Jerash for Research and Studies Journal مجلة جرش للبحوث والدراسات

Volume 22 | Issue 1 Article 24

2021

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Cover Page Footnote

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Jerash for Research and Studies **Vol. 22 No 1, 2021, pp 615-627**

Determination of the Total Phenolic Content, Total Antioxidant Capacity and Radical Scavenging Activity of Jordanian Royal Jelly and Honey Samples

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Received Date: 6/7/2020 **Acceptance Date: 29/9/2020**

Abstract

Honey and royal jelly are two functional bee-hive products with antioxidant activity.This study aimed at investigating the total phenolic content, antioxidant capacity and radical scavenging activity of royal jelly, multiflora honey and citrus honey. Royal jelly, multiflora honey, and citrus honey samples were analyzed for the total phenolic content using Folin-Ciocalteau method, for total antioxidant capacity using FRAP assay. Also, DPPH assay was used for assessing radical scavenging activity. The results revealed that the total phenolic contents, the total antioxidant capacities, as well as DPPH scavenging activities of RJ were higher than those of Multifloral and citrus honey,. Furthermore**,** there were positive correlations between total antioxidant capacity and between DPPH and total phenolic content in all samples. It is concluded that royal jelly is of superior total phenolic content and antioxidant capacity.

Keywords: Honey, Royal jelly, Total phenolic content, Antioxidant capacity; FRAP, Radical scavenging activity; DPPH.

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تحديد المحتوى الكلي للفينولات والقدرة المضادة للتأكسد والقدرة على كبح الجذور الحرة في غذاء الملكات والعسل المنتج في الأردن

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ملخص

يعتبر العسل وغذاء الملكات من من منتجات خلية النحل ذات الخصائص الوظيفية والنشاط المضاد للتأكسد.

هدفت هذه الدراسة إلى تحديد المحتوى الكلي للفينولات والقوة المضادة للتأكسد والقدرة الكابحة للجذور الحرة في الغذاء الملكي ونوعين من العسل هما العسل متعدد الأزهار وعسل الحمضيات. تم قياس المحتوى الكلي للفينولات والسعة المضادة للتأكسد والقدرة على كبح الجذور الحرة في عينات الغذاء الملكي والعسل باستعمال طريقة Folin-Ciocalteau وطريقة FRAP وطريقة DPPH على التوالي. بينت النتائج أن محتوى الفينولات الكلي و السعة المضادة للتأكسد والقوة الكابحة للجذور الحرة للغذاء الملكي أعلى منها للعسل متعدد الأزهار وعسل الحمضيات. كما بينت الدراسة أن هناك علاقة إيجابية بين المحتوى الكلي للفينولات وكل من قوة منع التأكسد والقوة الكابحة للجذور الحرة في الغذاء الملكي ونوعي العسل. يستنتج من هذه الدراسة أن الغذاء الملكي متميز في محتواه العالي من الفينولات الكلية والقدرة المانعة للتأكسد.

الكلمات المفتاحية: العسل، الغذاء الملكي، الفينولات الكلية، القوة المضادة للتأكسد، القدرة الكابحة للجذور الحرة.

1. Introduction:

Honey is a sweet and flavorful natural product prepared by honeybees from the nectar and other sugary substances of many plants (Perez et al., 2006). Honey is composed of different kinds of phytochemicals with high content of polyphenols and flavonoids that provide the antioxidant capacity of honey (Pyrzynska and Biesage, 2009).

Royal jelly (RJ) is a thick substance secreted by the cephalic glands of nurse bees and given to the honeybee larvae as the most important part of their diet

(Pavel et al., 2011). RJ was reported to have large number of pharmacological activities as: antioxidant activity, neurotrophic, hypo-cholesterolemic, hypoglycemic, hepato-protective, hypotensive and blood pressure regulatory, antitumor, antibiotic, anti-inflammatory, immunomodulatory and anti-allergic characteristics (Pavel *et al.,* 2011).

Honey and royal jelly are considered as functional foods because they contain phenolic compounds that have the ability to scavenge free radicals, thereby protecting cells and living tissues against oxidative stress (Uthurry *et al*., 2011). Their content of phenolic compounds is gathered by the bees from the nectar of plants and most often provides color and taste (Martos *et al*., 2008).

Phenolic compounds are the major components that provide the functional properties of foods, such as antioxidant capacity, antibacterial capacity, antiviral capacity, anti-inflammatory capacity, cardio-protective effects, and enzymatic browning prevention (Martos *et al.*, 2008).

The major components of honey are almost the same in all honey samples, while the chemical composition and physical properties of natural honeys are not exactly the same; they differ according to many factors such as: the plant species, floral source, climate, soil type, genetic factors, and environment (Montenegro and Mejias, 2013). These authors stated that the antioxidant strength of the honey depends on the color intensity of honey, where the bioactive phenolic compounds are higher in darker honeys and lower in lighter honeys.

The association between polyphenols content and antioxidant properties of Malaysian honeys were studied by Aljadi and Kamaruddin (2004). Two of the most common Malaysian honeys were studied in relation to their phenolic content, antioxidant power, and total radical scavenging activity. The data showed a significant correlation between the antioxidant activity of the honeys and their total phenolic contents.

Liu *et al.* (2008) reported that RJ collected 24 hours after the larval transfer has the highest antioxidant capacity because delaying harvest declines the antioxidant properties of RJ [8]**.** So, it is rational to suggest that the anti-oxidative capacity of RJ is reduced with age and might therefore be utilized as a quality parameter. This study was carried out to investigate the total phenolic content and the antioxidant capacity of royal jelly (RJ), multiflora honey (MFH) and citrus honey (CH) collected from Jordanian bee-hives.

2. Materials and Methods:

This research was approved by the Department of Nutrition and Food Technology Committee and the Faculty of Graduate Studies at the University of Jordan.

2.1. Measurement of Antioxidant Capacity and Total Phenolic Content of Honey and Royal Jelly

2.1.1. Antioxidant Capacity by Ferric Reducing Antioxidant Power Assay (FRAP):

The antioxidant capacity values of honey and royal jelly were estimated by a method developed by Benzie and Strain (1996). The method is based on the reduction of a ferric 2,4,6-tripyridyl-s-traizine complex (Fe^{3+}-TPTZ) to its ferrous, colored form (Fe^{2+}-TPTZ) in the presence of antioxidants. The FRAP reagent was prepared by the addition of 2.5 ml of a 10 mM TPTZ solution in 40 mM HCl, 2.5 ml of 20 mM $FeCI_3$ and 25 ml of 0.3 M acetate buffer, (pH 3.6).

A sample aliquot of 50 µl was mixed with 2.5 ml of the FRAP reagent and incubated at 37 ˚C for 4 min. Then the absorbance of the reaction mixture was measured by spectrophotometer (SL 150, ELICO, India) at 593 nm against the reagent blank containing distilled water. The standard curve (linear, $R^2 = 0.961$) was constructed with aqueous standard solutions of $FeSO_4$ $6H_2O$ (2- 5 mM) and used for the calibration curve. The results were expressed as the FRAP value in mM Fe [II]. The experiment was performed in triplicate.

2.1.2. DPPH Free Radical Scavenging Activities (%)

The free radical scavenging activity of honey samples was determined by the 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay as described by Chua *et al* (2013). Stable DPPH radical reaches the absorbance maximum at 517 nm and its color is purple. Adding an antioxidant results in the decrease of absorbance and changes purple color into yellow.

In brief, the DPPH solution (20mg/L) was prepared by dissolving 2mg of DPPH in 100 mL of methanol. Then 0.5 ml dose at different concentrations of sample (50, 100, 200, 300, 400, 500 µg/mL) was mixed with 0.5 mL DPPH and 2 mL of methanol, shaken vigorously by vortex, and incubated in dark place for 30 min at room temperature.

Absorbance was measured using spectrophotometer (SL 150, ELICO, India) at 517 nm against a blank. The DPPH scavenging activity was calculated using equation described by uthurry *et al.,* (2011). Ascorbic acid was used as a standard to determine DPPH inhibition (%) in different treatments.

DPPH radical scavenging activity (%) = ((Control absorbance – Sample absorbance) / Control absorbance) ×100

2.1.3. Determination of Total Phenolic Compound Content

The Folin-Ciocalteau reagent was used to determine total phenolic compound content in honey and royal jelly based on the method described by Singleton *et al.* (1998). In brief, 0.1 g of sample was dissolved in 0.8 ml of deionized water in volumetric flask followed by the addition of 0.1 ml of the Folin-Ciocalteau reagent. After incubating the mixture for 3 min at room temperature, 0.3 ml of Na_2CO_3 (20% W/V) was added and further incubated for 30 min. The absorbance of the solution was measured at 765 nm by using a UV-Visible spectrophotometry (SL 150, ELICO, India). The standard curve (linear, $R^2 = 0.969$) was constructed with aqueous solution of known gallic acid concentration in the range of (0–25 µg/ml). The results were expressed as (mg GAE/ 100 g) of the sample. All measurements were done in triplicate.

2.2. Statistical Analysis

Statistical analysis of the data was performed using statistical package for the social science (SPSS). Data were expressed as mean ± standard error of the mean (SEM) and the levels of significance were at ($P<0.05$). Pearson's correlation and regression analysis were used to assess the strength of the association and the relationship between the antioxidant capacity, total phenolic content and DPPH inhibition of the royal jelly, citrus honey and multiflora honey samples.

3. Results:

3.1. Antioxidant Activities of Royal Jelly, Citrus and Multiflora Honey

3.1.1. Total Phenolic Compounds Content

Table 1 shows the mean value of total phenolic compounds content, as calculated from the standard curve of gallic acid against absorbance. The total phenolic compounds values of royal jelly, citrus honey and multiflora honey were expressed as mg GAE/100g. The results showed that there were significant differences (*P<0.05*) between RJ, CH, and MFH phenolic content.

Table 1: Average total phenolic compounds content (mg GAE/100g) of royal jelly, citrus honey and multiflora honey.[1]

Treatment	Phenolic Content
Royal Jelly	621.60 ± 9.30^a
Multiflora Honey	16.92 ± 1.13^b
Citrus Honey	12.45 ± 0.44^b

[1]Mean of phenolic content ± standard deviation. Values having different letters are significantly different ($P<0.05$).

3.1.2. Total Antioxidant Capacity

The total antioxidant capacities of royal jelly, citrus honey and multiflora honey are presented in Table 2. The results showed that there were significant differences ($P<0.05$) between RJ and honey types, while no significant difference was observed between multiflora honey and citrus honey.

Table 2: Average antioxidant capacity (mM Fe^{+2}/100g) of royal jelly, citrus honey and multiflora honey.[1]

Treatment	FRAP
Royal Jelly	16.68 ± 0.79^{a}
Multiflora Honey	0.150 ± 0.01^{b}
Citrus Honey	0.122 ± 0.016^{b}

[1]Mean of antioxidant capacity ± standard deviation. Values having different letters are significantly different ($P<0.05$).

3.1.3. DPPH Radical Scavenging Activity (%)

Table 3 shows the DPPH free radical scavenging activity (%) for different concentrations of royal jelly, citrus honey and multiflora honey. The samples with higher total phenolic content had the greater DPPH Radical Scavenging Activity (%).

Table 3: DPPH radical scavenging activity of royal jelly, citrus honey, and multiflora honey at different concentrations.[1]

Concentrations	Royal Jelly	Citrus Honey	Multiflora Honey
50 µg/mL	35.5 ± 0.71^{a}	14.07 ± 0.04^{c}	15.25 ± 0.26^{b}
100 µg/mL	46.27 ± 0.65^{a}	17.37 ± 0.46^{b}	18.10 ± 0.35^{b}
200 µg/mL	59.67 ± 0.83^{a}	26.91 ± 0.48^{c}	29.1 ± 0.38^{b}
300 µg/mL	67.07 ± 0.72^{a}	33.10 ± 0.45^{c}	37.99 ± 0.32^{b}
400 µg/mL	77.88 ± 0.76^{a}	43.96 ± 1.81^{b}	41.82 ± 0.76^{b}
500 µg/mL	79.30 ± 0.91^{a}	47.46 ± 2.6^{b}	45.07 ± 2.1^{c}

[1]The results are expressed as mean of three replicates ± standard deviation. Values having different letters within the same row are significantly different.

The results showed that royal jelly had a strong color inhibition (79.30 ± 0.91%) at 500 µg/mL concentration; this was related to the high content of phenolic compounds that provide the strong antioxidant capacity of royal jelly. Moreover, royal jelly had the highest DPPH radical scavenging activity (%) at all tested concentrations compared to citrus honey and multiflora honey.

DPPH radical scavenging activities of citrus honey and multiflora honey were significantly different ($P< 0.05$) at (50, 200, 300 and 500 µg/mL)

concentrations. DPPH inhibition values of citrus honey were higher than multiflora honey. Citrus honey values ranged from (14.07 to 47.46 %), while for multiflora honey values were (15.25 to 45.07 %) for different concentrations.

3.1.4. Correlations and Regression Analyses

3.1.4.1. Correlations Analysis

Table 4 shows the correlation between total phenolic content, antioxidant capacity and DPPH radical scavenging activity (%) of royal jelly. There was a strong positive correlation between total phenolic content of royal jelly and antioxidant capacity (r= 0.906, P≤0.01). The DPPH radical scavenging activity (%) at 50 µg/ml concentration of royal jelly showed a strong positive correlation with the antioxidant capacity (r= 0.860, P≤0.01), and a strong positive correlation with the total phenolic content (r= 0.871, P≤0.01). In addition, the DPPH radical scavenging activity (%) at 100 µg/ml concentration of royal jelly showed a very strong positive correlation with antioxidant capacity (r= 0.929, P≤0.01), and a very strong positive correlation with total phenolic content (r= 0.936, P≤0.01).

Table 4: Pearson correlation between antioxidant capacity (mM Fe+2), total phenolic content (mg GAE/100g) and DPPH inhibition at (50 and 100 µg/ml) concentrations of royal jelly

Parameter	Antioxidant Capacity (FRAP)	Total Phenolic Content	DPPH % (50 µg/ml)	DPPH % (100 µg/ml)
Antioxidant Capacity (FRAP)	1.00	0.906**	0.860**	0.929**
Total Phenolic Content	0.906**	1.00	0.871**	0.936**
DPPH % (50 µg/ml)	0.860**	0.871**	1.00	1.00
DPPH % (100 µg/ml)	0.929**	0.936**	1.00	1.00

FRAP: ferric reducing antioxidant power; DPPH: 1,1 diphenyl-2-picrylhydrazyl
*Correlation is significant at the P< 0.05 level.
**Correlation is significant at the P< 0.01 level.

There was a strong positive significant correlation (r= 0.913, P≤0.01) between total phenolic content of multiflora honey and total antioxidant capacity. As shown in Table 5. In addition, there was a strong positive correlation between total phenolic content and DPPH radical scavenging activity (%) at 200 µg/ml concentration of multiflora honey (r= 0.946, P≤0.05). Also, there was a strong positive correlation between total antioxidant capacity and

DPPH radical scavenging activity (%) at 200 µg/ml concentration of multiflora honey (r= 0.879, P≤0.01).

Table 5: Pearson correlation between antioxidant capacity (mM Fe+2), total phenolic content (mg GAE/100g) and DPPH inhibition at (200 µg/ml) concentration of multiflora honey

Parameter	Antioxidant Capacity (FRAP)	Total Phenolic Content	DPPH % (200 µg/ml)
Antioxidant Capacity (FRAP)	1.00	0.913**	0.879**
Total Phenolic Content	0.913**	1.00	0.946*
DPPH % (200 µg/ml)	0.879*	0.946*	1.00

FRAP: ferric reducing antioxidant power; DPPH:1,1 diphenyl-2-picrylhydrazyl
*Correlation is significant at the P< 0.05 level.

Table 6 shows that there was a strong positive correlation between total phenolic content of citrus honey and total antioxidant capacity (r=0.813, P≤0.01). Also, there was a positive correlation between total antioxidant capacity and DPPH radical scavenging activity (%) at 400 µg/ml concentration of citrus honey (r=0.796, P≤0.01). Furthermore, there was a positive correlation between total phenolic content and DPPH radical scavenging activity (%) at 400 µg/ml concentration of citrus honey (r=0.706, P≤0.05).

Table 6: Pearson correlation between antioxidant capacity (mM Fe+2), total phenolic content (mg GAE/100g) and DPPH inhibition at (400 µg/ml) concentration of citrus honey

Parameter	Antioxidant Capacity (FRAP)	Total Phenolic Content	DPPH % (400 µg/ml)
Antioxidant Capacity (FRAP)	1.00	0.813**	0.796**
Total Phenolic Content	0.813**	1.00	0.706*
DPPH % (400 µg/ml)	0.796**	0.706*	1.00

FRAP: ferric reducing antioxidant power; DPPH:1,1 diphenyl-2-picrylhydrazyl
*Correlation is significant at the P< 0.05 level.
**Correlation is significant at the P< 0.01 level.

3.1.4.2. Regression Analysis

Regarding regression analysis, total phenolic content of royal jelly showed a relatively high influence on the total antioxidant capacity (R^2 = 0.822, P≤0.001). Moreover, total phenolic content showed a significant influence on DPPH radical scavenging activity (%) at 50 µg/ml concentration of royal jelly (R^2 = 0.758, P≤0.01), and also a relatively high influence on DPPH radical

scavenging activity (%) at 100 µg/ml concentration of royal jelly (R^2 = 0.875, $P \leq 0.001$). The total antioxidant capacity showed a significant influence on DPPH radical scavenging activity (%) at 50 µg/ml concentration of royal jelly (R^2 = 0.740, $P \leq 0.001$), and a relatively strong influence on DPPH radical scavenging activity (%) at 100 µg/ml concentration of royal jelly (R^2 = 0.862, $P \leq 0.001$).

Furthermore, total phenolic content of multiflora honey showed a relatively high influence on total antioxidant capacity (R^2 = 0.834, $P \leq 0.01$). Total phenolic content showed a high influence on DPPH radical scavenging activity (%) at 200 µg/ml concentration of multiflora honey (R^2 = 0.896, $P \leq 0.01$) and the total antioxidant capacity also showed a significant influence on DPPH radical scavenging activity (%) at 200 µg/ml concentration of multiflora honey (R^2 = 0.772, $P \leq 0.01$).

The total phenolic content of citrus honey showed a significant influence on the total antioxidant capacity (R^2 = 0.661, $P \leq 0.05$). In addition, total phenolic content showed a significant influence on the DPPH radical scavenging activity (%) at 400 µg/ml concentration of citrus honey (R^2 = 0.499, $P \leq 0.05$), and the total antioxidant capacity showed a significant influence on DPPH radical scavenging activity (%) at 400 µg/ml concentration of citrus honey (R^2 =0.633, $P \leq 0.01$).

4. Discussion:

This study aimed mainly at investigating the total phenolic content, antioxidant capacity, and radical scavenging activity of royal jelly (RJ), multiflora honey (MFH) and citrus honey (CH). Phenolic compounds are aromatic secondary metabolites that provide the food with its color, sensory and antioxidant properties (Wu *et al.*, 1999). Honey is composed of different kinds of polyphenols that provide their antioxidant capacity (Pyrzynska and Biesage, 2009). Compared to CH and MFH, the RJ sample in this study has extremely higher total phenolic compounds content.

The results of the total phenolic content of MFH and CH in the present study were (16.92 and 12.45mg GAE/100 g) respectively. These values are strongly in agreement with the results obtained by Perna *et al.*, (2012) who reported that the total phenolic contents of CH and MFH, collected from southern Italy, ranged between (9 and 15 mg GAE/100 g). However, Meda *et al.* (2005) studied the total phenolic compounds content of Burkina Fasan CH and MFH and showed much higher values (32 and 93 mg GAE/100 g) than the values obtained by this study.

Up to now, little is known about the phenolic content of RJ. The results of the total phenolic compounds content of RJ obtained in the present study were (621.60 mg GAE/100g). These values were much lower than the results reported by Pavel *et al*., (2014) who studied the total phenolic content of Romanian RJ with values of (14 to 39 mg GAE/g). However, the present study values of RJ total phenolic content are greater than the results of West Anatolian RJ observed by Kanbur *et al*. (2009) who reported a value of (6.65 mg GAE/100g).

It has been reported that the total phenolic compound concentration of RJ and honey is affected by many factors, such as the kind of plant visited by the bees, health of the plant, season, and climate (Martos *et al.*, 2008; Liu *et al*., 2008). The total antioxidant capacity was measured by using FRAP assay. The reducing power of FRAP measures the ability of RJ and honey antioxidants to reduce Fe^{3+} to Fe^{+2} (Muniruzzaman *et al.*, 2012). A single type of test is not enough to give accurate results about the antioxidant activity of RJ, CH, and MFH. Thus, using FRAP coupled with other method such as DPPH radical scavenging activity (%) is preferred since they have the ability to reflect more accurate results of RJ and honey antioxidant properties (Muniruzzaman *et al*., 2012).

Total antioxidant capacity of RJ and honey were investigated in the current study, the results of CH total antioxidant capacity (0.122 mM Fe^{+2}) strongly agreed with that reported by Bertoncelj *et al.* (2007) which was (118.8 µM Fe^{+2}), while showed relatively lower values when compared to the value (152 µM Fe^{+2}) obtained by Perna *et al*. (2012). In contrast, total antioxidant capacity of MFH in this study (0.150 mM Fe^{+2}) did not agree with the results obtained by both Bertoncelj *et al.*, (2007) and Perna *et al*., (2012) who had results higher than the results of this study with values of (224 µM Fe^{+2}), and (208 µM $Fe^{+2)}$), respectively (Perna *et al*., 2012; Bertoncelj *et al.*, 2007).

The results of total antioxidant capacity of RJ were also higher than those of CH and MFH. Very few studies on the total antioxidant activity of RJ are available. Pavel *et al.*, (2014) reported that the total antioxidant capacity of RJ is (2.2 mM Fe^{+2}/g) [15], which is 14 times higher than the results of the present study (16.68 mM Fe^{+2}/100g). However, it seems that there is a high variability in the total phenolic content and total antioxidant capacity of RJ and till now there are no precise values that can be assured. The seasonal variations and harvesting time seem to highly affect the total phenolic content and the antioxidative activities of RJ (Liu *et al.*, 2008).

The DPPH radical scavenging activity (%) was used to assess the antioxidant activity of RJ, CH, and MFH in this study. DPPH is a free radical used to test the samples that have antioxidant activity. When the tested sample is

added to this free radical, it will be decolorized, due to the donation of H ions from the antioxidant compounds (Lim and Murtijaya, 2007).

The percent of DPPH radical scavenging activity of RJ was the highest if compared with CH and MFH. The explanation of the higher DPPH inhibition (%) of RJ could be due to the high phenolic compounds content. The maximum DPPH inhibition (%) for RJ, CH, and MFH was obtained at a concentration of 500 µg/mL with values of (79.30, 47.46, and 45.07%), respectively.

The CH radical scavenging activity in this research is found to be close to the values reported by AL *et al.* (2008), while Perna *et al.* (2012) obtained higher results with a mean value of (54.29 %). Furthermore, the MFH radical scavenging activity agreed with the results reported by Sagdic *et al.* (2013), but showed lower values compared to the mean value (62.26%) reported by Perna *et al.* (2012).

Regarding RJ radical scavenging activity, the results of the current study showed higher values than the values reported by Pavel *et al.* (2014), who reported that the DDPH inhibition (%) of RJ samples ranged between (17.57 and 48.38 %).

5. Conclusion:

According to the results of this study, it could be concluded that total phenolic content of CH and MFH was significantly lower than that of the RJ and the total antioxidant capacity of RJ was significantly higher than those of the CH and MFH. Furthermore, The DPPH scavenging activity of RJ showed strong color inhibition (79.3%) at concentration of 500 µg/mL, whereas the honey samples had lower color inhibition. There was a strong correlation between total phenolic compound content, total antioxidant capacity, and DPPH scavenging activity in all samples suggesting that phenolic compounds are the strongest contributing factor to the antioxidant activity of RJ and honey.

6. Acknowledgments:

The authors are grateful for the Deanship of Academic Research at The University of Jordan for the financial support, and Miss Dana Abdelrahim for helping in preparation of the manuscript.

7. Conflict of Interest:

The authors declare equal participation in conducting this research and claim that they do not have any conflict of interest.

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