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RESEARCH ARTICLE

Determination of *In vitro* Antioxidant Activities and Macro and Micro Elements Level in Different Extracts of *Cynara Scolymus* L. leaf

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ABSTRACT

Globe Artichoke (*Cynara scolymus* L.) belonging to the family of Astericaea, has antioxidant, hepatoprotective and hypoglycemic activities, its leaves have traditionally been used for diuretic and choleretic purposes. Therefore the main goal of this study is to determine the total phenolic content of artichoke leaf and its extracts with methanol, ethyl acetate and n- hexane, some *in vitro* antioxidant activities, selected macro (Na, K, Ca, Mg, P) and micro (Zn, Cu, Mn, Cr, Se, I) elements' levels. Accordingly the total phenolic content values for the methanol, ethyl acetate and N-hexane extracts found 5,375 mg, 0,917 mg, 0,167 mg Gallic acid (GAE)/g respectively. Methanol extract showed highest DPPH free radical scavenging activity (87,73%), ethyl acetate extract possessed the highest Superoxide radical scavenging activity (SRSA) (49,02 %) whereas N-hexane extract contained high level metal chelating ability (289,32µM Fe). In terms of macro and micro elements (except I and Cr levels), the highest concentrations are recorded in its leaves, which are considered as a natural mineral source. Accordingly, it is evaluated that artichoke leaves provide a potential natural sources of K and Zn, while methanol and N-hexane extracts are good sources of P and Zn.

Keywords: Antioxidant activity, artichoke, *Cynara scolymus* L., leafextracts, minerals

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Cynara scolymus L. Yaprağının Farklı Ekstraktlarda *In vitro* Aktioksidan Aktivitelerinin ve Makro ve Mikro Element Seviyelerinin Belirlenmesi

ÖZ

Papatyagiller familyasına ait olan Küre enginarın (*Cynara scolymus* L.) antioksidan, hepatoprotektif ve hipoglisemik etkilere sahiptir, yaprakları geleneksel olarak idrar söktürücü ve koleretik amaçlarla kullanılmaktadır. Bu nedenle bu çalışmanın temel amacı enginar yaprağının ve yaprağın metanollü, etil asetatlı ve n-hekzanlı ekstraktlarının toplam fenolik içeriğini bazı *in vitro* antioksidan aktiviteleri, seçilmiş makro (Na, K, Ca, Mg, P) ve mikro (Zn, Cu, Mn, Cr, Se, I) element düzeylerini, belirlemektir. Toplam fenolik içerik değerlerine göre metanol, etil asetatlı ve n-hekzan ekstraktları sırasıyla 5,375 mg, 0,917 mg, 0,167 mg Gallik asit (GAE)/g bulunmuştur. Metanol ekstraktı en yüksek DPPH serbest radikal süpürme aktivitesi (%87,73) gösterirken, etil asetat ekstraktı en yüksek süperoksit radikal süpürme aktivitesine (%49,02) sahip iken n-hekzan ekstraktı yüksek seviye metal şelatlama kapasitesi (289,32 µM Fe) içermektedir. Makro ve mikro elementler açısından (I ve Cr seviyeleri hariç) en yüksek konsantrasyonlar doğal mineral kaynağı olarak kabul edilen yapraklarında kaydedilmiştir. Buna göre enginar yapraklarının potansiyel bir doğal K ve Zn kaynağı sağladığı, metanol ve n-hekzan ekstraktlarının ise iyi P ve Zn kaynağı olduğu değerlendirilmektedir.

Anahtar Kelimeler: Antioksidan aktivite, *Cynara scolymus L.*, enginar, mineraller, yaprak ekstraktları

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INTRODUCTION

Artichoke (*Cynara scolymus* L.) is a plant belonging to the Asteraceae family that grows naturally in Mediterranean region countries and is a cultured variety of wild artichoke (*Cynara cardunculus* L.). Artichoke, whose head, flower and leaves can be eaten, is traditionally used in hepatobiliary diseases, indigestion (dyspepsia), obesity and hyperlipidemia. *In vitro* and *in vivo* studies have demonstrated that artichoke and its leaves have antimicrobial (Vamanu et al. 2011), antioxidant (Ben Salem et al. 2017a, Biel et al. 2020), hypocholesterolemic (Mocelin et al. 2016, Öcal et al. 2019), hypoglycemic (Ben Salem et al. 2017b), anticancer (Nadova et al. 2008, Sokkar et al. 2020), antifungal (Ben Salem at al.2017b), hepatoprotective (Colak et al. 2016) and choleretic Kraft (1997) effects. Artichoke head and leaves mainly contain phenolic compounds such as chologenic acid and cynarin, flavonoids such as luteolin and apigenin, polyphenolic compounds, carotenoids, inulin, fiber, vitamin C, E and minerals(Ben Salem et al. 2015, Biel et al. 2020, Salekzamani et al. 2019). Artichoke, which is a natural source of antioxidants in this respect, has been used in human and animal nutrition since ancient times, and at the same time, artichoke and leaf extracts are used in foods both as flavoring and to prolong the shelf life of foods by preventing lipid and protein oxidation(Biel et al. 2020). It is known that reactive oxygen species (ROS) such as O_2^-, $^*OH$, H_2O_2 which are released as aresult of ongoing metabolic reactions in the organism, induce/lead to oxidative stress that causes endotoxic shock, neurogenerative disorders and cancer (Ben Salem et al. 2015, Yazar et al. 2004).It has been shown in many studies that besides the cellular antioxidant mechanism, flavanoids, phenolic compounds as catechins, anthocynanins and the other minerals and vitamins that are naturally found in the structure of many plants such as artichoke, support the antioxidant defense system against oxidative stress (Avci et al. 2021, Ben Salem et al. 2017a, Biel et al. 2020, Erdogan et al. 2020). The presence of micro elements such as Mn, Cu, Zn and Se in plants as cofactors in the structure of enzymes such as superoxide dismutase and glutathione peroxidase in the antioxidant defense system (Sobirova and Murodova 2021, Tokalioglu 2012) is also important for the prevention of oxidative stress.

With the current increase in the use and consumption of raw plant materials, it is very important to monitor contaminants present in plants and set maximum standards for their concentrations in order to ensure safe use (Biel et al. 2020). Because of the bitter taste of artichoke leaves, extracts and tinctures, prepared with water ,are used rather than infusions, so quantitative determination of antioxidant activity and mineral concentrations in medicinal plants extracts which are prepared in different solvent are important in terms of determining their dosages and pharmacological effectiveness in the treatment of various diseases. Therefore, in this study, the total phenolic substances, *in vitro* antioxidant activities (DPPH radical scavenging activities, Superoxide radical scavenging activity, and metal chelating ability) and macro and micro elements levels determined.

MATERIALS AND METHODS

Plant Material

Leaves of artichoke (*Cynara scolymus* L.) were bought from an organic market in Ankara province, Turkey in May 2021. The voucher specimen of the plant was authenticated by Prof. Dr. EsraAkkol from Gazi University, Department of Pharmacognosy, Faculty of Pharmacy, Ankara, Turkey and the specimen was deposited in the Herbarium of Faculty of Pharmacy, Gazi University, Ankara, Turkey.

Total Phenolic Concentration (TPC)

The total phenolic substances of artichoke leaf extracts were measured using Folin-Ciocalteu's phenol reagent (FCR) according to procedure of Singleton and Rossi (1965). The principle of this method is based on the formation of a blue colored compound as a result of the phenolic substances reducing FCR.

The Gallic acid was used as standard solution. 0.5 mL of test extracts solution was mixed with 0.5 mL FCR and incubated for 3 minutes. Then, 2% Na_2CO_3 was added and was stored at room temperature for 2 h. After incubated, absorbance of reaction mixture was measured at 760 nm against blank as distilled water. The result was expressed as mg of Gallic acid equivalents (GAE)/g of extract by using an equation that was obtained from standard Gallic acid graph. All the experiment was conducted in three replicates.

Determination of DPPH Radical Scavenging Activity

The DPPH removal activity of artichoke leaf extracts was determined by using the method of Blois (1958). The method is based on the principle of removing DPPH, which is a stable free radical and has a dark purple color. When the DPPH radical is scavenged, the color of the reaction mixture changes from purple to yellow. Briefly, DPPH (2 mL, 0,1 mM) was added to 0.5 mL artichoke leaf extracts. Each mixture was kept in the dark for 30 min and the absorbance was measured at 517 nm against a blank ethanol. The solution without any extract and with DPPH and ethanol was used as control. The experiment was replicated in three independent assays. Ascorbic acid was used as positive controls. Inhibition of DPPH free radical in percentage was calculated by the formula:

DPPH radical scavenging activity (%)=(A blank - A sample/ A blank) × 100

Determination of Superoxide Radical Scavenging Activity

The superoxide radical removal activity is based on the principle that the superoxide radical produced by the NADH (nicotinamide adenine dinucleotide)/PMS (phenazinemethosulfate)/O_2complex reduces nitro blue tetrazolium (NBT) from yellow to purple-colored formazone (Nishimiki et al. 1972). The decrease of the absorbance values indicates consumption of superoxide radical anions.

According to method, 0.5 mL NBT (156μM) and NADH (468 μM) in the sodium phosphate buffer (20 mM, pH 7.4) were added to different concentrations of extract solutions (0.5 mL, 100-250-500-1000 µg/mL) in phosphate buffer. The reaction initiated by adding PMS (50µL, 60 µM) to the mixture and incubated at room temperature for 5 minutes. Then, the absorbance was measured at 560 nm against the corresponding distilled water as control. Inhibition of superoxide radical in percentage was calculated by the formula:

Inhibition % = (A control - A sample/ A control) × 100

Determination of Metal Binding Activity

Iron (II) ions of chelating activity of artichoke leaf extracts was performed according to the Carter (1971) and Dinis et al. (1994).

The principle of this method is based on the inhibition of the formationof ferrous iron-ferrozine complex of chelating agents in the test tube. The decreasing of red colour was determined by the decrease absorbance of the ferrous iron-ferrozine complex at 562 nm.

1 mL sample, 3.7 mL deionized water and 100 µL $FeCl_2$ solutions (2 mM, dissolved in distilled water) were then mixed thoroughly and incubated for 30 min. Then ferrozine (5 mM, 0.1 mL) was added to the mixture. After 10 min, absorbances of the test tubes and EDTA as standarts (50-250 µg/mL)were measured spectrophotometrically at 562 nm. The blank contains only distilled water except that $FeCl_2$ and ferrozine. The metal binding activity was calculated with this formula:

Metal binding (µM Fe) =[(A sample-A blank)/(A standart-A blank)]x 100

Mineral Composition

The elemental composition of mineral substances of the extracts have been analyzed using the ICP-MS device (inductively coupled plasma mass spectrometer) Perkin Elmer NexION 300. The ICP-MS operating conditions are shown in Table 1.

Table 1. ICP-MS Instrumental conditions

Auxiliary gasflow rate (L/min)	1.4
Plasma (Ar) gas flow rate (L/min)	18
Analog HV (V)	-1975
Pulse HV (V)	950
RF power output (W)	1600
Sample uptake rate (µL/min)	300
Dwell time (sec)	50
Sample run time (min/sample)	1,5
Nember of replicates	3

Dry plant powdered sample and its extracts were digested by microwave digestion system. 0.25 g sample extract was taken in a PTFE teflon vessel. After adding 5 ml of conc HNO_3 and 5 ml distilled water, the vessel was kept standing for a while at room temperature. Decomposition of the samples was carried out in a microwave system (CEM MARS, Italy, A five-step programme (see Table 2) was applied to the samples. There were no undissolved parts in vessels.

Table 2. Parameters for microwave digestion

Step	Effect,W	Time,Min
Step 1	250	1
Step 2	0	1
Step 3	250	5
Step 4	400	5
Step 5	650	5

After the digestion process the solution was filtered with a membrane filter (pore size 0,45 μm) which was subjected as the analysis solution to determine the total concentrations of diverse elements in globe artichoke leaf and its extracts by ICP-MS. The resulting solutions were quantitatively transferred into a volumetric flask and made up to 25 mL with doubled distilled water then used for direct injection into the spray chamber of device or diluted with doubled distilled water again. The internal standard elements (Ge, In and Re) were added for correcting matrix effect. Calibration was performed by external standards where the method of standard additions was used.

Statistical analysis

The data obtained in the research were analyzed with the SPSS 22.0 for Windows program. The statistical differences among groups were analysed through One way ANOVA. Least significant difference test (LSD) was used as a posthoc test. Statistical significance of all data was determined as $P < 0.05$.

RESULTS

Antioxidant activity results of artichoke leaves extracts in methanol, ethyl acetate and N-hexane solvents are showed in Table 3.

In this study, the total phenolic content values for the methanol, ethyl acetate and N-hexane extracts found 5,375 mg, 0,917 mg, 0,167 mg Gallic acid (GAE)/g respectively (Fig1).

Methanol extract showed highest DPPH free radical scavenging activity (87,73%) (Fig 2, ethyl acetate extract possessed the highest Superoxide radical scavenging activity (SRSA) (49,02 %) (Fig 3) whereas ethyl acetate extract contained high level metal chelating ability (289,32μM Fe) (Fig 4).

Table 3. Total phenolic substances concentration and invitro antioxidant activities of the extracts obtained from artichoke leaves.

Extracts	**Total phenolic substances (mgGAE/g extract)**	**DPPH radical removal activity (%)**	**Superoxide radical removal activity (%)**	**Metal binding activity(μM Fe)**
Methanol	5,735	87,73	44,15	133,01
Ethyl acetate	0,917	44,15	49,02	151,46
N-hexane	0,167	14,53	30,22	289,32

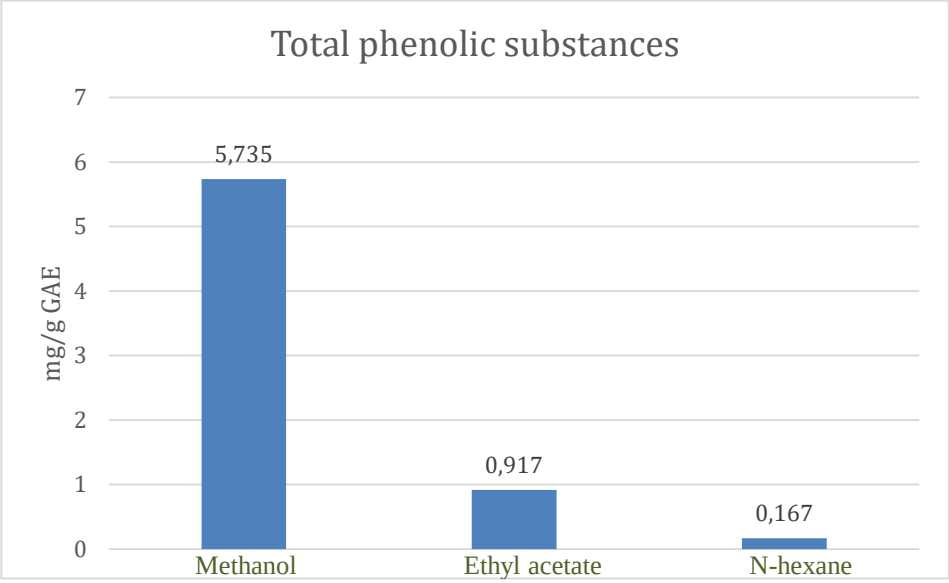

Figure 1: Total phenolic substances concentration (mg GAE/g) of the extracts obtained from artichoke leaves. Result represents means of triplicates of different concentrations analyzed.

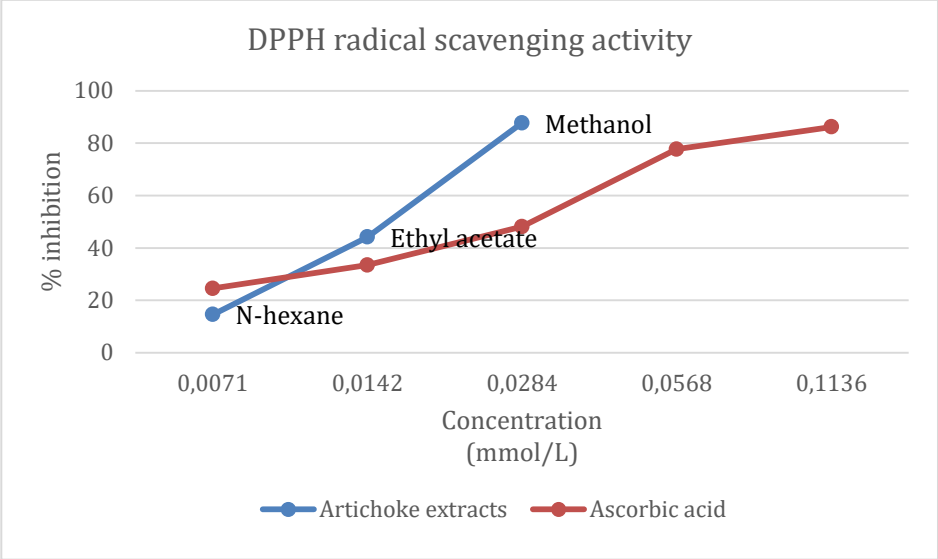

Figure 2: DPPH radical scavenging activities of the extracts of artichoke leaves and ascorbic acid. Result represents means of triplicates of different concentrations analyzed.

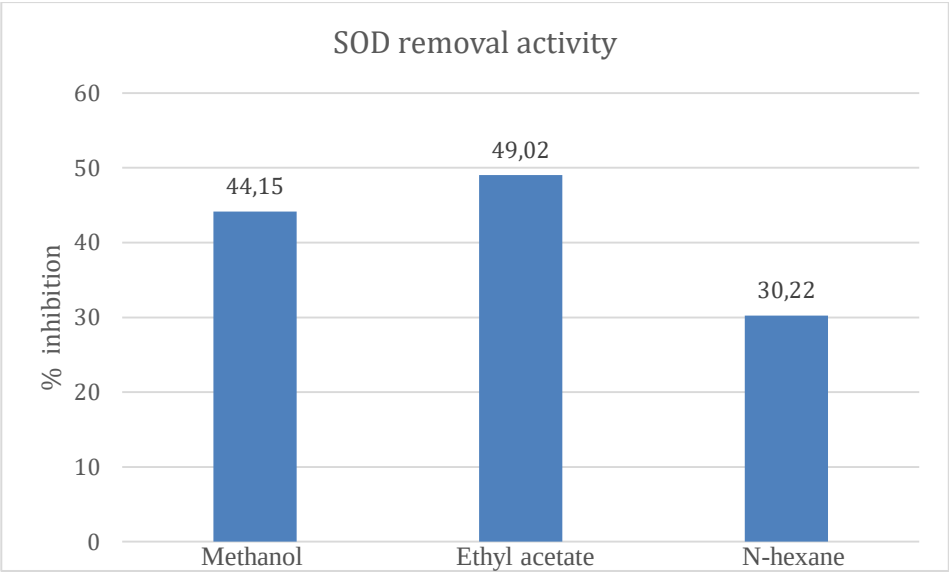

Figure 3: Superoxide radical removal activity (inhibition %) of the extracts obtained from artichoke leaves. Result represents means of triplicates of different concentrations analyzed.

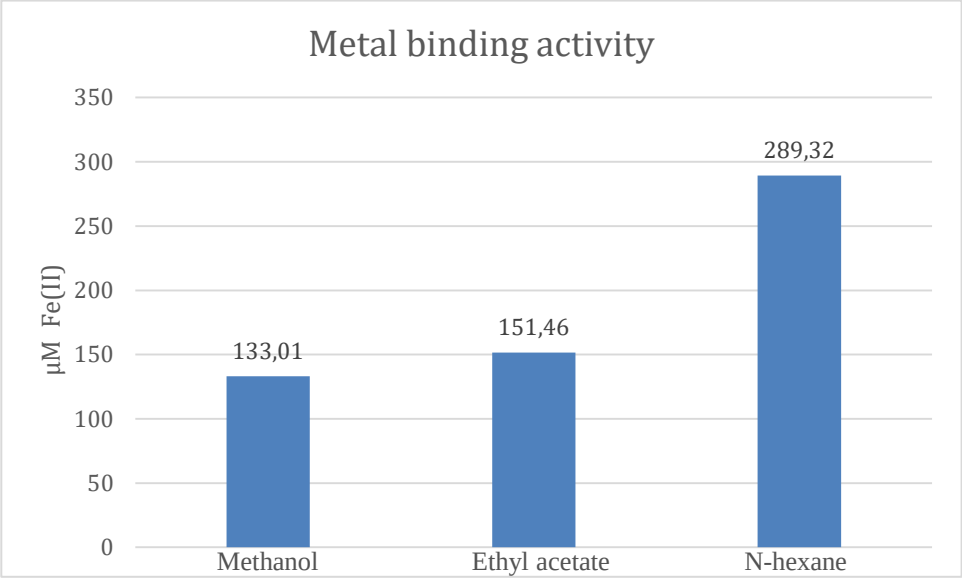

Figure 4: Metal binding activity of iron (II) ions(µM) of the extracts obtained from artichoke leaves. Result represents means of triplicates of different concentrations analyzed.

The macro element levels are statistically higher in the artichoke leaf compared to all other extracts, with the highest K and the lowest Na levels.
Among the extracts, it is seen that the methanol extract's macro minerals have the lowest level compared to the other extracts and P concentration (127.03±2.98 mg/g) is highest among macro minerals. As Mg is highest in ethyl acetate extract, Na, K and Ca levels are highest compared to other extracts. While P is found at the highest level in the N-hexane extract, it is determined as the extract containing the most macro minerals (Table 4).

Table 4. Levels of minerals in powdered leaf and organic solvents.

Macro elements(mg/100g)	**Artichoke Leaf X̄±S**	**Methanol X̄±S**	**Ethyl acetate X̄±S**	**N-hexane X̄±S**	**P**
Na (Sodium)	84.58±3.09^{a}	3.73±0.11^{d}	14.00±0.20^{b}	9.83±0.14^{c}	***
K (Potassium)	238.95±5.84^{a}	4.91±0.20^{d}	36.09±0.20^{b}	15.24±025^{c}	***
Ca (Calcium)	125.00±2.58^{a}	16.24±0.83^{d}	57.91±0.61^{b}	23.44±0.32^{c}	***
Mg (Magnesium)	175.68±18.60^{a}	33.58±0.44^{c}	99.17±1.36^{b}	91.88±0.77^{b}	***
P (Phosphor)	189.14±3.67^{a}	127.03±2.98^{b}	47.3±0.88^{c}	186.86±0.96^{a}	***
Micro elements (mg/100g)					
Fe (Iron)	5.25±0.45^{a}	1.57±0.91^{c}	Not detected	2.22±0.41^{b}	***
Zn (Zinc)	5.71±0.23^{a}	1.78±0.68^{c}	0.44±0.18^{d}	3.66±0.46^{b}	***
Cu (Copper)	1.52±0.30^{a}	0.92±0.32^{b}	0.72±0.32^{c}	0.28±0.08^{d}	***
Mn (Mangan)	2.18± 0.36^{a}	0.19±0.03^{d}	0.70±0.20^{c}	2.11±0.13^{b}	***
Cr (Chromium)	0.12±0.03^{a}	0.05±0.01^{c}	0.08± 0.00^{b}	0.11±0.08^{a}	***
Se (Selenium)	0.12±0.03^{c}	0.02±0.00^{b}	0.01±0.02^{ab}	0.006±0.00^{a}	*
I (Iodine)	0.04±0.00^{d}	0.03±0.01^{c}	0.06±0.00^{b}	0.21±0.01^{a}	*

X̄±S: mean ±std deviation, a,b,c,d: Different letters in the same row represent the statistically significant difference between the mean values ($p< 0,05$). *: $p < 0,05$ ***: $p < 0,001$

When all groups were examined in terms of microelements the microelements in the artichoke leaf (except I and Cr levels) were statistically at the highest level compared to all other extracts.
Accordingly, Zn and Fe levels were the highest in artichoke leaves and the lowest I was found. Fe and Zn were found to be highest in methanol extract, while ethyl acetate extract contained high levels of Cu and Mn, but it was determined as the extract containing the lowest level of micro minerals. While Fe, Zn and Mn levels were determined the highest in the N-hexane extract, it was also seen that it contained the micro minerals at the highest level compared to the other extracts (Table 2).

DISCUSSION

The interest in artichoke, a natural antioxidant, as well as its extracts is due to their versatile therapeutic effect (Biel et al. 2020). Oxidative stress is characterized by insufficient enzymatic (SOD, CAT, GPx and GSH) and non-enzymatic (Vitamin E, Vitamin C, carotenoids, flavonoids, polyphenols and others) antioxidant capacity due to excessive increase in reactive oxygen species (ROS), so shifting the balance towards oxidants (Salekzamani et al. 2019). While increasing oxidative stress has been reported to play a role in many disease such as aging, diabetes mellitus, hypertension, cardiovascular diseases, cancer, Parkinson's and Alzheimer's diseases (Valko et al. 2007), plants with strong antioxidant activity such as artichoke contain polyphenols, vitamins and minerals have a role in reducing the risk of disease that develop due to oxidative stress by inhibiting ROS production and reducing radicals (Salekzamani et al. 2019). Indeed Demir and Agaoglu (2021) have reported in many studies caffeoylquinic acid derivatives, luteolin and apigenin glucosides found in *C. scolymus* L. showed strong antioxidant properties. Phenolic compounds in plants are natural sources that show their antioxidant activities by binding free radicals or chelating with metals in proportion to the number and structure of –OH groups in the phenol ring (Falowo et al. 2014).

In addition, phenolic compounds have the ability to delay, slow or prevent oxidation at low concentrations and remain in a stable form when converted to free radicals (Kalogianni et al. 2020).

In studies carried out, Wand et al. (2003) report the total phenolic content (TPC) in Green Globe leaves as 8760-9561 mg CAE (Chlorogenic acid equivalents)/100 g DM and Salata and Gruszecki (2010) report 3168 mg caffeic acid equivalent/100 g DM. In our study the total phenolic content values, based on standard of Gallic acid, In the methanol, ethyl acetate and N-hexane extracts found 5,375 mg, 0,917 mg, 0,167 mg Gallic acid (GAE)/g, respectively. So, the reason why a value higher than the one reported by Biel et al. (2020) as 2795 mg CAE/100 g DM was found in the methanol extract of the leaf and may be due to the different standards used in the tests. Although not found enough studies on the total phenolic content of ethyl acetate and N-hexane extracts, it is seen that the highest phenolic content is in the methanol extract.

Considering the versatile mechanisms of antioxidant compounds, the need for various types of measurements to determine their activities has arisen (Biel et al. 2020). DPPH, widely used in the determination of the free radical scavenging activities of antioxidants, is a stable free radical (DPPH•) (Chen et al. 2007, Wojdylo et al. 2007), and when this purple-colored substance interacts with antioxidants, it transforms into a yellow-colored reduced form of DPPH (DPPH-H). Therefore, the lower the measured absorbance, the higher the free radical scavenging activity of the antioxidant (Chen et al. 2007).

Accordingly, DPPH radical removal activity being the highest in methanol extract (87.73% inhibition) in this study can be explained by the fact that the total amount of phenolic substances in this extract is higher than the others. These results are also compatible with the observation that the antioxidant properties of plant extracts originate in polyphenols, which donate hydrogen atoms or electrons, have the ability to neutralize free radicals (such as DPPH) (Sahoo et al. 2013). Thus, it is obvious that this activity is weakened due to the reduction in total phenolic content in ethyl acetate and N-hexane extracts. Biel et al. (2020) reported this activity in artichoke methanol extract at approximately 44% inhibition according to the trolox standard and the difference in the results of this study may be due to the evaluation based on the ascorbic acid standard.

When the solvents used in the extracts in the study are evaluated in terms of their polarity, the most non-polar solvent to the least is listed as n-hexane>ethyl acetate>methanol. Accordingly, it has been reported that the solvents used in the extraction affect the radical scavenging activity and this activity is higher in polar extracts (Hayouni et al. 2007,Ozcan et al. 2007). In their study, Erdogan et al. (2020) reported that the methanolic extract of olive leaf (*Olea europaea L.*) is more effective in terms of total phenolic substance content, the ferric thiocyanate method in the linoleic acid system, and reducing capacity with antioxidant activity compared to other extracts (ethyl acetate and n-hexane). As a matter of fact, Miliauskas et al. (2004) reported that the DPPH radical scavenging activity among plant extracts is the most effective in methanol extract compared to other extracts (acetone and ethyl acetate), while Shon et al. (2003) reported that hot water and methanol extracts are better than butanol, ethyl acetate, and chloroform extracts. Avci et al. (2021), on the other hand, stated that the different results found *in vitro* antioxidant activities in black cumin (*Nigella sativa* L.) essential oil obtained by water distillation may be due to the differences in the solvent and extraction methods used. Accordingly, apart from these observations, different species of the same plant, different agricultural practices, geographical conditions (daylight, climate), harvest time, and storage conditions are the factors affecting the phenolic content of plants (Heimler et al. 2007) —the reason for the differences in our results can be explained by considering these factors.

SOD, which forms the first line of defense against the harmful effects of superoxide radicals released in different parts of the cell, is one of the metalloenzymes that converts the superoxide radical into H_2O_2 and O_2 (Halliwell et al. 2000). Generally,

SOD has three different isoenzymes in higher plants, namely Mn-SOD, Fe-SOD, and CuZn-SOD Fridovlich (1986), which are found in different organelles of the cell such as mitochondria, peroxisome, and chloroplasts (Palma et al 2006). In this study, the superoxide radical removal activity was listed as ethyl acetate> methanol> N-hexane, and no other study was found on the subject. In a study using similar solvents, Erdogan et al. (2020) reported that the superoxide radical removal activity in olive leaf extracts was determined as ethyl acetate> methanol> N-hexane, which is similar in terms of extracts in the study.

In this study, the metal binding activity was the highest in the N-hexane extract (289.32 μM Fe), and the others were listed as ethyl acetate>methanol — there was not enough study on this subject as well. In studies on lipid peroxidation, Fe is used as an ion catalyst. Fe, a transition metal, acts as a catalyzer of lipid peroxidation due to its biological importance and ability to react directly with oxygen or with lipid peroxides promoting the reaction to form species that can initiate peroxidative events (Dinis et al. 1994).

The antioxidant defense system is generally based on both dietary vitamins and minerals and the presence of essential amino acids required for the synthesis of antioxidant proteins, such as glutathione and albumin (Evans and Halliwell, 2001). It is known that minerals in the structure of tissues are involved in the regulation of acid-base balance and osmotic pressure, the functioning of hormones and enzymes, transcription, and energy metabolism (Connie 2011). As a matter of fact, Ca and K are essential elements for plants, and Mg is a chlorophyll component in plants as well as a cofactor of enzymes in reactions where ATP is used (Evans and Halliwell 2001, Silvaet al. 2016). In this study, the macro element levels were listed as K > P > Mg > Ca > Na in the artichoke leaf, and the highest K (238.95 mg/100g DM) and the lowest Na (845.8 mg/100g DM) levels were detected. At the same time, it is noteworthy that the macro element levels are higher in the artichoke leaf than in all other extracts, and the Na mineral is at the lowest (37.3-845.8 mg/100g DM) level in all the extracts. In this study, the macro minerals in the methanol extract were lined at the level of P > Mg > Ca > K > Na, and the high P level was found as considerably lower than the values of 890-980 mg P/100g DM in the methanol extract of the leaf and 414 mg P/100g DM in the artichoke leaf (127.0 mg/100g DM) as reported by (Biel et al. 2020, Colla et al. 2013) respectively. Although there is no study comparing different extracts in terms of minerals, Biel et al. (2020) reported the macro element levels in the methanol extract of artichoke leaves in order of K > P > Ca > Mg > Na, and this high level in K (506.3 mg/100g DM) is due to the fact that it is the most absorbed mineral during the growth cycle of the plant.This observation was found to be consistent with the finding of this study that the most abundant mineral in the artichoke leaf was K (238.9 mg/100g DM), which is much higher than the K found in the methanol extract (4.9 mg/100g DM).

The macro minerals were found as Mg > Ca > P > K > Na in the ethyl acetate extract, and as P > Mg > Ca > K > Na in the N-hexane extract. Although there is no comparable study for these two extracts, in this study, Ca (125.0-16.24 mg/100g DM) and Mg (175.6-33.5 mg/100g DM) valuesfound in artichoke leaves and all its extracts were lower than the values reported by Biel et al. (2020) and Orlovskaya et al. (2007) in leaf methanol extract and leaf. According to the results of this study, while artichoke leaf is a source of K, methanol and n-hexane extracts can be good sources of P. In a study on various medicinal plants (*Cynara scolymus* L., Harpagophytumprocumbens D.C., Maytenusilifolia (March) ex Reiss.), including artichokes, Ca, K, Mg, and Na levels were found to be high, according to which such plants can be a natural source in the treatment of diseases related to the deficiency of these elements (Tannus et al. 2021).

Trace elements not only play an important role in the formation of active chemical ingredients in medicinal plants but also are responsible for their medicinal and toxic effects (Tokalioglu 2012). While trace elements act as cofactors of enzymes for their conversion to products in enzymatic reactions, some of them are responsible for taking or giving electrons in redox reactions which are important in the production and use of metabolic energy (Al-Fartusie and Mohssan 2017). Zn, which is a cofactor of more than 200 enzymes in the central nervous system, immune, skeletal and reproductive systems, is included in the structure of SOD (CuZnSOD) together with Cu. Cu is also required for ceruloplasmin, which transfers iron to transferrin, and deficiencies in Cu affect the activity of SOD more than Zn (Roohani et al. 2013).While Cu acts as a cofactor in the structure of many enzymes such as cytochrome c oxidase, tyrosinase, and dopamine beta-hydroxylase, contrarily, excess Cu causes peroxidative damage in membrane lipids. Indeed, in the presence of reducing agents such as superoxide radical, ascorbic acid, and glutation, Cu^{+2} is reduced to Cu^{+1}, which can catalyze the formation of hydroxyl radicals from hydrogen peroxide, and these radicals cause the oxidation of biological molecules (Gaetke and Chow 2003). Zn prevents the peroxidation of membrane lipids and has a stabilizing effect on membranes, possibly by replacing bound transition metal ions (Evans and Halliwell 2001). In this study, when microelements were examined in terms of all groups, the highest level of microelements was determined in artichoke leaf compared to all other extracts (except I and Cr levels). When the microelements in the artichoke leaf were evaluated, the order of Zn > Fe >Mn> Cu > Cr =Se > I was seen, with the highest level being Zn (5.71 mg/100g DM) and the lowest level I (0.004 mg/100g DM). Colla et al. (2013) reported the Zn

level (2.07-2.35 mg/100 g DM) in artichoke leaves, and in this study, Zn levels were found higher than in the relevant report.

Biel et al. (2020) reported the micro-minerals in the methanol extract of the leaf in the order of Zn > Fe > Cr >Mn, and the Zn and Fe levels are 2.10 mg/100g DM and 1.6 mg/100g DM. In the same study, it was stated that Cd, Pb, and Ni levels could not be determined. In this study, the ordering of micro minerals in Methanol as Zn > Fe > Cu >Mn> Cr > Se > I was found to be consistent with the reports, but the Zn level (1.78 mg/100g DM) was found to be lower than the previous reports yet consistent with the Fe level. Zn > Fe >Mn> Cu > Cr > I > Se sequence was seen in the N-hexane extract, and the Zn level was found higher at 3.66 mg /100g DM. In the study, while Fe remained below the detection level in the ethyl acetate extract, the minerals were listed as Cu >Mn> Zn >Cr > I > Se. When evaluated in terms of extracts, the ethyl acetate was the extract containing the lowest level of micro minerals and Se was found at the lowest level in all the extracts (Table 2).

The Mn level was found to be between 2.18-0.19 mg/100g DM in all groups, and Mn, which is the cofactor of the enzymes involved in the biosynthesis of fatty acids and cholesterol, is an essential element in the structure of mitochondrial Mn-SOD (Evans and Halliwell, 2001). While this element helps regulate oxidative phosphorylation, mucopolysaccharide and cholesterol metabolism, and the urea cycle, the excessive amounts are toxic to the organism and cause neurological effects (Rehnberg et al. 1982). Cr is the most important factor that increases insulin activity in the organism and enables the use of glucose by activating the phosphoglucomutase enzyme. The literature suggests that its use is especially beneficial against type-2 diabetes and insulin resistance. And in its deficiency, glucose tolerance decreases, which can lead to cardiovascular diseases and diabetes (Hua et al 2012, Tokalioglu 2012). In this study, Cr levels were found to be between 0.12-0.05 mg/100gDM in all groups and were higher than the reports of Biel et al. (2020) in leaf methanol extract and Tannus et al. (2021) in artichoke.

Se, which presents itself in the structure of glutathione peroxidase in the organism, also acts as an antioxidant against different abiotic stresses in plants, including salinity, drought, overtemperature, and toxic metals/metalloids stresses (Hasanuzzaman et al.· 2020). In this study, Se levels were found to be between 0.12-0.006 mg/100g DM in all groups, and it was found to be lower than the 0.37 mg/100g DM level reported by Tannus et al. (2021) in artichokes. Also, I levels were found to be between 0.21-0.03 mg/100g DM in all groups, and no comparable study was found for artichoke.

CONCLUSION

The results of this study show that the herbal extraction methods and the solvents affect the total phenolic substance content, antioxidant activity, and mineral levels. While methanol and ethyl acetate extracts stand out in terms of antioxidant activity and total phenolic substances, it is obvious that artichoke leaf and N-hexane extract are more valuable in terms of mineral source. Accordingly, the conclusion is that the leaf can be a good source of K and Zn, while methanol and N-hexane extracts can be a good source of P and Zn.

Ethics Committee Information: [1] This study is not subject to HADYEK's permission in accordance with Article 8 (k) of the "Regulation on Working Procedures and Principles of Animal Experiments Ethics Committees".

[2] The data, information and documents presented in this article were obtained within the framework of academic and ethical rules.

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REFERENCES

Al-Fartusie FS, Mohssan SN. Essential trace elements and their vital roles in human body. Indian J Adv Chem Sci. 2017; 5(3):127-136.

Avci G, Denk B, Bulbul A. Research on the antioxidant efficiacy of black seed essential oil using by *in vitro* method. Eurasian Journal Of Health Sciences. 2021; 4(3):154-161

Ben Salem M, Affes H, Ksouda K, Dhouibi R, Sahnoun Z, Hammami S, Zeghal KM. Pharmacological studies of artichoke leaf extract and their health benefits. Plant Foods for Hum Nutr. 2015; 70:441-453.

Ben Salem M, Ben Abdallah Kolsi R, Dhouibi R, Ksouda K, Charfi S, Yaich M, Hammami S, Sahnoun Z, Zeghal KM, Jamoussi K, Affes H. Protective effects of *Cynara scolymus* leaves extract on metabolic disorders and oxidative stress in alloxan-diabetic rats. BMC Complement Altern Med. 2017;17(1):328.

Ben Salem M, Affes, H. Chemicals compositions, antioxidant and anti-inflammatory activity of Cynara scolymus leaves extracts, and analysis of major bioactive polyphenols by

HPLC. Evid Based Complement Alternat Med. 2017; 2017:4951937.

Biel W, Witkowicz R, Piątkowska E, Podsiadło C. Proximate composition, minerals and antioxidant activity of artichoke leaf extracts. Biological Trace Element Research. 2020; 194:589–595.

Blois MS. Antioxidant determinations by the use of a stable free radical. Nature. 1958; 181:1199-1200.

Carter P. Spectrophotometric determination of serum iron at the submicrogram level with a new reagent (ferrozine). Anal Biochem. 1971; 40(2): 450-458.

Chen HY, Yen GC. Antioxidant activity and free scavenging capacity of extracts from guava *(Psidium guajava L.)* leaves. Food Chemistry. 2007; 101(2):686-694.

Colak E, Ustuner MC, Tekin N, Colak E, Burukoglu, D, Degirmenci I, Gunes HV. The hepatocurative effects of *Cynara scolymus L.* leaf extract on carbon tetrachloride-induced oxidative stress and hepatic injury in rats. Springerplus. 2016; 5: 216.

Colla G, Rouphael Y, Cardarelli M, Svecova E, Reac E, Lucini L. Effects of saline stress on mineral composition, phenolic acids and flavonoids in leaves of artichoke and cardoon genotypes grown in floating system. J Sci Food and Agric. 2013; 93(5):1119–1127.

Connie KL. Role of trace minerals in animal production. What do I need to know about trace minerals for beef and dairy cattle, horses, sheep and goats? http://www.progressivedairy.com/index.php?option= comcontent&view=article &id; Accessien date: 26.11.2011.

Demir T, Agaoglu S. Antioxidant, antimicrobial and metmyoglobin reducing activity of artichoke (*Cynara scolymus* powder extract added minced meat during frozen storage. Molecules. 2021; 26(18):5494.

Dinis TCP, Madeira VMC, Almeida LM. Action of phenolic derivatives (acetaminophen, salicylate, and 5-aminosalicylate) as inhibitors of membrane lipid peroxidation and as peroxyl radical scavengers. Archives of Biochemistry and Biophysics. 1994; 315(1):161-169.

Erdogan SM, Akkol E, Avci G. Research on the antioxidant efficiacy of olive (*Olea europaea* L.) leaf using by *in vitro* methods. Kocatepe Vet J. 2020; 13(3):319-326.

Evans P, Halliwell B. Micronutrients: oxidant/antioxidant status. British Journal of Nutrition. 85, Suppl. 2001; 2: 67-74.

Falowo AB, Fayemi PO, Muchenje V. Natural antioxidants against lipid–protein oxidative deterioration in meat and meat products: A review. Food Res. Int. 2014; 64:171–181.

Fridovich I. Superoxide dismutase. Adv. Enzymol. Relat. Areas Mol. Biol. 1986; 58:61-97.

Gaetke LM, Chow CK. Copper toxicity, oxidative stress, and antioxidant nutrients. Toxicology. 2003; 189 (1-2): 147-163.

Halliwell B, Clement MV, Lonh LH. Hydrogen peroxide in human body. FEBS letters. 2000; 486(1):10-13.

Hasanuzzaman M, Bhuyan MHMB, Raza A, Hawrylak-Nowak B, Matraszek-Gawron B. AlMahmud J, Nahar K, Fujita M. Selenium in plants: Boon or bane? Environmental and Experimental Botany. 2020; 178: 104170.

Hayouni EA, Abedrabba M, Bouix M, Hamdi M. The effect of solvent and extraction method on the phenolic contents and biological activities *in vitro* of Tunisian *Quercus coccifera* and *Juniperus phoenicea*L. fruit extracts. Food Chemistry. 2007; 105(3): 1126-1134.

Heimler D, Isolami L, Vignolini P, Tombell S, Romani A. Polyphenol content and antioxidative activity in some species of freshly consumed salads. J. Agric. Food Chem. 2007; 55:1724-1729.

Hua Y, Clark S, Ren J, Sreejayan N. Molecular mechanisms of chromium in alleviating insulin resistance. The Journal of Nutritional Biochemistry. 2012; 23(4): 313-319.

Kalogianni AI, Lazou T, Bossis I, Gelasakis, AI. Natural phenolic compounds for the control of oxidation, bacterial spoilage, and foodborne pathogens in meat. Foods. 2020; 9(6):794–822.

Kraft K. Artichoke leaf extract - Recent findings reflecting effects on lipid metabolism, liver and gastrointestinal tracts.1997; 4(4):369-78.

Miliauskas G, Venskutonis PR, van Beek TA. Screening of radical scavenging activity of some medicinal and aromatic plant extracts. Food Chemistry. 2004; 85(2):231-237.

Mocelin R, Marcon M, Santo GD, Zanatta L, Sachett A, Schönell AP, … Roman Junior WA. Hypolipidemic and antiatherogenic effects of *Cynara scolymus* in cholesterol-fed rats. Brazilian Journal of Pharmacognosy. 2016; 26: 233–239.

Nadova S, Miadokova E, Mucaji P, Grancai D, Cipak L. Growth inhibitory effect of ethyl acetate-soluble fraction of *Cynara cardunculus* L. in leukemia cells involves cell arrest, cytochrome c release and activiation of caspases. Phytother Res. 2008; 22:165–168.

Nishimiki M, Appaji N, Yagi K. The occurance of superoxide anion in the reaction of reduced phenazine methosulfate and molecular oxygen. Biochem Biophys Res Commun. 1972; 46(2):849-54.

Orlovskaya TV, Luneva IL, Chelombit'ko VA. Chemical composition of *Cynara scolymus* leaves. Chemistry of Natural Compounds.2007; 43: 239-240.

Ocal N, Askar TK, Buyukleblebici O, Tok D, Dolarslan M, Guleryuzlu Z. The effect of artichoke supplement on lipid metabolism in rats subjected to experimental acute exercise model. Avrasya Sağlık Bilimleri Dergisi. 2019;2(3):114-119.

Ozcan MM, Baydar H, Sagdıc O, Ozkan G. Türkiye'de ticari açıdan önemli *Lamiaceae* familyasına ait baharat veya çeşni olarak kullanılan bitkilerin fenolik bileşenleri ile

antioksidan ve antimikrobiyal etkilerinin belirlenmesi. *TÜBİTAK Projesi*, No: TOGTAG-3319, Konya, 2007.

Palma JM, Jimenez A, Sandalio LM, Corpas FJ, Lundqvist M, Gomez M, Sevilla F, Rio LA. Antioxidative enzymes from choloroplast mitochondria and peroxisomes during leaf senescence of nodulated pea plants. J of Experimental Botany. 2006; 54(8):1747-1758.

Rehnberg GL, Hein JF, Carter SD, Linko RS, Laskey JW. Chronic ingestion of Mn_3O_4 by rats: tissue accumulation and distribution of manganese in two generations. J Toxicol Environ Health. 1982; 9(2):175–188.

Roohani N, Hurrell R, Kelishadi R, Schulin R. Zinc and its importance for human health: an integrative review. J Res Med Sci. 2013; 18(2):144–157.

Sahoo S, Ghosh G, Das D, Nayak S. Phytochemical investigation and *in vitro* antioxidant activity of an indigenous medicinal plant *Alpinia nigra* B.L. Burtt. Asian Pac J Trop Biomed. 2013; 3(11): 871-876.

Sałata A, Gruszecki R. The quantitative analysis of polyphenolic compounds in different parts of the artichoke (*Cynara scolymus* L.) depending on growth stage of plants. Acta Sci Pol Hortorum Cultus. 2010; 9(3):175–181.

Salekzamani S, Ebrahimi-Mameghani M, Rezazadeh K. The antioxidant activity of artichoke (*Cynara scolymus*): A systematic review and meta-analysis of animal studies. Phytotherapy Research. 2019; 33 (1): 55–71.

Shon MY, Kim TH, Sung NJ. Antioxidants and free radical scavenging activity of Phellinus baumii (Phellinus of Hymenochaetaceae) extracts. Food Chemistry, 2003; 82(4): 593- 597.

Silva PSC, Francisconi LS, Goncalves RDMR. Evaluation of major and trace elements in medicinal plants. J Braz Chem Soc. 2016; 27(12):2273–2289.

Singleton VL, Rossi JA. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. American Journal of Enology and Viticulture. 1965; 16: 144-158.

Sobirova M, Murodova S. Effects of biopraparites on cynara scolymus L., micro and macroelements, and quantity of flavonoids. E3S Web of Conferences 258, 04025 (UESF-2021).

Sokkar HH, Dena ASA, Mahana NA, Badr A. Artichoke extracts in cancer therapy: Do the extraction conditions affect the anticancer activity?. Future Journal of Pharmaceutical Sciences. 2020; 6(1):1-21.

Tannus CA, Dias FS, Santana FB, Santos DCMB, Magalhães HIF, Dias FS,Júnior AFS. Multielement determination in medicinal plants and herbal medicines containing *scolymus* L., Harpagophytum procumbens D.C., and Maytenusilifolia (Mart.) ex reiss from Brazil using ICP-OES. Biological Trace Element Research. 2021; 199:2330–2341.

Tokalioglu S. Determination of trace elements in commonly consumed medicinal herbs by ICP-MS and multivariate analysis. Food Chemistry.2012; 134(4):2504-2508.

Vamanu E, Vamanu A, Niţă S, Colceriu S. Antioxidant and antimicrobial activities of ethanol extracts of *Cynara scolymus* (Cynarae folium, Asteraceae Family). Trop J Pharm Res. 2011; 10(6):777-783.

Valko M, Leibfritz D, Moncol J, Cronin MTD, Mazur M, Telser J. Free radicals and antioxidants in normal physiological functions and human disease. The International Journal of Biochemistry & Cell Biology. 2007; 39:44–84.

Wojdylo A, Oszmianski J, Czemerys R. Antioxidant activity and phenolic compounds in 32 selected herbs. Food Chemistry. 2007; 105(3):940-949.

Yazar E, Konyalioglu S, Col R, Birdane YO, Bas AL, Elmas M. Effects of vitamin E and prednisolone on some oxidative stress markers in endotoxemic rabbits. Revue Méd. Vét. 2004; 155(11):538-542.