

THE FATTY ACID COMPOSITION, PHYTOCHEMICALS AND ANTIOXIDANT POTENTIAL OF WILD EDIBLE *Smilax excelsa* L. SHOOTS

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ABSTRACT

The present study examines the fatty acid composition, phytochemicals content and antioxidant potential of *Smilax excelsa* L. shoots, which grows spontaneously and is consumed as food in rural areas of Düzce (Türkiye) province, was investigated. DPPH, ABTS and OH radical scavenging tests were utilized to put forth the antiradical properties of the extracts of this plant. In addition, the metal chelating potential of this plant was also evaluated. The higher the inhibition % value calculated in these tests, the higher the antioxidant activity was considered and the results were evaluated. The average ABTS radical cleaning test results of *Smilax excelsa* extracts prepared in different concentrations of methanol, ethanol and pure water were found to be 98.14%, 98.16%, 90.20%, respectively. The average DPPH radical cleaning test results of the extracts of this plant prepared in different concentrations of methanol, ethanol and pure water were determined as 87.48%, 76%, 46.53%, respectively. The OH radical cleaning test results of methanol, ethanol and pure water extracts of the *Smilax excelsa* were determined as 54.79%, 72.54%, none, respectively. In addition, the metal chelation test results of methanol, ethanol and pure water extracts of this plant were determined as 87.26%, 89.36%, 53.70%. The highest protein (85.91 mg BSA/g), proanthocyanidin (55.39 mg CE/g) and phenolic (4957.57 µg GAE/g) content of *Smilax excelsa* pure water extract was determined. It has been determined that gallic acid (117.33 µg/g), vanillic acid (33.89 µg/g), caffeic acid (4.55 µg/g), ferulic acid (93.78 µg/g), rosmarinic acid (0.33 µg/g) and hydrocyanic acid (0.33 µg/g) are found in different proportions in the *Smilax excelsa*. It has been stated that *Smilax excelsa* is an important source of palmitic acid (20.52%), stearic acid (4.95%), oleic acid (4.74%), linoleic acid (20.99%), γ -linolenic acid (2.26%), alpha-linolenic acid (34.29%) and docosahexaenoic acid (2.79%). It has been found that this plant has a low content of fat-soluble vitamins and phytosterols, with the exception of β -sitosterol (6.43 µg/g).

Key words: *Smilax excelsa*, fatty acids, phenolic acids, fat-soluble vitamins, phytosterols, antioxidant activity

INTRODUCTION

In many regions of the world, self-grown vegetables are still widely used and have a vital role in the nutrition of people living mainly in rural areas. In addition to acting as an important resource for humanity as a cultural heritage that must be protected around the world edible wild plants are also a cheap source of nu-

trients, vitamins, antioxidants and minerals [Harumi Iyda et al. 2019]. The chemical compositions of edible wild plants are of increasing interest in the scientific community as well as the food industry and consumers. Also recently, as a new trend in Europe and some developed countries, it is increasingly observed that

local wild plants are used in contemporary cuisines both as an element of cultural identity and for health related advantages [Geraci et al. 2018, Harumi Iyda et al. 2019].

It is seen that research aimed at determining the nutritional and phytochemical content of edible wild plants has come to the fore in recent years [Mzoughi et al. 2019]. Today, edible wild plants, not only to people's attention because of their nutritional content pays, but also diversification of your eating habits, for reasons such as to promote biodiversity and ecological sustainability has been a growing interest of people is subject to these plants [Brito et al. 2021]. *Smilax excelsa* is a species of perennial, climbing shrub-shaped wild plant belonging to the family *Smilacaceae* native to tropical and temperate regions. It has been found that this plant spreads in woodlands and shrublands up to 800 m in height. *Smilax aspera* and *Smilax excelsa* species of this plant grow in Türkiye. *Smilax aspera* grows intensively in western and southern Anatolia, and *Smilax excelsa* grows mainly in northern Anatolia regions [Demir et al. 2017, Efe et al. 2019]. Especially in the Black Sea region of Türkiye, young shoots of this plant are consumed as food during the spring months [Demir et al. 2017]. In studies on the pharmacological activities of *Smilax* species, it has been reported that these species exhibit anti-tumor, anti-mutagenic, anti-bacterial, anti-fungal, antioxidant, and anti-inflammatory properties [Lee et al. 2001, Jiang and Xu 2003, Ozsoy et al. 2008, Salas-Coronado et al. 2017].

The Black Sea region of Türkiye is in a remarkable position in terms of plant diversity. It can be seen that the people living here use the plants growing on their own at different times of the year for both nutritional and therapeutic purposes. One of these plants is *Smilax excelsa*. A previous study reported some information about the antioxidant potential of the leaves of this plant [Ozsoy et al. 2008, Miser-Salihoglu et al. 2010]. It seems that information on the phytochemical content of this plant is not in the literature. Therefore, the objective of the present study was to examine the phytochemical composition of the *Smilax excelsa* L. shoots such as fatty acid composition, fat-soluble vitamins, phytosterol and phenolic acid content, as well as its antioxidant potential.

MATERIAL AND METHODS

Chemical agents. All chemicals used in this study were obtained from Sigma-Aldrich Company.

Extraction procedures. Fresh *Smilax excelsa* (shoots) was obtained at a market in Düzce in the spring. After being rinsed with water, the plants were dried in a cool location. Then, using a mechanical grinder, it was processed into powder. Then, in 10 ml of solvent, 1 g of powder sample was extracted (methanol, ethanol and pure water). It took two hours to remove all of the samples. The samples were then centrifuged at +4°C for 5 minutes at 5000 rpm. As a result, the supernatant for the experiments (ABTS, hydroxyl, DPPH, metal ion chelation, and phenolic acids) was obtained [Keser et al. 2014]. A mixture of 3/2 (v/v) hexane isopropyl alcohol was used to homogenize the powdered samples of the *Smilax excelsa* plant for the analysis of fatty acids, fat-soluble vitamins and phytoosterols [Hara and Radin 1978]. The samples were then subject to centrifugation at 5000 rpm at +4°C. The supernatant portion was used in the analyses.

Determination of antiradical activities. The radical scavenging activities (RSAs) of ABTS^{•+} (2,2'-azino-bis(3-ethyl-benzothiazoline 6-sulfonate)), hydroxyl, DPPH (2,2-Diphenyl-1-picrylhydrazyl), and metal ions were measured using the methods of [Halliwell et al. 1987, Decker et al. 1990, Brand-Williams et al. 1995, Re et al. 1999], respectively. All tests were repeated three times, and then the average values were calculated. The following equation was used for calculating the radical scavenging activity percentages (RSA %) for each sample:

$$\text{RSA \%} = [(A_0 - A_1)/A_0] \times 100$$

A₀: control absorbance; A₁: sample absorbance.

Total phenolic contents (TPC). TPC was determined using the method outlined by Singleton and Slinkard [1977]. Gallic acid (GAE) was used as a standard.

Total proanthocyanidin content (TP). TP was calculated according to the approach published by Amaeze et al. [2011]. The catechin (CE) was used as a standard.

Total protein content (TPR). The method developed by Lowry et al. [1951] was used for determining TPR. The bovine serum albumin (BSA) was used as a standard.

Analysis of phenolic acids. The method set forth by Zu et al. [2006] was utilized for determining the phenolic acids via high performance liquid chromatography (Shimadzu-HPLC) in *Smilax excelsa* HPLC was used to measure gallic acid, vanillic acid, caffeic acid, ferulic acid, rosmarinic acid and hydrocinnamic acid in the *Smilax excelsa* extract.

Analyses of fatty acids. The method devised by Christie [1990] and Christie [1992] was employed for the analyses of fatty acids in the *Smilax excelsa* extract using gas chromatography (Shimadzu-GC). Percent of samples was used for expressing the results of the fatty acid analyses.

Lipophilic vitamins and phytosterols analyses. Lipophilic vitamins and phytosterols were extracted from *Smilax excelsa* according to the method described by Sanchez-Machado et al. [2002] and López-Cervantes et al. [2006]. The analyses were performed on the HPLC device. The analyses' results were represented as µg/g.

Statistical analyses. For statistical analysis, SPSS Statistics 18.0 was employed. The antiradical outcomes (DMRT) were investigated using analysis of variance (ANOVA) and Duncan's multiple range test.

RESULTS AND DISCUSSION

Antiradical properties. Secondary metabolites, phenolic compounds, vitamins, carotenoids and fatty acids in plants play a key role for the health of the human body. It has been reported that these compounds increase the anti-oxidant ability of the human body, moreover, these compounds have a significant effect on health and life, providing suppression of oxidative stress and inflammation-related diseases [Jia et al. 2011, Song et al. 2020, Gok et al. 2021]. Epidemiological data illustrated that the consumption of diets rich in fruits and vegetables can reduce the risk of various chronic diseases in humans [Kongkachuichai et al. 2015]. Therefore, the research of antioxidant potentials of plants with their phytochemical content is an important field of study today. In this study, anti-

oxidant and phytochemical content of *S. excelsa* shoot extract were investigated. The antiradical property results of *Smilax excelsa* shoot extracts are presented in Table 1.

The higher the calculated % inhibition value, the higher the antioxidant activity was considered and the results were evaluated. The average DPPH radical scavenging activity test results of the extracts of *Smilax excelsa* prepared from methanol, ethanol and pure water were determined to be methanol (87.48%), ethanol (76%) and pure water (46.53%). The average ABTS radical scavenging activity test results of *Smilax excelsa* extracts prepared from methanol, ethanol and pure water were determined to be methanol (98.14%), ethanol (98.16%) and pure water (90.20%) – Table 1. DPPH is a stable free radical that is commonly used to assess the ability of plants to scavenge free radicals [Ozsoy et al. 2008]. In a previous study, it was observed that information was shared about the antioxidant potential of *Smilax excelsa* samples in Istanbul [Ozsoy et al. 2008]. In this study, it was determined that the extracts of this plant prepared in different solvents have a strong cleaning property on the DPPH radical [Ozsoy et al. 2008]. DPPH is a stable free radical that is frequently used to evaluate a plant's capacity to scavenge free radicals [Efe et al. 2019]. It has been reported that a different species of this plant (*Smilax sebeana* Miq) has antioxidant potential [Ao et al. 2011]. It has been determined that the information about the ABTS radical scavenging potential of *Smilax excelsa* is not in the literature. In our study, it was determined that this plant has a strong ABTS radical scavenging potential.

The average metal chelation results of *Smilax excelsa* shoot extracts prepared from methanol, ethanol and pure water were determined to be methanol (87.26%), ethanol (89.36%) and pure water (53.70%) – Table 2. In a previous study, it was reported that extracts of *Smilax excelsa* prepared in different solvents have the potential to chelate metals. In addition, in this study, it was reported that the extract prepared in ethyl alcohol showed a fairly high chelation compared to other extracts [Ozsoy et al. 2008]. Similar results were revealed in this study.

The OH radical cleaning results of the methanol, ethanol and pure water extracts of *Smilax excelsa* shoot

Table 1. ABTS^{•+} and DPPH[•] radicals scavenging activities of *Smilax excelsa* L. shoot extracts

<i>Smilax excelsa</i> L. shoot extract	Concentration	DPPH [•] scavenging (%)	ABTS ^{•+} scavenging (%)
Methanol	25 µg/ml	88.99 ±1.27 ^a	97.19 ±0.41 ^b
Ethanol		47.51 ±1.55 ^c	98.70 ±0.09 ^a
Pure water		62.56 ±1.19 ^b	92.21 ±1.09 ^c
Methanol	50 µg/ml	92.94 ±0.36 ^a	98.54 ±0.09 ^a
Ethanol		62.15 ±1.39 ^b	96.68 ±0.59 ^a
Pure water		42.94 ±2.80 ^c	86.91 ±2.06 ^b
Methanol	100 µg/ml	90.66 ±1.24 ^a	99.63 ±0.09 ^a
Ethanol		90.40 ±0.36 ^a	99.53 ±0.16 ^a
Pure water		25.49 ±8.95 ^b	91.84 ±0.33 ^b
Methanol	150 µg/ml	88.84 ±0.77 ^a	98.55 ±0.09 ^a
Ethanol		89.68 ±0.76 ^a	98.65 ±0.09 ^a
Pure water		44.03 ±3.12 ^b	81.98 ±0.55 ^b
Methanol	200 µg/ml	82.19 ±0.24 ^b	98.18 ±0.09 ^a
Ethanol		83.59 ±0.36 ^a	98.44 ±0.16 ^a
Pure water		45.48 ±0.62 ^c	95.64 ±0.46 ^b
Methanol	250 µg/ml	81.26 ±0.23 ^a	96.73 ±0.54 ^a
Ethanol		82.66 ±0.24 ^a	96.99 ±0.09 ^a
Pure water		58.67 ±2.00 ^b	92.63 ±0.32 ^b
Average %	<i>Smilax excelsa</i> L. (methanol)	87.48	98.14
	<i>Smilax excelsa</i> L. (ethanol)	76	98.16
	<i>Smilax excelsa</i> L. (pure water)	46.53	90.20

were determined to be methanol (54.79%) and ethanol (72.54%). However, it was not evaluated because the pure water values were higher than the control group's values (Tab. 3). The hydroxyl radical (OH) is the most reactive of the oxygen radicals and it may harm biomolecules including proteins, nucleic acids, and lipids [Ozsoy et al. 2008]. Ozsoy et al. [2008] reported that ethyl acetate and ethanol extracts of *Smilax excelsa* have high OH radical cleaning properties, but the OH radical cleaning potential of their aqueous extract is low. In this study, it was determined that ethanol and methanol extracts of *Smilax excelsa* have a high OH radical cleaning potential, but pure aqueous extract does not have an OH radical cleaning potential.

Phytochemical composition. The total protein (TPR), total phenolic compounds (TPC), and total proanthocyanidin (TP) contents of *Smilax excelsa* L. shoot extracts are summarized in Table 4. *Smilax excelsa* methanol, ethanol, and pure water extracts of TPC amounts were 4939.16, 4957.57, and 4708.34 µg GAE/g extract, respectively; TP amounts were 4.42,

5.88, and 55.39 mg CE/g, extract respectively; TPR amount was 85.91mg BSA/g extract. According to the literature review, it was found that there is not enough information about the protein content of *Smilax excelsa*. In a previous study, information on the protein content in the shoots of this plant was shared. In this study, it was reported that the shoots had a protein content of 7.28% [Özbucak et al. 2007]. The current study investigated the protein content of this plant using a different method, and it was determined that the protein content was 85.91 mg/g. It has been reported that the protein content of leafy vegetables such as spinach, lettuce, cabbage and arugula is 26, 12, 12 and 36 mg/g, respectively [Demir et al. 2020]. Compared with these plants, it can be said that *Smilax excelsa* is an excellent source of plant protein. Ozsoy et al. [2008] found that the extracts of this plant prepared using different solvents have a phenolic content equivalent to GAE ranging from 35.7–8.8 mg/g. According to Efe et al. [2019] reported that the fruit extract of this plant has a phenolic content equivalent to 11.9 mg GAE. According to

Miser-Salihoglu et al. [2010] found that the aqueous extract of this plant has a phenolic content equivalent to 645.3 µg/ml GAE. It has been illustrated that extracts of *Smilax excelsa* in different solvents have a flavonoid content equivalent to catechin ranging from 28.7–22.9 mg/g [Ozsoy et al. 2008]. According to Efe et al. [2019] reported that the fruit extract of this plant had a flavonoid content equivalent to 0.79 mg QE. The leaves of this plant have an anthocyanin level of 0.32 mg/g, according to Ozsoy et al. [2008]. In the current study, it was determined that the especially aqueous extract of this plant has dense anthocyanin content.

Table 5 summarizes the phenolic acid concentration of *Smilax excelsa* L. shoot extracts. The phenolic acid amounts of *Smilax excelsa* were gallic acid (117.33 µg/g), vanillic acid (33.89 µg/g), caffeic acid (4.55 µg/g), ferulic acid (93.78 µg/g) rosmarinic acid (0.33 µg/g), and hydrocinnamic acid (0.33 µg/g). According to the literature review, it has been determined that there is no information about the phenolic acid content of this plant. In the present study, it was determined that *Smilax excelsa* contains gallic acid, vanillic acid, caffeic acid, ferulic acid, rosmarinic acid and hydrocinnamic acid. In a previous study, it was determined that *Smilax sebeana* contains phenolic com-

Table 2. Metal chelation activity of *Smilax excelsa* L. shoot extract

<i>Smilax excelsa</i> L. shoot extract	Concentration	Metal chelation activity (%)
Methanol	75 µg/ml	85.11 ±1.36 ^a
Ethanol		85.10 ±0.54 ^a
Pure water		57.16 ±0.51 ^b
Methanol	100 µg/ml	89.41 ±0.16 ^b
Ethanol		93.63 ±2.24 ^a
Pure water		50.25 ±0.67 ^c
	<i>Smilax excelsa</i> L. (methanol)	87.26
Average %	<i>Smilax excelsa</i> L. (ethanol)	89.36
	<i>Smilax excelsa</i> L. (pure water)	53.70

Table 3. Hydroxyl radical (OH[•]) radicals scavenging activities of *Smilax excelsa* L. shoot extracts

Concentration 100 µg/ml	OH [•] scavenging (%)
<i>Smilax excelsa</i> L. (methanol)	54.79 ±14.65
<i>Smilax excelsa</i> L. (ethanol)	72.54 ±7.07
<i>Smilax excelsa</i> L. (pure water)	none

Table 4. Total protein, total phenolic, and total proanthocyanidin contents of *Smilax excelsa* L. shoot extracts

<i>Smilax excelsa</i> L. shoot extracts	Total protein (mg BSA/g)	Total phenolic (µg GAE/g)	Total proanthocyanidin (mg CE/g)
Methanol	–	4939.16 ±939.74	4.42 ±1.05
Ethanol	–	4957.57 ±331.46	5.88 ±2.05
Pure water	85.91 ±4.57	4708.34 ±1213.65	55.39 ±13.57

Total proanthocyanidin content was measured in milligrams of catechin equivalent per gram of extract, while total phenolic content was measured in milligrams of gallic acid equivalent per gram of extract. Total protein is measured in milligrams of BSA per gram of extract

Table 5. Vitamins, phytosterols, fatty acids and phenolic acids content and composition in *Smilax excelsa* L. shoot extracts

Vitamin and phytosterols	($\mu\text{g/g}$)
δ -Tocopherol	0.04 \pm 0.02
Vitamin D ₂	0.16 \pm 0.15
α -Tocopherol	0.13 \pm 0.09
Ergosterol	0.34 \pm 0.14
Vitamin K ₁	0.02 \pm 0.00
Sitosterol	0.76 \pm 0.12
β -sitosterol	6.43 \pm 3.11
Vitamin K ₂	0.02 \pm 0.00
Retinol	0.01 \pm 0.01
Vitamin D ₁	not detected
Fatty acids (FA)	(%)
Myristic acid (C14:0)	0.47 \pm 0.07
Myristoleic acid (C14:1)	not detected
Palmitic acid (C16:0)	20.52 \pm 1.12
Palmitoleic acid (C16:1n7)	1.78 \pm 0.62
Margaric acid (C17:0)	0.79 \pm 0.11
Stearic acid (C18:0)	4.95 \pm 0.28
Oleic acid (C18:1)	4.74 \pm 0.90
Linoleic acid (C18:2)	20.99 \pm 1.47
γ -linolenic acid (C18:3, n-6)	2.26 \pm 0.36
Alpha-linolenic acid (C18:3, n-3)	34.29 \pm 2.26
Gadoleic acid (C20:1)	0.87 \pm 0.30
Eicosapentaenoic (EPA) acid (C20:5 n-3)	1.14 \pm 0.16
Docosapentaenoic acid (C22:5 n-6)	4.23 \pm 0.38
Heptadecanoic acid (C17:1)	0.27 \pm 0.24
Lignoceric acid (C24:0)	not detected
Docosahexaenoic acid (C22:6)	2.79 \pm 2.25
Behenic acid (C22:0)	1.11 \pm 0.00
Trichosanoic acid (C23:0)	not detected
Nervonic acid (C24:1)	not detected
Phenolic acids	($\mu\text{g/g}$)
Gallic acid	117.33 \pm 132.20
Vanillic acid	33.89 \pm 12.82
Caffeic acid	4.55 \pm 4.22
Ferulic acid	93.78 \pm 88.69
Rosmarinic acid	0.33 \pm 0.00
Hydrocinnamic acid	0.33 \pm 0.00

pounds such as chlorogenic acid, 4-formylphenol, epicatechin, cinchonain IIa, cinchonain Ia and cinchonain Ib [Ao et al. 2011]. Khaligh et al. [2016] isolated and explained three phenol compounds trans-resveratrol, 5-O-caffeoylshikimic acid and 6-O-caffeoyl- β -D-fructofuranosyl-(2 \rightarrow 1)- α -D-glucopyranoside from ethyl acetate extract of *S. excelsa*. In previous studies, it has been stated that this plant is an important source of natural antioxidants [Ozsoy et al. 2008, Efe et al. 2019]. In this study, it can be emphasized that the shoots of this plant have potential as an important source of natural antioxidants.

Table 5 shows the phytosterols, lipophylic vitamins, and fatty acids composition of *Smilax excelsa* shoot extracts. *Smilax excelsa* has α -tocopherol (0.13 μ g/g), δ -tocopherol (0.04 μ g/g), vitamin K₁ (0.02 μ g/g), and vitamin D₂ (0.16 μ g/g) lipophylic vitamin levels, and phytosterol levels of ergosterol (0.34 μ g/g), sitosterol (0.76 μ g/g), and β -sitosterol (6.43 μ g/g).

Smilax excelsa shoots contained 0.47% myristic acid (C14:0), 20.52% palmitic acid (16:0), 1.78% palmitoleic acid (C16:1n7), 0.79% margaric acid (C17:0), 0.27% heptadecanoic acid (C17:1), 4.95% stearic acid (18:0), 4.74% oleic acid (18:1), 20.99% linoleic acid (18:2), 2.26% γ -linolenic acid (C18:3, n-6), 34.29% alpha-linolenic acid (C18:3, n-3), 1.14% eicosapentaenoic (EPA) acid (C20:5 n-3), 4.23% docosapentaenoic acid (C22:5 n-6), 0.87% gadoleic acid (C20:1), 2.79% docosahexaenoic acid (C22:6), 1.11% behenic acid (C22:0). It has been established that there is no literature with information on the fatty acid composition of this plant, the content of fat-soluble vitamins and phytosterols. In the current study, it is seen that *Smilax excelsa* shoots are an important source of fatty acids such as palmitic acid, stearic acid, oleic acid, linoleic acid, alpha-linolenic acid and docosapentaenoic acid. The content of polyunsaturated fatty acids, such as alpha-linolenic acid, has been shown to be particularly high. It has been found that fat-soluble vitamins such as *Smilax excelsa* shoots δ -tocopherol, vitamin D₂, α -tocopherol, vitamin K₁, vitamin K₂ and retinol are present in different proportions. In addition, it has been found that this plant contains different amounts of phytosterols such as ergosterol, β -sitosterol and sitosterol.

CONCLUSIONS

In this study, important information has been obtained regarding the antiradical potential and phytochemical composition of *Smilax excelsa* shoot extracts prepared using methanol ethanol and pure water. In the current study, the first information about the content of phenolic acids, fatty acids, fat-soluble vitamins, phytosterols and proteins of this plant was obtained.

In this study, it was found that *Smilax excelsa* shoots contains fatty acids such as palmitic acid, stearic acid, oleic acid, linoleic acid, alpha-linolenic acid and docosapentaenoic acid. It has been determined that the level of alpha-linolenic acid, which is one of the polyunsaturated fatty acids, is quite high. It has been found that fat-soluble vitamins such as *Smilax excelsa* shoots δ -tocopherol, vitamin D₂, α -tocopherol, vitamin K₁, vitamin K₂ and retinol are found in different proportions. It has also been established that this plant contains different amounts of phytosterols such as ergosterol, β -sitosterol and sitosterol. In addition, it has been determined that this plant has six phenolic acids such as gallic acid, vanillic acid, caffeic acid, ferulic acid, rosmarinic acid and hydrocinnamic acid. It is observed that gallic acid, vanillin acid and ferulic acid are the dominant phenolic acids. It has been determined that the proanthocyanidin content is quite high, especially in aqueous extract.

Furthermore, the antioxidant ability of this plant was assessed utilizing antiradical assays such as DPPH, ABTS, OH, and metal chelation. According to the results, it was determined that this plant has significant antioxidant potential. Also especially compared to pure aqueous extract, methyl alcohol and ethyl alcohol extracts of high DPPH, ABTS, OH radical scavenging potential, and moreover showed high chelating feature. As a result, according to the data obtained, we can state that *Smilax excelsa* L. shoot has significant potential in terms of food and health.

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