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Research Article

Antioxidant properties of *Lycianthes rantonnetii* and contents of vitamin and element

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Abstract: The aim of this study was to investigate the antioxidant, antiradical activity of the *Lycianthes rantonnetii* (Solanacaeae) plant, to determine levels of element (Fe, Zn, Mn, Cu, Cr, Mo, Cd, As, Pb, Co, V, Tl, Li, Ti, Sr, Be), mineral (Mg, P) and vitamin (A, E, C). In the study, metals and mineral analyses were carried out using dry ashing method with ICP-OES, vitamin A and vitamin E analyses were carried out using the HPLC method and vitamin C, total phenol, flavonoids, antioxidant capacity, DPPH, ABTS, hydrogen peroxide, hydroxyl radicals scavenging activity and anti-hemolytic activity of the methanol extract *Lycianthes rantonnetii* (*L. rantonnetii*) plant was determined spectrophotometrically. According to the results, Cu, Zn, Co, Mg, retinol, α-tocopherol, ascorbic acid, total antioxidant activity, phenolic and flavonoid contents of *L. rantonnetii* were 57.60 ± 4.83 µmol/kg, 0.14 ± 0.01 mmol/kg, 2.23 ± 0.10 µmol/kg, 39.13 ± 1.76 mmol/kg, 12.22 ± 3.37 µmol/kg, 104.55 ± 7.44 µmol/kg, 80.61 ± 9.31 mg/100 g, 11.45 ± 0.60 mM ascorbic acid/g, 5.33 ± 0.41 mg GA/g and 3.76 ± 0.29 mg QE/g, respectively. The results of this study showed that the content of antioxidant vitamins, minerals of the *L. rantonnetii* plant was at high levels and it contains a reasonable amount of total phenol, and flavonoids also, it was determined that the plant had a high scavenging activity for free radicals. It is believed that *L. rantonnetii* can be used as an additive for food products in the various food sectors with appropriate antioxidant activities.

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1. INTRODUCTION

Nature is permanently a vital and abundant origin of various health-promoting ingredients. Some of these natural products contain fruit, vegetables, herbs, and spices that are widely used. Therefore, there are many medicinal plants that have shown important potential for health enhancement. The potential antioxidant properties of natural sources are one of the most useful impacts (Slavin & Lloyd, 2012).

Solanaceae family also called the nightshade or potato family, is one of the biggest and economically most significant families of angiosperms, including significant food, spice, and

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medicine plants, comprises of around 90 genera and 3000 species. Nearly 50% of the species in the family are included in the widespread, morphologically diverse, and economically significant genus Solanum. The family is almost cosmopolitan in distribution, discovered in entire tropical and temperate districts, but with a concentration of variety in Latin America and Australia. (D'Arcy, 1979; Jacobs & Eshbaugh, 1983).

Trace elements play an important role in the metabolic regulation of the human body and the excess of elements in food in the etiology of some diseases (Eken *et al*., 2017). Medicinal plants containing toxic elements such as Pb, Cd and As can have a harmful effect (Güvenç *et al*., 2007).

Reactive oxygen species (ROS) is produced as a natural direct consequence of regular oxygen metabolism. In common, harmful effects such as DNA damage, polyunsaturated fatty acid oxidation in lipids, and amino acid oxidation in proteins oxidatively prevent the growth of particular enzymes by co-factor oxidation (Khan *et al*., 2018). Free radicals affect the formation of atherosclerosis, ischemic heart diseases, old age, inflammation, diabetes, neurodegenerative diseases, and many other diseases (Jain *et al*., 2008).

The oxidant-antioxidant balance is related to maintaining a balance between the useful and damaging results of ROS (Gutteridge, 1995). Both endogenous and exogenous antioxidants are active against oxidative stress, and endogenous antioxidants cannot fully protect cells from ROS (Tanaka *et al*., 2011). A significant source of natural antioxidants could be the use of medicinal plants (Chandra *et al*., 2013), including anthocyanins, flavonoids, flavones, isoflavones, coumarins, catechins, and vitamins C and E, β-carotene and α-tocopherol, which are considered to have possible antioxidants (Jain *et al*., 2008).

The aim of this study was to analysis the antioxidant and antiradical activities in methanol extracts of *L. rantonnetii* by different analytical methods; total antioxidant activity**,** DPPH, ABTS, hydrogen peroxide, and hydroxyl radicals scavenging activity and anti-hemolytic activity, total phenolic and flavonoid contents, and, in addition, to investigate elements (Fe, Zn, Mn, Cu, Cr, Mo, Cd, As, Pb, Co, V, Tl, Li, Ti, Sr, Be), mineral (Mg, P) and vitamin (A, E, C).

2. MATERIAL and METHODS

2.1. Plant Material and Extraction

Lycianthes rantonnetii (Carrière ex Lesc.) Bitter (Solanacaeae) plant used in the study was collected from Ranya city, Sulaymaniyah, Iraq, on 10 July 2019. Plant species identification was made by Prof. Dr. Fevzi ÖZGÖKÇE, University of Van Yüzüncü Yil, Science Faculty, Biology Department. Witness plant specimen, stored with the code VANF-164103 in Van Yüzüncü Yil University Herbarium. The plant samples were dried in the shade without exposure to sunlight and stored for analysis. After drying the plant in the shade, crushed it to powder, 20 g of the powdered plant was weighed and transferred to a colored bottle, and 400 ml of 75% methanol was added. The sample stored in the colored bottle was extracted for 48 hours at room temperature with stirring. The solution is then separated from the precipitate by filtration. The methanol in the filtrate was removed using a rotary evaporator and aqueous extracts were obtained by lyophilization with a freeze-drier (-86 °C).

2.2. Determination of Vitamin (A, E)

2.2.1. Standard solutions and calibration

α-tocopherol and all-trans-retinol stock solutions were prepared at 500 µg/mL in methanol. Stock solutions were appropriately diluted with the mobile phase for the preparation of the standard solutions. Calibration was calculated by linear regression analysis of the peak area versus the concentrations of the standard solutions.

2.2.2. Extraction procedure

In our study, vitamin A and vitamin E amounts of *L. rantonnetii* plant were determined by modifying the method used by (Sahin *et al*., 2005; Al-Saleh *et al*., 2006). From the plant samples which were dried and ground in the shade, 5 g was weighed and extracted with n-hexane and ethanol. 0.01 % BHT was added to them, vortexed, and kept in the dark for 24 hours. Then centrifuged at +4 °C and 4000 rpm for 10 minutes. The supernatant was filtered using Whatman filter paper and 0.5 mL of n-hexane was added and dried at 37 °C under nitrogen gas. After drying, the residue was dissolved in a mixture of 200 µL methanol+tetrahydrofuran and made ready for analysis.

2.2.3. Chromatographic conditions

Analyzes, Gl Science C_{18} reverse-phase HPLC column (250 x 4.6 mm ID), methanol+tetrahydrofuran (80:20) mobile phase, at a flow rate of 1.5 mL/min, at a temperature of 25 °C. Thermo Scientific Finnigan Surveyor model in high-performance liquid chromatography, using a PDA array detector, applications in a volume of 100 µL in dark-colored vials in tray autosampler (-8 °C) were performed at 325 and 290 nm (α-tocopherol and all-trans-retinol). The chromatographic analysis was performed at 40 ºC with isocratic elution.

2.3. Vitamin C Analysis

Vitamin C stock solution was prepared at 4000 mg/mL in metaphosphoric acid. The stock solutions were appropriately diluted correctly with double distilled water to prepare standard solution preparation. Calibration was calculated by linear regression analysis of the absorbance versus the concentration of the standard solution. Vitamin C content of *L. rantonnetii* was measured spectrophotometrically (Shimadzu UV 1800, Japan) at 521 nm using 2,4-dinitrophenyl hydrazine method.

2.4. Element Determination

Detection of mineral content of *L. rantonnetii* plant was performed by using dry ashing method. 1 g of sample was weighed and placed in the crucible, put in an oven for 4-5 hours at 105 °C, the dried samples were crushed in thoroughly cleaned porcelain mortars. 2 mL of ethyl alcohol-sulfuric acid mixture (95:5) was added to each sample. Then it was left in the ash furnace set at 250 °C. The temperature was increased by 100 °C per hour until the temperature of the oven reached 550 °C. 5 mL of prepared 3N hydrochloric acid solution was added on the samples taken out from the ash furnace and it was completed to 50 mL with distilled water. The metal analysis was conducted using multi-element (NIST SRM) reference materials (inorganic ventures IV-Stock-1643). The concentration ranges of the standards for the elements (0.1-7 mg/L). Element analysis of P, Mg, Fe, Zn, Cu, Cr, Mn, Mo, Cd, As, Pb, Co, V, Tl, Li, Ti, Sr and Be were used inductively coupled plasma-optical emission spectrometry. ICP-OES (Thermo ICP-OES iCAP 6300 Duo, England) with ASX-520 autosampler was used for the analyses. Its operating conditions are reported as; spectral range (166-847 nm), plasma viewing (Dual), Plasma and shear gas (Argon), Nebulizer (Concentric glass), sample flush time (40 seconds), analysis pump rate (50 rpm), auxiliary gas flow (0.5 L/min), nebulizer gas flow (0.65 L/min), RF power (1150 W).

2.5. Determination of Total Phenol Content

The total phenol content of *L. rantonnetii* was carried out by utilizing reagent (Folin-Ciocalteu) (Yi *et al*., 1997; Gamez-Meza *et al*., 1999). 0.3 mL of 20 % Na_2CO_3 was added to the plant samples prepared by diluting with methanol, then combined with 100 µL of water diluted Folin reagent (1:1) and 2 hours incubated at room temperature. At 765 nm wavelength, samples absorbance read against a control sample. The phenol contents are expressed as gallic acid equivalents per gram (mg GAE/g) of extract.

2.6. Determination of Total Flavonoid Content

In this study, in order to determine the flavonoid content of the extract of *L. rantonnetii*, 0.1 mL of $Al(NO_3)_3$ solution mixed with 0.2 mL of the samples diluted with methanol and incubated at room temperature for 40 min. At 415 nm wavelength samples absorbance was recorded versus the control sample (Urgeova & Polivkau, 2009). Flavonoid concentration was given as mg/g equivalent of quercetin.

2.7. Determination of Total Antioxidant Capacity

In this study, the method progressed by (Prieto *et al*., 1999) was used to detect the total antioxidant capacity of *L. rantonnetii* plant. The assay depends on the reduction of acidic Mo (VI) to (V) at acidic pH which leads to the form green phosphate/Mo (V) complex. Added 0.2 mL of reagent (0.6 M, 28 mM, and 4 mM of sulfuric acid, sodium phosphate, and ammonium molybdate, respectively) to 0.2 mL samples of different concentrations of plant extract diluted with methanol, and kept for 90 minutes at 95 °C. Before reading the absorbance of samples versus the control sample at 695 nm wavelength, the samples were cooled to room temperature. Antioxidant capacity was given as mM ascorbic acid/g.

2.8. DPPH Radical Scavenging Capacity

DPPH radical scavenging capacity determination assay depends on the principle of spectrophotometric determination of the characteristic purple lightening by scavenging the stable DPPH free radical, by these chemicals in the presence of antioxidant chemicals that donate electrons or hydrogen atoms (Cuendet *et al*., 1997; Chen *et al*., 2009). In this study, in order to determine *L. rantonnetii* ability to scavenge DPPH radical, 5 mL of 0.004% DPPH solution was added to the methanol diluted different concentrations of plant extract and incubated for 30 min. the absorbance was measured against a blank at 517 nm. Antioxidant activity of the samples was expressed in term of IC_{50} values (µg/mL required to inhibit the formation of DPPH radicals by 50%) calculated from the graph in which the percentage inhibition was plotted against extract concentration. The synthetic antioxidant BHT was included in experiments as a positive control. The percentage inhibition of the free radical DPPH was calculated according to the following formula.

$$\text{Inhibition (\%)} = \left\{ \frac{A_{Blank} - A_{Sample}}{A_{Blank}} \right\} X\ 100$$

2.9. ABTS Radical Scavenging Capacity

In this study, The ABTS radical scavenging effect of *L. rantonnetii* plant was done with 0.1 M phosphate buffer with pH 7.4. Prepared 2 mM ABTS solution with 2.45 mM solution of potassium persulfate. Then these two solutions were prepared mixed up by magnetic stirrer at room temperature/dark for 12-16 hours. The absorbance of the prepared solution was recorded at 734 nm. The strength of molecules to scavenge stable free radicals was done with a synthetic antioxidant (Trolox) (Arnao *et al*., 2001).

$$\text{Inhibition (\%)} = \left\{ \frac{A_{Blank} - A_{Sample}}{A_{Blank}} \right\} X\ 100$$

2.10. Hydroxyl Radical Scavenging Capacity

In this study, in determining the hydroxyl radical removal capacity of *L. rantonnetii* plant extract, the hydroxyl radicals formed by the (Fe^{+3}/ascorbate/EDTA/H_2O_2) system. The method

based on the spectrophotometric measurement of thiobarbituric acid, reactive products released by the degradation of deoxyribose (Kunchandy & Rao, 1990). *L. rantonnetii* plant extract was added to the samples prepared in different concentrations from 3.0 mM deoxyribose, and 1 mM for each of the $FeCl_3$, EDTA, ascorbic acid, and H_2O_2 solutions, after that added 20 mM phosphate buffer (pH 7.4) until the volume reached 1 mL, kept the reaction for 1 hour at 37 °C. Before recording the absorbance added 1 mL for both the TBA, and TCA, and boiled for 30 minutes. The absorbance at 532 nm wavelength of the colored complex formed by TBA of the released malondialdehyde was read against the control sample. The IC_{50} values were determined by plotting the % inhibition values obtained against different concentrations of the plant extract.

$$\text{Inhibition (\%)} = \left\{ \frac{A_{Blank} - A_{Sample}}{A_{Blank}} \right\} X\ 100$$

2.11. Determination of Hydrogen Peroxide Scavenging Activity

Hydrogen peroxide (H_2O_2) scavenging activity of *L. rantonnetii* plant methanol extract was measured spectrophotometrically at 230 nm. For this, phosphate buffer (50 mM, pH 7.4) utilized to preparative 43 mM of H_2O_2 solution. *L. rantonnetii* plant extracts obtained from different concentrations were used. The volume of plant extract and standard antioxidant substances used in the study BHT solutions completed with buffer solution up to 0.4 ml. Before incubation for 10 minutes at the normal temperature inside the room added 0.6 ml of H_2O_2 solution. The decreasing amount of H_2O_2 at 230 nm was read as the reduced absorbance (Ruch *et al*., 1989).

2.12. Phenylhydrazine method

In this method. 1 mL phenylhydrazine, 0.1 mL 20% PCV, 1.85 mL buffer were added to the samples prepared with different concentrations of the plant methanol extract. Then, before centrifuging them for 10 min at 4000 rpm incubated at 37 °C for 1 hr. Finally, the supernatant portion was transferred to other tubes; the absorbance at 540 nm was read against the control sample (Valenzuela *et al*., 1985).

3. RESULTS

Total antioxidant capacity, total phenolic and total flavonoid contents, the radical scavenging activities of DPPH, ABTS, hydroxyl radical, and hydrogen peroxide as well as its anti-hemolytic activity were measured to determine the antioxidant properties of *L. rantonnetii*. In addition, the levels of vitamin, element, and minerals in *L. rantonnetii* were determined and the results were shown in Table 1 and 2.

Table 1. Vitamin A, E, C, total antioxidant capacity, total phenolic and flavonoid, element (P, Mg, Fe, Zn, Cu, Cr, Mn, Mo, Cd, As, Pb, Co, V, Tl, Li, Ti, Sr and Be) content in L. rantonnetii.

Parameters	*Lycianthes rantonnetii* $\bar{X}$ ± SEM
α-tocopherol (µmol/kg)	104.55 ± 7.44
Retinol (µmol/kg)	12.22 ± 3.37
Vitamin C (mg/100g)	80.61 ± 9.31
Total phenolic content (mg GA/ g)	5.33 ± 0.41
Total flavonoid content (mg QE/g)	3.76 ± 0.29
Total antioxidant capacity (mM A.A/ g)	11.45 ± 0.60
Be (µmol/kg)	0.09 ± 0.00
Li (µmol/kg)	0.66 ± 0.29
V (µmol/kg)	1.57 ± 0.13
Ti (µmol/kg)	3.07 ± 0.26
Cr (µmol/kg)	4.61 ± 0.33
Cu (µmol/kg)	57.60 ± 4.83
Sr (µmol/kg)	14.41 ± 3.24
As (µmol/kg)	0.67 ± 0.01
Tl (µmol/kg)	0.16 ± 0.01
Cd (µmol/kg)	0.75 ± 0.02
Co (µmol/kg)	2.23 ± 0.10
Pb (µmol/kg)	0.83 ± 0.11
Mo (µmol/kg)	1.14 ± 0.01
Mg (mmol/kg)	39.13 ± 1.76
Fe (mmol/kg)	0.63 ± 0.01
Mn (mmol/kg)	0.42 ± 0.02
P (mmol/kg)	34.60 ± 0.42
Zn (mmol/kg)	0.14 ± 0.01

Values are expressed as mean ± standard error of the mean ($\bar{X}$ ± SEM). Samples were carried out in triplicate.

Table 2. Values of % Inhibition and IC_{50} (µg/mL) in methanol extracts of *L. rantonnetii* compared with a positive controls.

	Control	% Inhibition $\bar{X}$ ± SEM	IC_{50} (µg/mL) $\bar{X}$ ± SEM
DPPH$^{\bullet}$		67.25 ± 0.08	96.52 ± 0.13
	BHT	74.78 ± 3.04	66.04 ± 13.55
$OH^{\bullet}$		61.18 ± 1.39	32.46 ± 0.81
	BHT	81.32 ± 0.13	84.53 ± 6.03
H_2O_2		54.72 ± 0.13	34.32 ± 0.05
	BHT	54.17 ± 0.03	23.57 ± 0.29
ABTS		57.75 ± 1.11	112.81 ± 0.41
	Trolox	96.56 ± 0.01	53.11 ± 0.70
$PhNHNH_2$		67.18 ± 7.33	191.13 ± 52.51
	Trolox	50.77 ± 0.58	16.17± 6.22

Values are expressed as mean ± standard error of the mean ($\bar{X}$ ± SEM). Samples were carried out in triplicate. DPPH: 2,2-diphenyl-1-picrylhydrazyl, OH**:** Hydroxyl, H_2O_2**:** hydrogen peroxide, ABTS: 2.2-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid), $PhNHNH_2$: Phenylhydrazine.

Figure 1 and 2 shows at 325 and 290 nm α-tocopherol and all-trans-retinol chromatograms**.** While evaluating the results, it was observed that *L. rantonnetii* contained vitamins A, and E in significant levels.

Figure 1. Chromatogram of the vitamin A (mobile phase: methanol/tetrahydrofuran (20/80 v/v), flow rate: 1.5 mL min^{-1}. Column: GI Science C18 5µL (250/4.6 mm), wavelength: 325 nm).

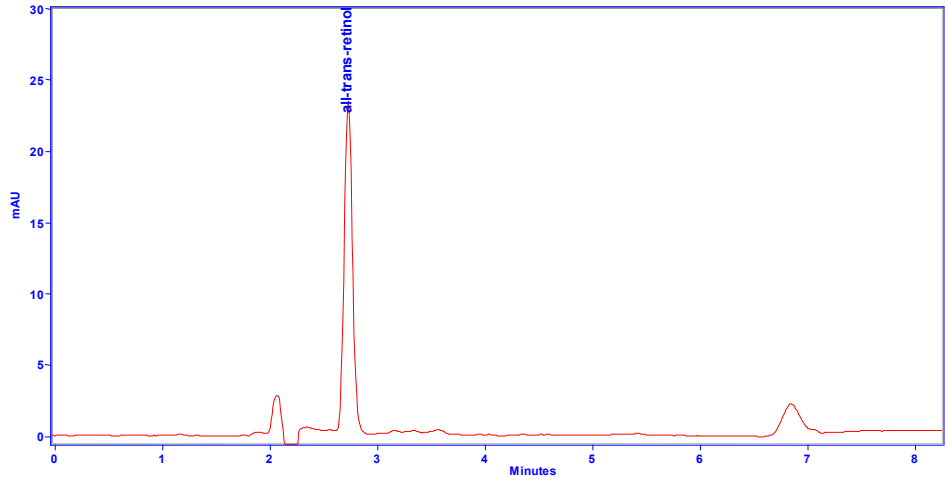


Figure 2. Chromatogram of vitamin E (mobile phase: methanol/tetrahydrofuran (20/80 v/v), flow rate: 1.5 mL min^{-1}. Column: GI C18 5µL (250/4.6 mm), wavelength: 290 nm).

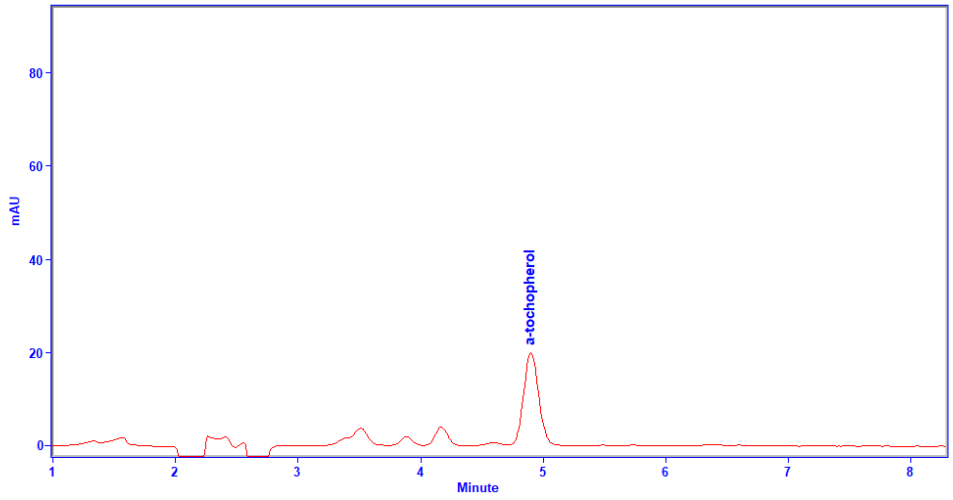


Figure 3 shows % inhibition and IC_{50} values of DPPH radical and BHT and ABTS radical for *L. rantonnetii* and trolox.

Figure 3. % inhibition and IC_{50} values of DPPH radical and BHT and ABTS radical for *L. rantonnetii* and trolox.

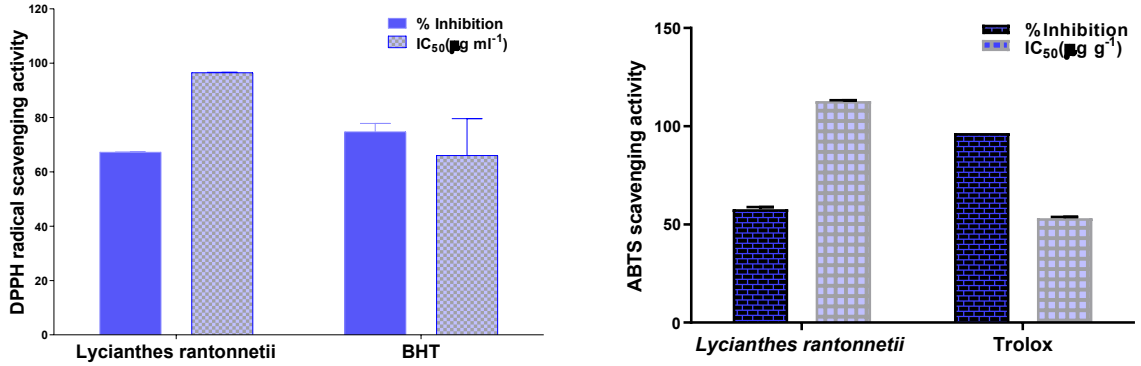


Figure 4 demonstrates % inhibition and IC_{50} values of the hydroxyl radical scavenging activity and BHT and hydrogen peroxide radical scavenging activity and BHT of *L. rantonnetii*

Figure 4. Graph showing the change in % inhibition and IC_{50} values of the hydroxyl radical scavenging activity and BHT and hydrogen peroxide radical scavenging activity of *L. rantonnetii* and BHT.

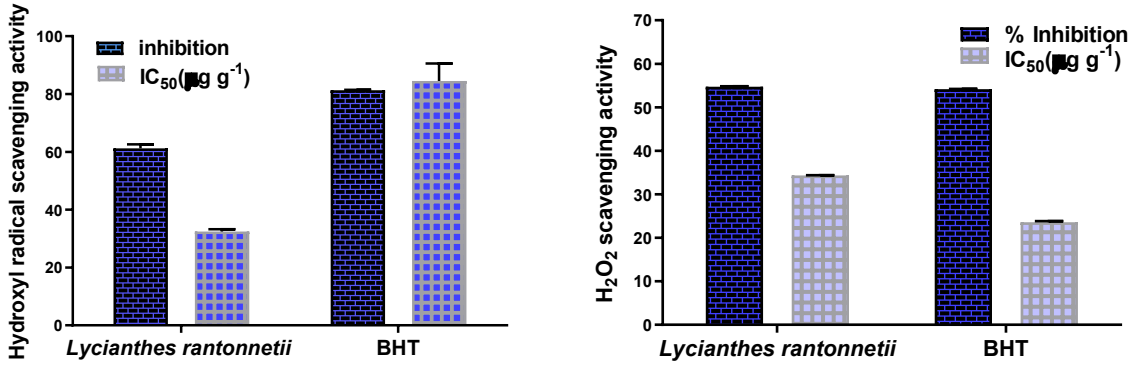
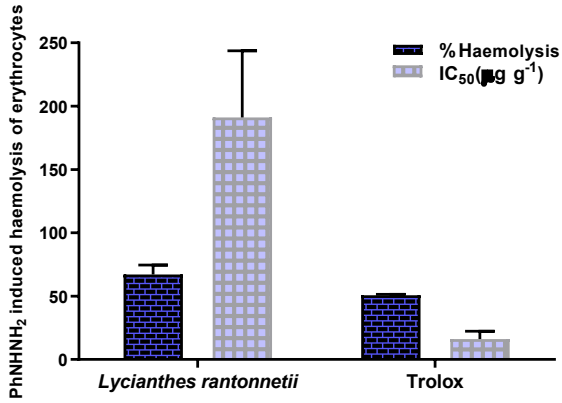


Figure 5 illustrated % haemolysis and IC_{50} values of the phenylhydrazine for *L. rantonnetii* plant and trolox.

Figure 5. Graph showing the change in % haemolysis and IC_{50} values of the phenylhydrazine for *L. rantonnetii* plant and trolox.



4. DISCUSSION and CONCLUSION

Oxidative stress occurs when an increase in ROS levels affects the balance between ROS development and detoxification and contributes to impaired cellular activity (Imlay, 2003). Therefore, many defense mechanisms, including various antioxidants and detoxifying enzymes, must regulate ROS compounds (Duarte & Lunec, 2005). Deficiencies or degradation in these antioxidants could contribute to oxidative stress, which can trigger cell harm and lysis (Valko *et al*., 2007). The pathways observed by antioxidant protection are; preventing the formation of free radicals, scavenging of oxidants, turning toxic free radicals into less toxic compounds, disrupting the production of secondary toxic metabolites and inflammatory mediators, restricting the secondary oxidants chain propagation, restoring and initiating harmed molecules, and improving the endogenous antioxidant synthetic (Halliwell, 2007).

In this work, first of all, the amount of vitamins A, E, and C in *L. rantonnetii* plant was determined. In terms of minimizing the risk of illness and develop positive health, vitamins play a significant part. Vitamins are necessary for all splitting body cells to operate normally and proliferate. (Piccardi & Manissier, 2009).

The vitamin C level of *L. rantonnetii* plant extract included in the study was determined as 80.61 ± 9.31 mg/100g. The vitamin A level was determined as 12.22 ± 3.37 μmol/kg and the vitamin E level as 104.55 ± 7.44 μmol/kg. In the literature review, no study was found on the levels of vitamins A, C, and E of *L. rantonnetii* plant. At the same time, there is no study on these vitamin levels in *Lycianthes* species. However, the vitamin C level of *Capsicum* genus (chili pepper) plant, are important species belonging to the Solanaceae family, also has the same tribe with *L. rantonnetii* plant, was determined to be 27.13 ± 0.05 mg/100g. In a study conducted by Emmanuel-Ikpeme *et al*., the vitamin E level of the *Capsicum* genus (chili pepper) plant is 0.41 ± 0.02 mg/100g, and the vitamin A level is 1.26 ± 0.03 μg/100g (Emmanuel-Ikpeme *et al*., 2014). When the *L. rantonnetii* plant was compared with the chili pepper plant, it was determined that the vitamin C, A, and E levels were higher than the chili pepper plant. This shows that the plant is a better source of vitamins than the chili pepper plant. Considering the results, it was determined that the *L. rantonnetii*. Plant had a significant vitamin A, C, and E content, and especially vitamin C and E were at a high level.

Polyphenols are organic compounds found abundantly in the plant (Lecour & Lamont, 2011). They are necessary for plant growth, reproduction, color, and aroma formation (Guiné *et al*., 2015). Polyphenols consists of phenolic compounds, phenolic acids, and flavonoids (Ratnam *et al*., 2006). These compositions have various pathways for practicing their antioxidant powers, including free radical scavenging, strengthening endogenous enzymatic protections, reducing lipoperoxidation (Watson *et al*., 2014). The antioxidant function of some phenolic substances in the work by (Servili *et al*., 2014), depends largely on their degree of hydroxylation. They also act as H atom donors, singlet oxygen, and superoxide radical scavenger, and metal chelator (Heleno *et al*., 2015). It is well-established that oxidative processes are related to diverse pathologies (Watson *et al*., 2014), polyphenols contribute to the prevention of cardiovascular disease, cancer and osteoporosis reduction and play a role in combating neurodegenerative diseases and diabetes mellitus (Scalbert *et al*., 2005).

In this study, total phenol, total flavonoid, and total antioxidant capacities of *L. rantonnetii* plant were determined. The total phenol amount was determined using the gallic acid standard curve graph, and the total phenol amount of the plant was calculated as 5.33 ± 0.41 mg gallic acid/g. Total flavonoid contents were considered using the typical quercetin curve, and the total flavonoid content of the plant was determined as 3.76 ± 0.29 mg quercetin/g. Furthermore, the overall antioxidant potential was calculated in line with the standard for ascorbic acid, and the total antioxidant value of the plant was 11.45 ± 0.60 mM/g ascorbic acid. These findings indicate that phenolic and flavonoid compounds are responsible for the higher rates of antioxidant activity.

In the literature review, no study was found on the total amounts of phenols and flavonoids of the *L. rantonnetii* plant. The overall amounts of phenols and flavonoids of methanol extract of *Capsicum* genus (chili pepper) was determined the phenol content; 480 mg gallic acid/g, flavonoid content; 240 mg quercetin/g (Helmja *et al*., 2007). The results indicate that the total phenol and flavonoid levels of *L. rantonnetii* plant were close to the chili pepper plant.

Elements are also essential to plants. If a plant cannot obtain an element from the soil, its life process is disrupted. Medicinal plants differ in trace element content as in all living organisms. All elements found in soil, air, and water enter the plant tissue, but in general, trace element intake varies according to the plant's need for an element, the amount of the element in the soil, and the soil type. The pharmacotherapy effects of pharmaceutical products obtained from plants are significantly affected by trace element factors in plant substances. Plants synthesize organic compounds in photosynthesis, including pharmacologically active, from various mineral constituents. More than 20 elements are involved in the synthesis of organic

compounds. Besides, all elements play important roles in the photosynthesis process (Suchacz & Wesołowski, 2006).

When the element and mineral levels of *L. rantonnetii* plant were examined in our study, it was found that it had content, 0.63 ± 0.01 mmol/kg Fe, 0.14 ± 0,01 mmol/kg Zn, 57.60 ± 4.83 µmol/kg Cu, 0.42 ± 0.02 mmol/kg Mn, 4.61 ± 0.33 µmol/kg Cr, 2.23 ± 0.10 µmol/kg Co, 39.13 ± 1.76 mmol/kg Mg, 34.60 ± 0.42 mmol/kg P, 14.41 ± 3.24 µmol/kg Sr, 3.07 ± 0.26 µmol/kg Ti, 1.57 ± 0.13 µmol/kg V, 1.14 ± 0.01 µmol/kg Mo, 0.83 ± 0.11 µmol/kg Pb, 0.75 ± 0.02 µmol/kg Cd, 0.67 ± 0.01 µmol/kg As, 0.66 ± 0.29 µmol/kg Li, 0.16 ± 0.01 µmol/kg Tl, 0.09 ± 0.00 µmol/kg Be.

Comparing the element and mineral levels of the *L. rantonnetii* plant, magnesium (Mg) > phosphorus (P) > iron (Fe) > manganese (Mn) > zinc (Zn) > copper(Cu) > strontium (Sr) > chromium (Cr) > titanium (Ti) > cobalt (Co) > vanadium (V) > molybdenum (Mo) > lead (Pb) > cadmium(Cd)> arsenic (As) > lithium (Li) > thallium (Tl) > beryllium (Be).

In the literature search, no study was found on the element and mineral levels of *L. rantonnetii* plant. However, in the studies conducted on the *Capsicum* genus (chili pepper), one of the important species belonging to the Solanaceae family, mineral levels were found to be 7l.4l ± 0.04 mg/100g Fe, 38.02 ± 0.05 ppm Zn, 18.22 ± 0.01 ppm Cu, 274.88 ± 0.02 mg/100g Mg, 13.27 ± 0.09 ppm Co, 28.75 ± 0.04 mg/100g P (Emmanuel-Ikpeme *et al*., 2014). Comparing *L. rantonnetii* plant to chili pepper plant, it was determined that Fe, Zn, Mg, and Co levels in chili pepper plant were higher, and Cu and P in *L. rantonnetii* plant were higher.

Spectrophotometric assays using DPPH and ABTS are the most popular methods for the *in vitro* evaluation of antioxidant ability in foods (Becker *et al*., 2019). The extract's free radical scavenging behavior was calculated using the constant free radical DPPH regarding hydrogen donation or radical scavenging capacity. Modifications that occur in the radical scavenging by hydrogen donations are attributable to the reaction between radicals and antioxidant molecules (Aadesariya *et al*., 2017). The sample concentration necessary to reduce the initial DPPH, ABTS concentration by 50% (IC_{50}) which calculated from inhibition plot under the experimental conditions are determined.

The scavenging capacity of the methanol extract of *L. rantonnetii* to scavenge DPPH, ABTS, and hydroxyl radicals, which are important free radicals, was determined, and the antiradical capacity of the plant was evaluated and compared with BHT, a synthetic antioxidant. The highest inhibition value of the DPPH radical of plant was determined as 67.25 ± 0.08% and 74.78 ± 3.04% for BHT. A smaller IC_{50} value means that antioxidant effect is greater. The specimen concentration in need to minimize the original concentration of DPPH, by 50 percent (IC_{50}) under the test conditions was calculated. For 96.52 ± 0.13 µg/mL and 66.04 ± 13.55 µg/mL for BHT, scavenging activity was recorded. It is safe to assume that *L. rantonnetii* plant's methanolic extract showed less potent antioxidant activity, with a lower inhibition percentage than BHT. In scavenging DPPH radical of plant was thus found to be less efficient than BHT.

In this study, the plant extract's scavenging capacity on ABTS radical was determined and compared with trolox, a synthetic antioxidant. The highest inhibition value of ABTS radical of plant was determined as 57.75 ± 1.11% and 96.56 ± 0.01% for trolox. A lower IC_{50} value means that antioxidant activity is larger. The specimen concentrations needed to decrease the initial ABTS intensity by 50 percent (IC_{50}) under the test conditions was calculated. Concentrations that inhibited the ABTS radical by 50 percent were determined as 112.81 ± 0.41 µg/mL in the plant and 53.11 ± 0.70 µg/mL for trolox. The inference can be drawn that the *L. rantonnetii* plant showed less potent antioxidant activity, with a lower inhibition ratio proportion than the BHT. Thus plant is less effective than trolox in scavenging ABTS radical.

In the study scavenging capacity of the hydroxyl radical of the *L. rantonnetii* plant was determined. The highest inhibition value of the plant extract's hydroxyl radical was determined as 61.18 ± 1.39% and 81.32 ± 0.13% for BHT. The concentrations that inhibit the hydroxyl radical by 50 percent were determined as 32.46 ± 0.81 µg/mL in plant and 84.53 ± 6.03 µg/mL for BHT. It has been determined that the plant is more effective than BHT in scavenging the hydroxyl radical. As a result of this study, it was found that *L. rantonnetii* plant effectively scavenges the hydroxyl radical.

Hydrogen peroxide is not a free radical but is called a ROS because it can be converted into other free radicals, such as hydroxyl radical, that facilitate much of the toxic results of H_2O_2 (Halliwell & Gutteridge, 1995). In this study, *L. rantonnetii* plant's hydrogen peroxide removal activity was also determined. Results indicated that the highest inhibition value of the hydrogen peroxide of both plant extract and BHT was determined, and the values close each other were 54.72 ± 0.13%, 54.17 ± 0.032% for plant extract and BHT, respectively. The concentrations that inhibit the hydrogen peroxide by 50 percent were determined as 34.32 ± 0.052 µg/mL in plant extract and 23.57 ± 0.29 µg/mL for BHT. It has been determined that the plant is less efficient than BHT in scavenging the hydroxyl peroxide.

Several hemolytic agents are known that induce hemolysis in animal models. One of these agents is phenylhydrazine (PHZ), a hydrazine derivative. The PHZ hemolytic action pathway has been linked to its association with RBCs. This reaction creates hydrogen peroxide, which produces oxidized compounds and hydrazine free radicals, eliminating the hemoglobin pigment. PHZ also causes the development of ROS, lipid peroxidation, and protein oxidation due to the plasma membrane's reaction (Allahmoradi *et al*., 2019).

In the present study, the anti-hemolytic activity of the methanol extract of *L. rantonnetii* plant was determined. The highest haemolysis value of the plant extract's phenylhydrazine was determined as 67.18 ± 7.33% and 50.77 ± 0.58% for trolox. The concentrations that inhibit the phenylhydrazine haemolysis by 50 percent were determined as 191.13 ± 52.51 µg/mL in plant and 16.17± 6.22 µg/mL for trolox. *L. rantonnetii* plant and trolox showed a differential pattern of anti-hemolytic activity. Their IC_{50} results indicate that the anti-hemolytic action of plant methanol extracts weaker than trolox.

The analysis result was showed that the plant is very rich in mineral content and contains high amounts of magnesium and phosphorus. The overall phenol and flavonoid composition of the plant was noted, and its antioxidant potential was also at a reasonable level. Moreover, it was concluded that the plant is effective in the inhibition of ROS, scavenging ABTS and DPPH radicals, which are stable free radicals, hydrogen peroxide and has a good activity of antioxidants and antiradical through hindering the hydroxyl radical, which in biological systems is the most responsive species. In conclusion, the *L. rantonnetii* plant has very high total phenolic, flavonoid and vitamin E, A, C content, mineral content, antioxidant, and antiradical activity. It may be considered for the treatment and prevention of many diseases caused by ROS. Moreover, reasonable amounts of total phenol and total flavonoids, and our research will consider future research as well. The results of the study revealed that *L. rantonnetii* can be used as an additive for food products in the various food sectors with appropriate antioxidant activities.

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Declaration of Conflicting Interests and Ethics

The authors declare no conflict of interest. This research study complies with research and publishing ethics. The scientific and legal responsibility for manuscripts published in IJSM belongs to the authors.

Authorship contribution statement

Abdalla Ali Amin: Plant collection, Laboratory analysis, and Writing in the study. **Suat Ekin:** Investigation, Designing and Writing. **Ahmet Bakir:** Laboratory analysis. **Damla Yildiz:** Laboratory analysis.

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