi. Studies have shown that β-Carotene and lycopene are powerful antioxidants and that their dietary intake is very beneficial for health. As a result of our analysis, it was determined that *Lemna minor* extract contained 0.116 mg/100 ml of β-carotene. It is known that β-carotene has antioxidant activity due to its free radical scavenging ability. In addition, β-carotene is the precursor of vitamin A, which has skin rejuvenation, visual functions, reproduction, anmmune-enhancing effects. Lycopene is not a vitamin A precursor, but is the strongest antioxidant in the carotenoids. In addition, in many scientific studies, it has been reported that lycopene induces apoptosis on many cancer cell lines such as prostate, lung, colon, and also has an effect on cardiovascular diseases, bone, skin and eye health. It was determined that the *L. minor* extract we studied contains 0.091 mg/ 100ml lycopene. As a result of the study, it was observed that the methanol extract of *L. minor* had high carotene and lycopene [28].

TABLE 1. Contents of total phenols, β-carotene, lycopene and IC_{50} (μg /ml) values of DPPH and Fe^{+2} in the methanolic extract.

Species	Total Phenol (mg/GAE g)	DPPH IC_{50}	Fe^{+2} IC_{50}	β-carotene mg/100 ml	Lycopene mg/ 100ml
L. minor	20,44±1,03	159,08±5,14	103,76±3,26	0.116±0,03	0.091±0,01

4. CONCLUSION

Within the scope of this study, the chemical content and antioxidant properties of *Lemna minor* macrophyte obtained from inland waters of Turkey were investigated. The results of the study showed that the *L. minor* extract has high phenolic content and these results were also confirmed by GC-MS analysis. At the same time, it was determined that our extract had high DPPH scavenging and iron chelating efficiency in parallel with its phenol content. Considering these results, it is thought that our L.

minor extract is a strong natural antioxidant and its usability in food and pharmacy areas can be investigated.

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Within the scope of this study, the chemical content and antioxidant properties of Lemna minor macrophyte obtained from inland waters of Turkey were investigated.

Author Contribution Statements MBE- Collection, identification and development of *Lemna minor*. SA- project development, manuscript editing. SYD- data analysis, manuscript writing and manuscript editing. All authors have read and approved the manuscript.

Declaration of Competing Interests The authors declare no conflict of interest

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