JAP&T TPA-Timişoara

Available online at http://journal-of-agroalimentary.ro

Journal of Agroalimentary Processes and Technologies 2020, *26*(4), 347-352

Journal of Agroalimentary Processes and Technologies

The effect of sowing dates on total phenol, antioxidant activity and phenolic compounds of orache garden leaves harvested from plants growing garden house and field conditions

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Abstract

Total phenol contents of leaves of garden orache (*A.hortensis* L.) growing in greenhouse and field conditions changed between 130.14 mgGAE/100g and 281.11 mgGAE/100g to371.94 mgGAE/100g and 477.50 mgGAE/100g, respectively. In addition, while antioxidant activity values of leaves of garden orache growing in greenhouse are determined between 8.37% and 30.40%, antioxidant activity values of garden orach leaves growing in field changed between 71.86% and 82.97%. Gallic acid, 3,4-dihydroxy benzoic acid, (+)-catechin and 1,2-dihydroxybenzene were the key phenolic constituents of garden orach leaves growing in garden house and field conditions. In all phenolic compounds studied, gallic acid was determined at the highest ratio, its value changed between 35.64 mg/100g (3rd harvest) and 284.0 mg/100g (1st harvest) for field and 132.81 mg/100g (1st harvest) and 218.0 mg/100g (4th harvest).garden orache leaves are rich in Ca, P, S, Mg, Na and K elements. Calcium contents of leaves harvested from greenhouse and field conditions changed between 12810.5 and 14787 mg/kg to 10623.8 and 15515.3 mg/kg, respectively. In addition, while K contents of leaves harvested from greenhouse vary between 46057.4 mg/kg and 57909.1 mg/kg, K contents of leaves harvested from field changed between 34678.2 and 42214.3 mg/kg.

Keywords: Garden orache (*A.hortensis* L.), sowing dates, leaves, total phenol, antioxidant activity, phenolic compounds, HPLC

1.Introduction

Atriplex hortensis L. Known as Garden Orache is a member of Chenopodaceae family, and used as an annual herb at the most place of World [1] . It is called as garden orach, mountain spinach, sea purslane, unluca and salt bush, and is considered to be one of the oldest wild edible plants, valued primariliy for its leaves, Usable parts of garden orache are the young stems and leaves. Leaves of garden orache can be consumed either fresh or boiled, separately or together with other vegetables [2-5]. Garden orach is rich in several nutrients such as protein (25.7%), total dietary fiber (12.1%), vitamin A and C. The leafy vegetables are a popular complement to a daily diet for their low energy content and presence of nutritionally important substances (vitamins of B-group and C, carotenoids and minerals). These plants represent also an important source of antioxidants such as polyphenols [6]. Garden orach has been used both as a potherb for traditional medicinal purposes and as a soil erosion control. In traditional medicine, it is used as tonic effect, helps in nutrition absorption, digestion and enhance the metabolism [5-9]. Garden orach is characterized by a high content of flavonoids, mineral components and amino acids [10,11]. Limited studies were conducted on bioproperties of garden orache (*Atriplex hortensis*) leaves. However, the aim of this study was to determine the effect of sowing on total phenol, antioxidant activity and phenolic compounds of orache garden leaves harvested from plants growing garden house and field conditions.

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2. Material and Methods

2.1.Growing Experimental

The research was carried out at the Selçuk University, Faculty of Agriculture in the greenhouse area and open air conditions in Konya-Turkey. Dibbling was carried out on 8 March 2017 in both greenhouse and outdoor area with 5x50 cm distances. In the study, cultural practices (weeding and thinning) were made on equal conditions and nothing was done except the irrigation. As the early harvesting occurred under the greenhouse conditions due to better grow, the outdoor area were slower to develop. Greenhose soil is in a loamy structure, the content of organic matter is medium, alkaline, low in salt level, excess lime. In addition, open land is clayey loamy structure, organic matter is low, alkaline, salt level is low, is over-calcified. The greenhouse and open field experiments were harvested for 15 days in 4 equal time periods. Due to the climatic conditions, the first harvest of the greenhouse and open air was carried out on April 14th and May 14 th, respectively. According to this situation, while the last harvest in the greenhouse is completed on 29 May, it was completed on 12 June for open air conditions. The leaves harvested were dried in the shade for analysis.

2.2. Methods

Sample extraction: For phenolic compounds and antioxidant activity, leaves of garden orache were dried, ground and extracted according to Talhaoui et al. [12]. The sample (0.5 g) was added to 10 ml of methanol: water mixture (80/20, v/v). The mixture was sonicated for 30 min, followed by centrifugation at 6000 rpm for 10 min. These steps were repeated twice and the supernatants were collected. The extract was concentrated at 37°C under the vacuum. After its volume was completed to 25 ml by methanol, it was filtered with 0.45 µm filter.

Total phenolic content: The total phenolic content of garden orache leave extracts was determined by the Folin-Ciocalteu (FC) reagent according to Yoo et al. [13]. FC (1 ml) was added to the samples and mixed for 5 min. Following the addition of 10 ml of Na_2CO_3, the solution in the tubes was mixed again, and the final volume was adjusted to 25 ml with deionized water.

At the end of 1 h, the total phenol content was determined at a wavelength of 750 nm in a spectrophotometer with a calibration curve made using gallic acid (0–200 mg/ml) as the standard. The results are given as mg gallic acid equivalent (GAE)/100 g of dry weight.

Antioxidant activity: The free radical scavenging activities of garden orache leave extracts were determined using DPPH (1,1-diphenyl-2-picrylhydrazyl) according to Lee et al. [14]. The extract was mixed with 2 mL methanolic solution of DPPH. After shaking vigorously, it was stored at room temperature for 30 min. The absorbance was recorded at 517 nm by using a spectrophotometer.

Determination of phenolic compounds: Phenolic compounds of garden orache extracts were determined using a Shimadzu-HPLC equipped with a PDA detector and an Inertsil ODS-3 (5 µm; 4.6 × 250 mm) column. Gradient elution was performed for separation and a mixture of 0.05% acetic acid in water (A) and acetonitrile (B) as the mobile phase was used. The flow rate of the mobile phase was 1 ml/min at 30°C, and the injection volume was 20 µl. The peaks were recorded at 280 and 330 nm using a PDA detector. The total running time per sample was 60 min. Phenolic compounds were determined according to the retention time and absorption spectra of peaks of standard compounds. The total area under peak was used to quantify the each phenolics.

2.3. Statistical Analysis

A complete randomized split plot block design was used, and analysis of variance (ANOVA) was performed by using JMP version 9.0 (SAS Inst. Inc., Cary, N.C.U.S.A). All analyses were carried out three times and the results are mean±standard deviation (MSTAT C) of independentgarden orache leave samples [15].

3.Results and Discussion

Total phenol and antioxidant activity values of leaves of garden orach (*Atriplex hort*ensis) harvested at certain intervals and growing in greenhouse and field conditions are given in Table 1. While total phenol contents of leaves of garden orach growing in greenhouse change between 130.14 mgGAE/100g and 281.11 mgGAE/100g, total phenol contents of garden orach leaves growing in field varied between 371.94 mgGAE/100g and 477.50 mgGAE/100g.

In addition, antioxidant activity values of leaves of garden orach growing in greenhouse are determined between 8.37% and 30.40%, antioxidant activity values of garden orach leaves growing in field ranged between 71.86% to 82.97%.

Table 1. Total phenol and antioxidant activity values of garden orache leaves

	Total phenol (mgGAE/100g)			**Antioxidant activity (%)**		
Greenhouse 1	190.56	±	0.02*bc	18.95	±	0.01c
Greenhouse 2	130.14	±	0.02d**	8.37	±	0.00d
Greenhouse 3	281.11	±	0.05a	30.40	±	0.01a
Greenhouse 4	219.86	±	0.01b	25.64	±	0.00b
Field 1	428.06	±	0.01b	82.97	±	0.01a
Field 2	477.50	±	0.01a	82.88	±	0.01a
Field 3	371.94	±	0.00d	71.86	±	0.02c
Field 4	382.50	±	0.01c	76.33	±	0.02b

*mean±standard deviation;** Values in each column with different letters are significantly different ($p<0.05$)

The highest total phenol contents (281.11 and 477.50 mgGAE/100g) of garden orach leaves growing in green house and field conditions were found at the 3rd harvest and 2nd harvest of plant, respectively. But, the highest antioxidant activity values of leaves harvested from greenhouse and field were determined at 3rd harvest and 1stharvest periods, respectively. The total phenol and antioxidant values of leaves harvested from both greenhouse and field showed fluctuations depending on harvest time. These fluctuations may possibly be attributed to leaves and stem forms, nutrient accumulation, growing conditions of plant, and analytic conditions. But, generally, both total phenol and antioxidant activity values of garden orach leaves harvested from field were found higher then those of results of leaves harvested from greenhouse. This may have been due to the synthesis of phenolic compounds in large quantities, possibly by direct use of daylight in field conditions. Total phenol and antioxidant activity of garden orache leaves harvested from plants grown at the third date of sowing was significantly higher in comparison with the other sowing dates, and were found low in field conditions. A statistically significant difference was found between total phenol content and antioxidant activities of orchard leaves depending on harvesting time ($p<0.05$). In the present study [16], the results showed inhibition percentage and IC_{50} values ranges from 14.3 to 98.6 and 2.0 to 9.9 respectively. Among the four different concentrations of standard Trolox (100, 200, 300, 400 µg/ml) used in the study, showed 72.70%, 78.04%, 83.83% and 91.72 % scavenging activity respectively, where highest scavenging activity of trolox was recorded 91.72% at 400 µg/ml concentration. Among the plants studied the highest ABTS free radical scavenging activity in terms of inhibition percentage was observed in *A. hortesnsis* ethanol extract i.e. 98.6 % µg/ml with IC_{50} value 2.0 µg/ml. The lowest inhibition percentage was observed in C. album chloroform extract i.e. 29.3 µg/ml with IC_{50} value 7.3 µg/ml [16]. Grzeszczuk et al. [3] reported that total phenol and antioxidant activity values of garden orache leaves changed between 181.3 and 194.5 mgGAE/100g and 23.61 and 31.13% depending on harvest times, respectively. Fernandez et al. [17] determined on average 1500 mg/100 g (dw) polyphenols in leaves of *Atriplex lampa*. The leaves methanolic extract of *Atriplex halimus* contained higher levels of total phenolic (10.12 mgGAE/g (dw)) than stems methanolic extract (3.77 mgGAE/g (dw)) [18].

The phenolic compounds of garden orach leaves harvested from greenhouse and field at certain intervals are presented in Table 2. Gallic acid, 3,4-dihydroxy benzoic acid, (+)-catechin and 1,2-dihydroxybenzene were the key phenolic constituents of garden orach leaves growing in garden house and field conditions, and followed by syringic acid, isorhamnetin, quercetin, kaempferol and apigenin 7 glucoside. In all phenolic compounds studied, gallic acid was determined at the highest ratio, its value changed between 35.64 mg/100g (3rd harvest) and 284.0 mg/100g (1st harvest) for field and 132.81 mg/100g (1st harvest) and 218.0 mg/100g (4th harvest).

Table 2. Phenolic compounds of garden orache leaves (mg/100g)

	Field 1	Field 2	Field 3	Field 4	Greenhouse 1	Greenhouse 2	Greenhouse 3	Greenhouse 4
Gallic Acid	284.04 ± 1.78*a	171.15 ± 28.52c	35.64 ± 1.53d	187.50 ± 6.18b	132.81 ± 2.12d	139.43 ± 1.17c	180.23 ± 2.71b	218.09 ± 6.40a
3,4-Dihydroxybenzoic Acid	113.61 ± 9.29a**	28.27 ± 4.71c	21.44 ± 1.32d	37.85 ± 0.51b	7.53 ± 0.98d	8.84 ± 0.89c	13.24 ± 0.63b	15.05 ± 0.59a
(+)-Catechin	6.12 ± 0.52d	48.96 ± 8.16b	146.69 ± 6.33a	30.05 ± 0.00c	2.66 ± 0.15c	1.40 ± 0.06d	2.88 ± 0.29b	141.23 ± 8.57a
1,2-Dihydroxybenzene	35.20 ± 2.51c	23.34 ± 3.89d	43.40 ± 4.06b	85.93 ± 6.44a	2.21 ± 0.25c	0.47 ± 0.00d	6.03 ± 0.59b	95.87 ± 1.43a
Syringic Acid	2.60 ± 0.37d	10.40 ± 1.73c	11.81 ± 0.47b	31.35 ± 2.97a	0.61 ± 0.06d	1.46 ± 0.05c	2.59 ± 0.19b	4.30 ± 0.46a
Caffeic Acid	2.00 ± 0.15d	9.92 ± 1.65b	2.94 ± 0.27c	24.85 ± 0.98a	2.19 ± 0.18b	1.96 ± 0.16c	1.47 ± 0.21c	6.55 ± 0.78a
Rutin trihydrate	2.52 ± 0.13c	4.32 ± 0.72a	2.65 ± 0.05c	3.48 ± 0.10b	2.04 ± 0.25a	0.75 ± 0.11c	1.11 ± 0.08b	0.84 ± 0.04c
p-Coumaric Acid	0.43 ± 0.02a	0.27 ± 0.05c	0.18 ± 0.01d	0.37 ± 0.02b	0.72 ± 0.12a	0.21 ± 0.03b	0.12 ± 0.02c	0.22 ± 0.02b
trans-Ferulic Acid	0.63 ± 0.07c	3.49 ± 0.58b	3.61 ± 0.31b	10.23 ± 0.09a	2.32 ± 0.36a	0.62 ± 0.03b	0.96 ± 0.08b	0.97 ± 0.07b
Apigenin 7 glucoside	2.68 ± 0.16d	13.55 ± 2.26b	7.94 ± 0.81c	51.86 ± 0.85a	1.44 ± 0.18b	2.10 ± 0.04a	1.09 ± 0.03b	1.99 ± 0.04b
Resveratrol	2.09 ± 0.21b	2.39 ± 0.40b	3.01 ± 0.17a	2.48 ± 0.08b	0.70 ± 0.05b	0.79 ± 0.06b	0.97 ± 0.02a	0.73 ± 0.04b
Quercetin	3.98 ± 0.31c	12.07 ± 2.01a	4.50 ± 0.48b	1.34 ± 0.04d	1.82 ± 0.01b	1.32 ± 0.04b	5.22 ± 0.50a	1.15 ± 0.07b
trans-Cinnamic Acid	0.35 ± 0.02d	0.48 ± 0.08c	0.94 ± 0.11b	1.35 ± 0.19a	0.30 ± 0.03c	0.23 ± 0.03d	0.38 ± 0.02b	0.41 ± 0.07a
Naringenin	2.29 ± 0.11a	1.31 ± 0.22b	2.88 ± 0.11a	2.92 ± 0.05a	1.76 ± 0.14a	0.91 ± 0.06c	1.24 ± 0.18b	0.76 ± 0.00d
Kaempferol	4.24 ± 0.14a	2.23 ± 0.37b	2.94 ± 0.51b	1.83 ± 0.06c	1.65 ± 0.16c	1.60 ± 0.08c	2.19 ± 0.15a	1.72 ± 0.12b
Isorhamnetin	4.79 ± 0.28a	2.18 ± 0.36b	4.81 ± 0.47a	2.19 ± 0.19b	1.39 ± 0.10c	2.22 ± 0.04a	1.74 ± 0.16b	1.04 ± 0.08d

*mean±standard deviation;** Values in each row with different letters are significantly different ($p<0.05$)

Table 3. Macro and micro elements of garden orache leaves (mg/kg)

Growing area	Ca	P	S	Mg	Na	K	Cu	Fe	Mn	Zn	B
Greenhouse 1	12810.5 ±514.09*c	8070.9 ±323.33a	3659.8 ±181.20d	7108.2 ±29.26c	13358.0 ±95.51cd	57909.1 ±108.21a	19.6 ±0.46c	145.6 ±9.86b	41.9 ±1.51d	54.2 ±2.30a	21.2 ±1.16d
Greenhouse 2	15660.6 ±99.05a**	5861.6 ±80.68b	3877.3 ±56.56c	7085.1 ±308.27d	13706.7 ±776.35c	51496.4 ±372.68c	19.8 ±2.48c	128.6 ±21.37c	44.7 ±0.45c	49.3 ±0.95b	28.8 ±1.42c
Greenhouse 3	14753.0 ±26.14b	4587.7 ±154.43c	4456.4 ±7.09b	7310.9 ±348.05b	14334.0 ±909.38b	52269.1 ±106.59b	91.2 ±0.61b	96.7 ±2.25d	59.3 ±0.61b	49.2 ±0.41b	31.2 ±0.35b
Greenhouse 4	14787.1 ±80.56b	4524.2 ±242.49c	4830.0 ±61.80a	7572.7 ±83.42a	14625.2 ±283.36a	46057.4 ±634.31d	92.3 ±1.50a	152.3 ±5.97a	91.8 ±0.91a	49.0 ±1.09b	38.0 ±1.61a
Field 1	11634.1 ±183.19c	5307.4 ±58.39a	4126.1 ±58.75d	6583.4 ±550.40b	12484.3 ±122.64b	41994.3 ±162.63b	19.8 ±1.71a	119.2 ±7.77b	68.1 ±0.40d	52.7 ±1.04b	28.7 ±1.79c
Field 2	15515.3 ±153.16a	3906.4 ±21.75c	4421.3 ±1.40c	7232.5 ±207.40a	13611.0 ±210.49a	42214.3 ±426.79a	18.0 ±1.67ab	143.8 ±2.25a	73.5 ±0.20c	49.6 ±1.32c	27.1 ±0.36cd
Field 3	12741.9 ±649.75b	3733.0 ±115.41d	6998.1 ±256.43a	6449.1 ±244.73c	11856.2 ±532.06c	38884.9 ±897.74c	17.4 ±1.62b	86.3 ±11.36c	100.1 ±3.65b	55.2 ±3.48a	39.2 ±0.32b
Field 4	10623.8 ±152.12d	4137.4 ±72.71b	5701.8 ±127.14b	6364.4 ±202.58cd	12020.9 ±355.93bc	34678.2 ±737.60d	15.8 ±0.35c	72.2 ±3.52cd	108.5 ±2.01a	38.7 ±0.80d	41.1 ±0.41a

*mean±standard deviation;** Values in each column with different letters are significantly different ($p<0.05$

Also, while 3,4-dihydroxybenzoic acid contents of leave extracts harvested from field change between 21.44 mg/100g (3rd harvest) and 113.61 mg/100g (1st harvest), 3,4-dihydroxybenzoic acid contents of leaves harvested from greenhouse varied between 7.53 mg/100g (1st harvest) and 15.05 mg/100g (4th harvest). In addition, (+)-catechin contents of samples obtained from field vary between 6.12 mg/100g (1st harvest) and 146.69 mg/100g (3rd harvest), (+)-catechin contents of leave harvested from greenhouse changed between 1.40 mg/100g (2nd harvest) and 141.23 mg/100g (4th harvest). 1,2-dihydroxybenzene contents of leaves harvested from field changed between 23.34 mg/100g (2nd harvest) and 85.93 mg/100g (4th harvest). The highest 1,2-dihydroxybenzene (95.87 mg/100g) was found in leave harvested at 4th harvest period in greenhouse. Also, the highest apigenin 7 glucoside content of leaves harvested from both gardenhose and field conditions was found at 4th harvest in field conditions (51.86 mg/100g). Generally, phenolic constituents of garden orach leaves harvested from field were found higher than those of results of leaves harvested from greehouse. Phenolic constituents of garden orach leaves harvested from field and greenhouse showed fluctuations depending on harvest time. But, gallic acid and 3,4-dihydroxy benzoic acid contents of garden orah leaves harvested from greenhouse gradually increased. Phenolic compounds of leaves showed differences depending on growing conditions, sowing and harvest times. A statistically significant difference was found between phenolic compounds of orchard leaves depending on harvesting time ($p<0.05$). But, trans-ferulic acid contents showed similar in Greenhose 2 and Greenhouse 4 harvesting periods. Total quercetin and rutin contents of *Atriplex hortensis* and *A.patula* leaves were determined as 4240 mg/kg and 2180 mg/kg, respectively [19]. For comparison, Hertog et al. [20] reported that the total quercetin content in endive and lettuce was 1.3 and 14 mg kg–1 of fresh matter, respectively. According to Trichopoulou et al. [21] the total quercetin content in traditional Greek wild greens ranged from 104 mg/kg of dry matter (*Allium schoenoprasm*) to 862 mg/kg of dry

matter (*Rumex obtusifolius*). Rutin was presented relatively seldom in our experimental plants. It was found only in *Chenopodium album* L. (868 mg/kg of dry matter) and in *Chenopodium polyspermum* L. (244 mg kg–1 of dry matter). The results showed that, the total phenolic contents in the selected plants species for the study varied considerably and ranged from 3.71 to 32.33 mg GAE/g. The highest phenolic content was observed in *A. hortesnsis* leaf Methanolic extract i.e. 32.33 mg GAE/g and lowest phenolic content was observed in *C. album* ethyl acetate extract i.e. 3.71 mg GAE/g [16]. It is also reported that phenolics compounds and flavonoids are natural products which have been shown to possess various biological properties related to antioxidant mechanisms [22].

Macro and micro element contents of garden orache leaves harvested in greenhouse and field conditions are shown in Table 3. As seen in Table 3, garden orache leaves are rich in Ca, P, S, Mg, Na and K elements. While Ca contents of leaves harvested from greenhouse change between 12810.5 and 14787 mg/kg, Ca contents of leaves harveted from field varied between 10623.8 and 15515.3 mg/kg. In addition, while K contents of leaves harvested from greenhouse vary between 46057.4 mg/kg and 57909.1 mg/kg, K contents of leaves harvested from field changed between 34678.2 and 42214.3 mg/kg. Also, P contents of garden orache growing in greenhouse changed between 4524.2 and 8070.9 mg/kg, P contents of leaves harvested from field ranged from 3733.0 and 5307.4 mg/kg. Generally, macroelement contents of leaves harvested from greenhouse were found high compared to results of leaves harvested from field. Among microelements, Fe was determined as the highest element. While Fe contents of garden orache leaves harvested from greenhouse are determined between 96.7 and 152.3 mg/kg, Fe contents of leaves harvested from field changed between 72.2 and 143.8 mg/kg. In addition, Mn contents of leaves growing in field were found partly higher compared to results of garden orache leaves growing in greenhouse. A statistically significant difference was found between total phenol content and antioxidant activities of orchard leaves depending on harvesting time ($p<0.05$). But, there was no statistically significant difference between Zn contents in orachenin 2-4 harvesting period in greenhouse conditions. Leafy vegetables, among the other vegetable species, contain also a very high content of iron, magnesium and selenium, among the highest recorded [6]. Van Niekerk et al. [23] reported that *Atriplex canescens* and *Atriplex halimus* leaves contained 20.6 and 21.5 g/kg Ca, 1.9 and 1.92 P, 16.1 g/kg and 20.3 g/kg Mg, 39.0 and 22.0 µg/kg Se, 110 and 103 mg/kg Zn and 170 and 395 mg/kg in Hatfield location. *Atriplex* spp leaves contained 0.21-5.57% Na, 2.48-4.95% K, 1.12-2.48% Ca, 0.18-0.28% P, 0.66-0.84% Mg, 250-485 µg/Fe, 54-70 µg/g Zn, 20-26µ g/g Cu and 74-89µg/g Mn [24]. The macro- and micro element contents of leaves showed differences depending on sowing and harvest times, and growing conditions. The high mineral content of the leaves in the greenhouse conditions is probably due to the temperature difference between day and night. For this reason, the plant may be sourced by taking more elements from the soil due to more respiration.

4.Conclusion

1-Total phenol and antioxidant activity of garden orache leaves harvested from plants grown at the third date of sowing was significantly higher in comparison with the other sowing dates.

2-Gallic acid, 3,4-dihydroxy benzoic acid, (+)-catechin and 1,2-dihydroxybenzene were the key phenolic constituents of garden orach leaves growing in garden house and field conditions, and followed by syringic acid, isorhamnetin, quercetin, kaempferol and apigenin 7 glucoside.

3-Phenolic compounds of leaves showed differences depending on growing conditions, sowing and harvest times.

4-Garden orache leaves are rich in Ca, P, S, Mg, Na and K elements.

5-The total phenol, antioxidant activity, phenolic compounds, macro- and micro element contents of leaves showed differences depending on sowing and harvest times, and growing conditions.

Compliance with Ethics Requirements. Authors declare that they respect the journal's ethics requirements. Authors declare that they have no conflict of interest and all procedures involving human or animal subjects (if exist) respect the specific regulation and standards.

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