Antimicrobial and Antioxidant Activities of Different Spice Extracts

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Abstract

Up to day, very little work has been done on the antimicrobial activity of spices extracts against food pathogens. Additionally, there is very little information about the antioxidant activities of spices. The aim of this work was to investigate the antimicrobial effects of spices extracts against food pathogens, and its non-enzymatic antioxidant potentials. The plants have been provided from C2 region of Mugla and local herbalists. Antimicrobial activities of plants were evaluated using disc diffusion method. The extracts showed maximum inhibition zone against various microorganisms, and the zone was 8 mm. Candida albicans and Listeria monocytogenes showed the lowest sensitivity to different extracts (3250 µg/ml). In addition, the extracts were tested against the DPPH [2,2-Diphenyl-1-picrylhydrazyl] free-radical for antioxidant activity. A lot of extracts were displayed a high antioxidant activity. The highest antioxidant activity was determined on Nigella sativa (84%). In this study determined that the spices extracts have antimicrobial and antioxidant properties.

Keywords: Coriandrum, Mentha, Ribes, Nigella, Thymus, Origanum, Crocus, food pathogen, biological activity

Farklı Baharat Özütlerinin Antimikrobiyel ve Antioksidan Aktivitelerinin Saptanması

Öz

Günümüzü kadar, gıda patojenlerine karşı baharat özütlerinin antimikrobiyel aktivitesi üzerine çok az çalışma yapılmıştır. İlave, baharatların antioksidan aktiviteleri hakkında çok az bilgi vardır. Bu çalışmanın amacı, gıda patojenlerine karşı baharat özütlerinin antimikrobiyel etkilerini ve bunların enzimatik olmayan antioksidan potansiyellerini araştırmaktır. Bitkiler, Muğla’nın C2 bölgesinde ve yerel aktarıldan sağlanmıştır. Bitkilerin antimikrobiyel aktiviteleri disk difüzyon metodu kullanılarak ölçülmüştür. Buna ek olarak, özütler antioksidan aktivite için 2,2-Difenil-1-pikrilhidrazil (DPPH*) serbest radikaline karşı test edilmiştir. Özütler, çeşitli mikroorganizmalarla karşı maksimum inhibisyon zonu göstermiştir ve bu zon 8 mm’dir. Candida albicans ve Listeria monocytogenes, farklı bitki özütlerine en düşük duyarılılığı göstermiştir (3250 µg/ml). Özütlerin birçoğu yüksek antioksidan aktivite sergilemiştir. En yüksek antioksidan aktivite Nigella sativa’da saptanmıştır (%84). Bu çalışmada, baharat özütlerinin antimikrobiyel ve antioksidan özelliklerine sahip olduğu saptanmıştır.

Anahtar Kelimeler: Coriandrum, Mentha, Ribes, Nigella, Thymus, Origanum, Crocus, gıda patojeni, biyolojik aktivite

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1. Introduction

Foodborne diseases emerge as an increasingly serious public health problem all over the world. The control of pathogens can significantly reduce foodborne disease outbreaks (Kiran et al., 2008). In 2005, it was reported that 1.8 million people died from diarrheal diseases (WHO, 2005), this is substantially due to contaminated food and drinking water. Antimicrobial resistance is a particular problem for bacterial pathogens in the food chain. For more than 50 years, the use of antimicrobial agents in both human and veterinary has been an important factor in the treatment of infectious diseases, but as a result, the development of antimicrobial resistance has increased and become widespread. The consequences of this resistance in terms of public health are a failure in treatment, long-lasting illnesses, the progression of systemic infections, an increased number of hospitalizations, and increased mortality (WHO, 2008).

Medicinal plants are very powerful and effective sources of drugs, they are also used for medicinal purposes in different countries (Srivashta et al., 1996). Nowadays, the importance of using spices and extracts for food preservation has increased because consumers are directed to foods with minimal processing and no chemical additives. It is known that extending the shelf life of perishable foods with natural additives is of great importance. In parallel with the emergence of various harms of chemical additives on human health and various studies that demonstrate the benefits of spice ingredients, the use of spices in foods has gained more importance (Üner et al., 2000).

*Crocus sativus* (Safran), one of the oldest known spices, is from the family Iridaceae, it is a 20–30 cm tall *Crocus* plant that blooms in autumn, and is a spice obtained from this plant. Saffron is used among people as a sedative and pain medication in gastrointestinal diseases. It is also used in the treatment of diseases such as heart palpitations, shortness of breath, gout, impotence, anemia (Rios, et al., 1996; Baytop, 1999). Modern pharmacological studies show that saffron extracts and their active components have antioxidant (Verma, 1998; Martinez, et al., 2001) antioxidant effects (Escribano et al., 1996). Moreover, saffron has anti-inflammatory activity in various trials (Declume, 1989; Garbacki et al., 1999). The essential bioactive component of black seed essential oils, thymoquinone has been used as an antioxidant, anti-inflammatory, and antineoplastic drug for a long time (Trang et al., 1993; Hosseinzadeh et al., 2004).

*Mentha* (Nane), a genus of the Labiatae (Bailbbagiller) family, has about 7 species in Anatolia. Of these, the smell of *Mentha piperita* is special and strong, and the taste is cooling. It is used to relieve nausea, degassing, and odor. The essential oil obtained by water vapor distillation from the flowering and leafy branches of the *Mentha piperita* species is called peppermint. This essential oil is used for a mild antiseptic, refreshing, fragrance, and nausea.

*Ribes nigrum* (Kuş üzümü, siyah frenk üzümü) is a deciduous and aromatic shrub that can develop up to 2 m tall (Rehder, 1986). Plant buds are a rich source of aroma-volatile compounds, the majority of which consisted of oxygenated fractions of hydrocarbons and terpenes (Dvaranauskaite et al., 2009). As a result of studies on buds, total phenolics, and antioxidants are extracted and then used in most applications (Tabart et al., 2006; 2007; 2011; Dvaranauskaite et al., 2008). Traditionally, plant leaves are used in folk medicine in Europe to treat rheumatism, arthritis, and respiratory problems (Stević et al., 2010). The leaves have been reported to show anti-inflammatory activity in various trials (Declume, 1989; Garbacki et al., 2005). Besides, the composition and antimicrobial activity of essential oils of the plant buds and leaves have been reported in recent years (Opera et al., 2008; Stević et al., 2010).

*Origanum majorana* L. (Mercan köşkü) is known as the Cyprus medical endemic plant and "Sampsishiä", Johannes et al., (2002) reported the essential oils of this plant. Essential oils are used for sauces, seasonings, and other products (deVincenzi et al., 1997). The plant used in India as a diuretic, anti-asthma, and anti-paralytic (Yadava and Khare, 1995), and also is a common salad plant (Picton et al., 2000). Also, tea is made from herbal vinegar and leaves (Facciola et al., 1998). Used in cancer treatment (Leung et al., 2003), Stefanakis et al. (2013) reported that essential oil extracted from various types of *Origanum* can be used for the disinfection of rotifers, Abdel Massih et al. (2010) reported that it has antiproliferative and antioxidant activity. Koidis et al. (1996) reported their inhibitory effect on the development of *Campylobacter jejuni* in their study with 4 spices including marjoram.

The World Health Organization (WHO) reports that medicinal plants may be the top source for providing the...
diversity of drugs, however, the impact and safety of such plants should be better understood (Nascimento et al., 2000). Although there are studies on the biological activity of spices, Turkey’s Mugla at widely grown and used spices, there are no studies on antimicrobial activity and antioxidant activity against food pathogens. The goal of this study is to investigate the biological activities of different spices against food pathogens and to contribute to the lack of information on these activities in the literature.

2. Material and Method

2.1. Plant materials

Spice samples, which are research materials, were obtained from Muğla (C2) region and local akthars. There are 7 spices used in the study; Mentha piperita (mint), Ribes nigrum (currant), Nigella sativa (black seed), Origanum majorana (marjoram), Thymus serpyllum (thyme), Coriandrum sativum (coriander), Crocus sativus (saffron). Spice materials were identified by Dr. Olcay Ceylan and samples are stored in the herbarium of the Department of Biology, Mugla Sıtkı Kocman University, Turkey. The identification of plant materials was made according to Davis (1978).

2.2. Organisms

The four food pathogens were used in the study, 3 of them are bacteria and one is yeast. These; Escherichia coli ATCC11229, Salmonella Typhimurium RSKK19, and Listeria monocytogenes ATCC7644 are bacteria, and the yeast is Candida albicans RSKK02029. Bacterial cultures were developed at Mueller-Hinton Broth (Merck) environment at 37°C for 24 hours, and yeast at Sabouraud Dextrose Agar medium (at 30°C for 24 hours). Microorganisms were identified by Dr. Olcay Ceylan and samples are stored in the herbarium of the Department of Biology, Mugla Sıtkı Kocman University, Turkey. The identification of plant materials was made according to Davis (1978).

2.3. Preparation of spice materials

The samples were washed 2-3 times in flowing water and once in sterile distilled water. The spices are dried in the air and powdered in the slicer. All materials were held on at room temperature until sample preparation, then stored at 4°C till needed for analysis.

2.4. Preparation of spice extracts

Air-dried and powdered samples were extracted with methanol and ethanol using the Soxhlet apparatus. The essential extracts of spices with essential oil content were taking into the Cleveenger apparatus and essential oils were obtained. Th air-dried and powdered samples were extracted with methanol and ethanol using the Soxhlet apparatus. The essential extracts of spices with essential oil content were taking into the Cleveenger apparatus and essential oils were obtained. After the extracts in organic solvents were evaporated, each of them was preserved under refrigerator conditions until used in small sterile opaque bottles in its own solvent.

2.5. The Cultivation of organisms

There are 3 bacteria to be used in the study, Escherichia coli ATCC11229, Salmonella Typhimurium RSKK19, and Listeria monocytogenes ATCC7644. However the yeast agent is a 1 and that is Candida albicans RSKK02029. Microorganisms were produced by incubation at their own temperatures for 24 hours in Mueller-Hinton Broth (MHB, Merck) medium. The active cultures were used for all trials. Turbidite of all bacterial cultures was set to 0.5 McFarland.

2.6. Determination of Antimicrobial Activity

Antimicrobial activity studies were conducted using Kirby-Bauer (1966) disc diffusion method. The methanol, ethanol, and aqueous extracts of spice samples (150 mg/ml) were tested by disk diffusion method, cultures were incubated on Mueller-Hinton Agar plates (MHA, Merck) for 24 hours at their own temperature. Turbidite of bacterial cultures is set at 0.5 McFarland. After the incubation, the inhibition zones formed were recorded in mm.

2.7. Determination of Minimum Inhibitor Concentration (MIC)

In this study, the values of minimum inhibitory concentration (MIC) of spice extracts as another antibacterial activity was also determined. MIC was considered to be the lowest concentration that inhibits growth after incubation. The liquid dilution method has been tried as described in CLSI standards (CLSI, 2003; CLSI, 2006). The final concentrations of extracts were adjusted to 13000, 6500, 3250, 1625, 812.5, and 406.25 μg/ml.

2.8. Determination of Non-Enzymatic Antioxidant Activity

Non-enzymatic antioxidant activity was determined using DPPH [2,2-Diphenyl-1-picrylhydrazyl] as a free radical. Stable DPPH was used to determine the free radical scavenging activities of the extracts. The extract (0.1 ml) was added to 3.9 ml of 0.1 mM methanol DPPH solution. After 30 minutes of incubation, the absorbance of the extract was measured at 515 nm using a spectrophotometer. While methanol DPPH solution was used as a control, methanol was used as a blind. Trolox was used as a reference antioxidant. DPPH was calculated using the scavenging capacity formula and given as (%) (Brand-Williams et al., 1995).

3. Results and Discussion

In this study, antibacterial activity studies were conducted using the disk diffusion method reported by Kirby-Bauer (1966). The plant extracts (150 mg/ml) were tested by the disk diffusion method, and the cultures were incubated on Mueller-Hinton Agar plates for 24 hours at their own temperature. At the end of antimicrobial activity studies, methanol and aqueous extracts of Ribes nigrum (currants) showed effective antibacterial activity against Salmonella Typhimurium RSKK19. The antibacterial activities of other spice extracts against this bacterium have not been found. In another bacterium, Escherichia coli ATCC11229, only one extract showed effective activity. This is Nigella sativa ethanol extract (8 mm), 2 different spice extract showed antimicrobial activity against Listeria monocytogenes ATCC7644. These are Mentha piperita (7 mm) and Origanum majorana (7mm) spices, respectively. The antibacterial activities were not found in other spices extracts. 2 spices were effective against Candida albicans RSKK02029. While only ethanol extract of Nigella sativa (8 mm) was effective, Thymus serpyllum (8 mm) showed activity in both ethanol and methanol extracts. No antimicrobial activity was determined from either of the other

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spices studied. These are *Coriandrum sativum* and *Crocus sativus* (Table 1).

| Plant (150 mg/ml) | Solvent | Inhibition Zone Diameters (mm) |
|------------------|---------|--------------------------------|
| Mentha piperita  |         |                                |
| Ethanol          | -       | 7                              |
| Methanol         | -       | -                              |
| Aqueous          | -       | -                              |
| Ribes nigrum     |         |                                |
| Ethanol          | -       | 8                              |
| Methanol         | 8       | -                              |
| Aqueous          | 8       | -                              |
| Nigella sativa   |         |                                |
| Ethanol          | -       | 8                              |
| Methanol         | -       | -                              |
| Aqueous          | -       | -                              |
| Origanum majorana|         |                                |
| Ethanol          | -       | 7                              |
| Methanol         | -       | -                              |
| Aqueous          | -       | -                              |
| Thymus serpyllum |         |                                |
| Ethanol          | -       | 8                              |
| Methanol         | -       | 8                              |
| Aqueous          | -       | -                              |
| Coriandrum sativum|       |                                |
| Ethanol          | -       | -                              |
| Methanol         | -       | -                              |
| Aqueous          | -       | -                              |
| Crocus sativus   |         |                                |
| Ethanol          | -       | -                              |
| Methanol         | -       | -                              |
| Aqueous          | -       | -                              |

(-): No Zone

In this study, 3 reference antibiotics were used as the positive control. These are penicillin (10µg), chloramphenicol (30µg), and nystatin (100µg). Chloramphenicol strongly inhibited the growth of *Salmonella* and *Listeria*. Nystatin, on the other hand, weakly inhibited the growth of *Candida albicans* (Table 2).

| Microorganisms | Antibiotics | Solvent |
|----------------|-------------|---------|
|                | Penicillin (10 µg) | Chloramphenicol (30 µg) | Nystatin (100 µg) |
| *Salmonella Typhimurium RSKK19* | 9 | 22 | NT |
| *Escherichia coli ATCC11229* | 8 | 21 | NT |
| *Listeria monocytogenes ATCC7644* | 7 | 22 | NT |
| *Candida albicans RSKK02029* | NT | NT | 7 |

NT: Not Tested; E: Ethanol; M: Methanol; A: Aqueous; (-): No Inhibition

Another antimicrobial activity test in this study is MIC. It contains MIC values of different solvents of different spices provided by the broth dilution method. MIC test was not applied to *Coriandrum sativum* and *Crocus sativus*, which did not show antimicrobial activity. According to the results obtained from this study, the lowest MIC value was determined from *Origanum majorana* and *Mentha piperita* (3250 µg/ml) against *Listeria monocytogenes* ATCC7644, and *Candida albicans* RSKK02029, the lowest MIC value (325 µg/ml) was determined from *Thymus serpyllum* extract (Table 3).
In the current study, extracts of spices in different solvents were compared with standard antibiotics (Tables 1 and 2). In this study, various extracts of different spices were tested against 4 food pathogens. Antimicrobial activity was determined by inhibition zone diameter and MIC.

Today, consumers prefer more natural foods, the effects of toxic synthetic foodstuffs, and the increasing resistance of pathogenic microorganisms to antibiotics is pointed out as a positive bacteria (Farag et al., 1989). These studies show a similarity to our results. Arıdoğan et al. (2002) reported that *Nigella* showed activity against *L. monocytogenes* (Table 1). Similarly, Rauha et al. (2000) achieved the same result in their study.

In the current study, extracts of spices in different solvents were compared with standard antibiotics (Tables 1 and 2). In this study, various extracts of different spices were tested against test microorganisms, and their antimicrobial potentials were determined by inhibition zone diameter and MIC.

In this study, *Nigella sativa* showed activity against *Escherichia coli* and *Candida albicans* (Table 1). In a study, the inhibitory effects of sage, rosemary, black seed, cumin, clove, thyme, and their basic components were analyzed. In the study, it was determined that various essential oils prevent microbial growth even at a 0.25-12 mg/ml ratio, and essential oils and their basic components are more effective on Gram-negative bacteria than Gram-positive bacteria (Farag et al., 1989). These studies show a similarity to our results. Besides, another study on the effectiveness of seven spices (cumin, thyme, laurel, myrtle leaf, immortelle, marjoram, laurel) extracts on the growth of *Escherichia coli* O157:H7 was reported that thyme and marjoram show higher antimicrobial activity than other spice varieties (Sagdiç et al., 2003). Khalid et al., (2011) in their study, reported that *Nigella* showed antibacterial activity against the 5 test bacteria. Our results differ compared to those obtained from this literature. These differences may be due to environmental and genetic factors, as well as different chemotypes and nutritional status of plants and it is possible to be affected by the change of oil composition depending on other factors (Özcan and Chalchat, 2002). Andoğan et al. (2002) reported that *Mentha piperita* was ineffective against *Escherichia coli*. This study supports our results. Andoğan et al. (2002) reported that *Origanum onite* essential oil shows a 25 mm inhibition zone against *Escherichia coli*. As a result of our studies, no antibacterial effect of *Origanum majorana* extracts against *Escherichia coli* was detected (Table 1). In our study, the antimicrobial activity of *Rubes nigra* extracts against *Escherichia coli* was detected (Table 1). Similarly, Rauha et al. (2000) achieved the same result in their study.

In this study, the authors were determined that *Origanum majorana* extract show activity (7mm) against *Listeria monocytogenes* (Table 1). Based on the information in the literature, it is reported that *Origanum majorana* may be useful as an antimicrobial agent, and this oil has great potential in...
industrial applications (Ezzeddine et al., 2001). Ting and Deibel (1992) investigated the effect of some spices on the growth of *Listeria monocytogenes*, they found that the minimum inhibition concentration of cloves or dianthus, and wild marjoram was 0.5-0.7% a/h. They were the two most effective spices. In a part of the study, the effects of cloves, wild marjoram, and sage tea at 4°C and 24°C on the survival and growth of *Listeria monocytogenes* (Scott A) were investigated. At the end of this study, whereas clove showed bactericide, wild marjoram showed the bacteriostatic effect. These studies from the literature support our results.

According to the results obtained from the *Mentha piperita* studies, it was determined that ethanol extracts have effective antibacterial activity against the *Listeria* (7 mm), and the MIC value was determined as 3250 µg/ml (Tables 1 and 3). However, İçcan et al. (2002) reported a MIC value lower than the MIC value in this study. The reason for the difference between studies may be related to the fact that the mint used in the trials was collected from different regions and the menthol contents were different from each other (Özcan and Chalchat, 2002).

*Coriandrum sativum* (coriander) and *Crocus sativus* (saffron) extract were determined to not show antimicrobial activities against test microorganisms (Table 1). Soureshjian and Heidari (2014) reported that saffron did not show activity against *Escherichia coli* in their study. These results support our work. Acar et al. (2010) reported that different *Crocus* species have antimicrobial activity in their study. Asgarpanah et al. (2013) reported that *Crocus sativus* has antibacterial activity against *Salmonella* and *Escherichia coli*. Jastaniah (2014) reported that these plant extracts show antibacterial activity against *Escherichia coli* and *Salmonella*. Antimicrobial activity alters highly dependent on spice or plant species, test environment, and microorganisms (Indu and Hatha, 2006). As a result of our study, we were determined that *Thymus serpyllum* showed antifungal activity, and an 8 mm inhibition zone was determined by ethanol and methanol extracts (Table 1). These results support our work. In this study, the MIC value against *Candida albicans* was determined as 3250 and 13000 µg/ml. Nikolic et al. (2014) reported the MIC value as 2 µg/ml in their study.

The screening of plant extracts with the DPPH free-scavenging method to determine antioxidant activities is an effective selection method. The plant extracts can be rich in flavonoids for radical scavenging activity. The free radical scavenging and antioxidant activities of most medicinal plants have been reported in the literature. These properties of medicinal plants are effective against cancer, tissue inflammation, and cardiovascular diseases (Cai et al., 2004).

In our antioxidant activity studies, antioxidant activities of different solvents were scanned for all spices, and different results were obtained according to plants, and high antioxidant activities were determined (Table 4). The high antioxidant activities are associated with the number of phenolic ingredients and total flavonoids in the extracts. Flavonoids are known as powerful radical scavengers (Robak and Gryglewski, 1988). Burits and Bucar (2000) reported that spice has antioxidant activity and essential oil composition in their study with *Nigella sativa*. Another study reported that rosmanic acid content was very high in *Oregano* extracts (Chen and Ho, 1997). In a study with *Mentha*, strong radical scavenging compounds have been reported, such as monoterpen ketones and 1,8-cineole (menthone and iso menthone) (Mimica-Dukic et al., 2003). Zheng and Wang (2001) and İçcan et al. (2002) reported that there are phenolic compounds in the structure of the essential oils of *Mentha piperita*. Darugbe et al. (2012) reported that *Coriandrum sativum* essential oils contain phenolic compounds in their chemical composition. Wong and Kitts (2006) reported in their study with coriander, the antioxidant activity of root methanol extracts as 44%, however, the result obtained from our studies was 82.35% and higher. The essential oil composition of *Coriandrum sativum* leaves is monoterpenes, aldehydes, alcohols, and alkanes as reported (Mataysyoh et al., 2009). Oussalah et al. (2007) reported that *Coriandrum sativum* had linalool in its essential oil, terpene, terpinene, and thymol in the essential oil of *Origanum majorana*, and *Thymus serpyllum* oil had cymene and carvacrol. These results obtained from the literature support our studies. Ramadan et al. (2012) reported that ethanolic extracts (20, 40, 60, 80, and 100 mg/ml) of *Crocus sativus* at different concentrations did not show antioxidant activity, while Ferrara et al. (2014) studies showed 14-16% antioxidant activity. As a result of our studies, high antioxidant activity was found (Table 4). Acar et al. (2010) reported in their study that methanol extracts of different *Crocus* species showed high antioxidant activity. Okmen et al. (2016) reported that ethanol extract of *Crocus* leaves had 84% DPPH scavenging activity. These studies support our results. The reasons for this difference, as reported by Raina et al. (1996) and Maggi et al. (2009), differences in harvest time, processing temperature, storage, and packaging may enormously affect the quality of the end product. According to Fogden and Neuberger (2003), active ingredients of plants are affected by location and quality control, including harvest season, preparation methods, plant species, altitude, and climate.

In this study, the radical scavenging effect of *Thymus serpyllum* aqueous extract was determined as 60.8% (Table 4). Mata et al. (2007) studied the essential oil composition of the plant and found the terpenoids that they thought were responsible for antioxidant activity. Sokmen et al. (2004) studied the essential oil composition of *Thymus spathulifolius*, and gave the proportions of phenolic compounds, also demonstrating the presence of radical scavenging and antimicrobial activities. Adzet et al. (1988), in their study, reported the presence of polyphenols and methoxylated flavonoids in the structure of the *Thymus*. Kulisic et al. (2005) in their study, determined the essential oil composition of *Thymus vulgaris* and *Thymus serpyllum* and recorded DPPH scavenging activity as 38% in *Thymus vulgaris* and 30% in *Thymus serpyllum*. According to the literature, researchers were studied with *Thymus eugii*, which has phenolic monoterpenes thymol and carvacrol (Tepe et al., 2004). These studies support our results.

4. Conclusions and Recommendations

The results of the current study have shown that different spices have antimicrobial properties. Researchers expect that the development of natural antimicrobials to be help reduce the negative effects of synthetic drugs. Our results support the use of these herbs in traditional medicine, and some of these herbs show that they havcole good antibacterial and antifungal compounds, so they can be used as antibacterial and antifungal agents in the research of new drugs. The antioxidant activities were determined in all of the spices extracts used in the study.
and most of these activities were obtained from methanol extracts.

According to this study results, methanol is the most effective solvent for antioxidant compounds. The extracts of spices can be useful as an antioxidant protective system for the human body against oxidative stress. More studies are needed to investigate the bioactive compounds of spices. It is necessary to determine and characterize the fractions of bioactive compounds.

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