In vitro inhibitory effects of methanol extracts of four *Centaurea* species on α-amylase and α-glucosidase

Dört *Centaurea* türünün metanol ekstrelerinin α-amilaz ve α-glukozidaz üzerindeki *in vitro* inhibitör etkileri

Abstract

Aim: Medicinal plants have been used in traditional folk medicine for thousands of years against various diseases, including diabetes. Some *Centaurea* species are among these medicinal plants. The aim of this study was to scientifically and comparatively evaluate the antidiabetic activities of methanol extracts obtained from the capitulum and non-capitulum aerial parts of *Centaurea cuneifolia, C. kilaea, C. solstitialis* subsp. *solstitialis* and *C. stenolepis* against α-glucosidase and α-amylase for the first time except *C. kilaea*.

Materials and Methods: Methanol extracts from *Centaurea* species were obtained by maceration method. Antidiabetic activity was performed by two known *in vitro* methods such as alpha-glucosidase and alpha amylase inhibitory activity.

Results: Among the extracts, *Centaurea cuneifolia* capitula (CCC) and *C. solstitialis* subsp. *solstitialis* capitula (CSSC) methanol extracts with IC_{50} values of 164.30 and 463.70 µg/mL exhibited the best inhibitory activity against α-amylase and α-glucosidase enzymes.

Conclusion: This is the first study on anti α-amylase and anti α-glucosidase activity of three *Centaurea* species except *Centauera kilaea*. These results indicate that CCC and CSSC have inhibitory effects against alpha amylase and alpha glucosidase. At the same time, the extracts were generally found to be active against the alpha amylase enzyme. In addition, it was found remarkable that only the capitulum of *Centaurea* species were effective against alpha glucosidase enzyme. However, *in vivo* studies are needed to fully reveal the antidiabetic effect and bioactivity-directed fractionation, and isolation studies are needed to reveal the compounds responsible for the antidiabetic effect.

Keywords: *Centaurea* species, diabetes, antidiabetic activity, α-glucosidase, α-amylase

Özet

Amaç: Tıbbi bitkiler geleneksel halk tıbbında binlerce yıldır diyabet dahil çeşitli hastalıklara karşı kullanılagelmiştir. Bu tıbbi bitkiler arasında bazı *Centaurea* türleri de yer almaktadır. Bu çalışmada, *Centaurea cuneifolia, C. kilaea, C. solstitialis* subsp. *solstitialis* ve *C. stenolepis* türlerinin kapitulum ve kapitulum hariç toprak üstü kısımlarından elde edilen metanol ekstrelerinin α-glukozidaz ve α-amilaz enzimlerine karşı antidiyabetik aktivitelerinin *C. kilaea* hariç ilk kez bilimsel ve karşılaştırmalı olarak değerlendirilmesi amaçlanmıştır.

Gereç ve Yöntem: *Centaurea* türlerinden elde edilen metanol ekstreleri maserasyon yöntemi ile elde edilmiştir. Antidiyabetik aktivite, alfa-glukozidaz ve alfa amilaz inhibitör aktivite gibi bilinen iki *in vitro* yöntemle gerçekleştirilmiştir.

Bulgular: Ekstreler arasında, 164,30 ve 463,70 µg/mL'lik IC_{50} değerlerine sahip *Centaurea cuneifolia* capitula (CCC) ve *C. solstitialis* subsp. *solstitialis* capitula (CSSC) metanol ekstreleri, α-amilaz ve α-glukozidaz enzimlerine karşı en iyi inhibitör aktiviteyi göstermiştir.

Sonuç: Bu çalışma, *Centauera kilaea* türü hariç diğer üç *Centaurea* türlerinin anti

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Ali ŞEN[1]
AŞ: 0000-0002-2144-5741

[1] Marmara University, Faculty of Pharmacy, Department of Pharmacognosy, İstanbul, TÜRKİYE

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Sorumlu Yazar / Corresponding Author:
Ali ŞEN
E-posta:
ali.sen@marmara.edu.tr

α-amilaz ve anti α-glukozidaza aktivitesine yönelik ilk çalışmadır. Bu sonuçlar, CCC ve CSSC'nin alfa amilaz ve α-glukozidaz enzimlerine karşı inhibitör etkilere sahip olduğunu göstermektedir. Aynı zamanda, ekstrelerin genellikle α-amilaz enzimine karşı aktif olduğu bulunmuştur. Ayrıca, *Centaurea* türlerinin sadece kapitulumlarının α-glukozidaz enzimine karşı etkili olması dikkat çekici bulunmuştur. Bununla birlikte, antidiyabetik etkiyi tam olarak açığa çıkarmak için de *in vivo* çalışmalara ve antidiyabetik etkiden sorumlu bileşikleri ortaya çıkarmak için biyoaktivite rehberliğinde fraksiyonlama ve izolasyon çalışmalarına ihtiyaç vardır.

Anahtar kelimeler: *Centaurea* türleri, diyabet, antidiyabetik aktivite, α-glukozidaz, α-amilaz

1. Introduction

Type 2 diabetes mellitus is a disease that causes problems in regulating blood sugar levels, and effective treatments are based on blood sugar control and reducing side effects [1]. Inhibition of carbohydrate digestive enzymes such as alpha-amylase and alpha-glucosidase is a widely practiced strategy to manage diabetes mellitus, as both enzymes, responsible for the digestion of starch and glycogen, control postprandial glucose levels [2]. Currently, drugs used in the treatment of DM include acarbose, miglitol and voglibose, and among these drugs, acarbose can inhibit both diabetic enzymes, while the other two can only inhibit the α-glucosidase enzyme [3]. However, these drugs have been reported to have some undesirable side effects such as abdominal distension, flatulence, bloating, and possibly diarrhea [4]. Therefore, there is a need to investigate promising and safer potential inhibitors with low toxicity from natural products such as medicinal plants. In order to find these inhibitors, it is very important to conduct research on crude extracts obtained from medicinal plants.

Centaurea species, a member of the Asteraceae family, are represented by 194 taxa (159 species), more than half of which are endemic (110 of which are endemic) in Türkiye (and therefore the country of genetic source of these species) [5]. Various parts of *Centaurea* species are used internally and externally, usually in the form of infusion and decoction, in traditional folk medicine to treat many different diseases, including diabetes. In these studies, it was reported that the infusion obtained from the leaves of *C. iberica*, *C. glastifolia*, *C. pterocaula* and the flowers and leaves of *C. saligna* were used as a blood sugar lowering agent [6,7]. Scientific studies on *Centaurea* species have reported that these species have anti-inflammatory, antioxidant, antipyretic, antimalarial, antimicrobial, antiviral, antiphytoviral, antiulcerogenic, smooth muscle contraction, hypoglycemic, immunological, neurotoxic, cytotoxic (anticancer), vasodilator, wound healing, and antidiabetic activities [8-21]. Phytochemical analysis studies on *Centaurea* species have suggested that these species contain phenolic acids, flavonoids, terpenes (such as sesquiterpenes, sesquiterpene lactones, diterpenes, triterpenes), fatty acids [22-32].

In scientific studies on antidiabetics against alpha-glucosidase and/or alpha-amylase enzymes, *C. amanicola*, *C. antitauri*, *C. cadmea* subsp. *pontica*, *C. centaurium*, *C. depressa*, *C. drabifolia* subsp. *detonsa*, *C. karduchorum*, *C. kilaea*, *C. kotschyi var. persica*, *C. sivasica*, *C. patula*, *C. pulchella*, *C. tchihatcheffi*, *C. triumfettii*, *C. urvillei* subsp. *hayekiana* were found to have significant activities. The fact that *Centaurea* species have both ethnobotanical uses as blood sugar lowering and that these species exhibit good antidiabetic activity in scientific studies indicates that it may be important to carry out antidiabetic activity studies on different *Centaurea* species.

The aim of this study was to scientifically and comparatively investigate the antidiabetic activities of methanol extracts of different parts of four *Centaurea* species (*C. cuneifolia*, *C. kilaea*, *C. solstitialis* subsp. *solstitialis* and *C. stenolepis*) based on the inhibition of alpha amylase and alpha glucosidase enzymes for the first time except *C. kilaea*.

2. Materials and methods

2.1 Plant material

Centaurea species (*C. cuneifolia*, *C. kilaea*, *C. solstitialis* subsp. *solstitialis* and *C. stenolepis*) were previously collected from various districts of Istanbul, Türkiye and identified by Gizem Bulut, an expert botanist at Marmara University Faculty of Pharmacy. The specimens, which were given herbarium numbers (*C. cuneifolia*: 11690, *C. kilaea*: 11712, *C. solstitialis* subsp. *solstitialis*: 11965 and *C. stenolepis*: 11651), are kept in Marmara University Faculty of Pharmacy herbarium [12].

2.2 Preparation of plant extracts/fractions

Methanol extracts from the capitulum and aerial parts excluding the capitulum of *Centaurea* species were previously obtained in the study of Sen et al. (2013) [12]. Briefly, each sample was ground and after weighing 15 g, they were macerated with 180 mL of methanol for 24 h. Then, the samples were filtered, and the same volume of fresh methanol was added to the remaining residue. This process was carried out three times in total. The solvents of the combined filtrates were evaporated to dryness in a rotary evaporator at a temperature not exceeding 40 °C and extracts were obtained. The yields of the obtained extracts were calculated (*C. cuneifolia* capitulum: 12.69%, *C. cuneifolia* aerial part: 10.60%, *C. kilaea* capitulum: 11.33%, *C. kilaea* aerial part: 12.92%, *C. solstitialis* subsp. *solstitialis* capitulum: 10.90%, *C. solstitialis* subsp. *solstitialis* aerial part: 8.14%, *C. stenolepis* capitulum: 13.22% and *C. stenolepis* aerial part: 13.54%) and stored at +4 °C until analysis.

2.3 Alpha-amylase inhibitor activity

The α-amylase inhibitory activity of the extracts was evaluated according to the methods of Ramakrishna et al. (2017) and Şen et al. (2019) [33, 34]. 10 µL of extract, 15 µL of 0.02 M sodium phosphate buffer (pH 6.9, 0.006 M NaCl) and 25 µL of porcine-amylase (0.5 mg/ml-15 units) prepared in buffer were mixed. The mixture was incubated at 25°C for 10 minutes. Then, 25 µL of a 1 % starch solution prepared in buffer was added to each well. The mixtures were again incubated at 25°C for 10 mins. The reaction was stopped with 50 µL of dinitrosalicylic acid (DNSA) and incubated in boiling water bath for 10 mins. The solutions were cooled to room temperature and then were read absorbance at 540 nm diluting with 225 µL of ultra pure water. Acarbose was used as standard. The percentage inhibitory activity of the extract and standard against α-amylase enzyme were calculated according to the following:

$$\alpha\text{-amylase inhibitor activity (\%)} = [(A_0-A_1)/A_0]\times 100$$

where A_0 is the absorbance of the control (containing all reagents except the test compounds), and A_1 is the absorbance of the extract/standard. Extract or standard concentration providing 50% inhibition (IC_{50}) was calculated from the graph plotting inhibition percentage against extract or standard concentration. Tests were carried out in triplicate.

2.4 Alpha-glucosidase inhibitor activity

The α-glucosidase inhibitory activity of the extracts was evaluated according to the methods of Ramakrishna et al. (2017) and Şen et al. (2019) [33, 34]. 10 µL of extract was mixed with 40 µl of 0.1 M sodium phosphate buffer (pH 6.9), and 100 µL of α-glucosidase (obtained from *Saccharomyces cerevisiae*) prepared in buffer. The mixtures were incubated at 25°C for 10 minutes. Thereafter, 50 µL of 5 mM *p*-nitrophenyl-α-D-glucopyranoside (pNPG) prepared in buffer to the solutions was added. The mixtures were re-incubated at 25°C for 5 minutes, and their absorbance was recorded by reading in the microplate reader before and after incubation at 405 nm. Acarbose was used as standard. The percentage inhibitory activity of the extract and standard against α-glucosidase enzyme were calculated according to the following:

$$\alpha\text{-glucosidase inhibitor activity (\%)} = [(A_0-A_1)/A_0] \times 100$$

where A_0 is the absorbance of the control (containing all reagents except the test compounds), and A_1 is the absorbance of the extract/standard. Extract or standard concentration providing 50% inhibition (IC_{50}) was calculated from the graph plotting inhibition percentage against extract or standard concentration. Tests were carried out in triplicate.

2.5 Statistical analysis

Statistical analyses were performed with GraphPad Prims 5.0 software. Activity results were expressed as mean ± standard deviation. After one-way analysis of variance (ANOVA) was applied, differences between groups were determined by Tukey test. A significance level of $p < 0.05$ was accepted.

3. Results

Among the extracts, CCC exhibited the best α-amylase inhibitory activity with an IC_{50} value of 164.30 µg/mL, while CSSC showed the lowest activity with an IC_{50} value of 846.70 µg/mL (Table 1). All extracts showed low anti-α-amylase activity compared to standard acarbose (16.61 µg/mL). (Table 1). CCC showed the highest inhibition against the α-amylase enzyme with an inhibition rate of 47.86% at a concentration of 142.86 µg/mL, followed by CKC (43.79%), CKA (43.74%), CSA (39.96%), CSC (38.44%), CCA (36.38%), CSSC (14.65%) and CSSA (14.58%), respectively (Figure 1).

When the extracts were evaluated in terms of inhibitory activities on α-glucosidase enzyme, the best ac-

Table 1 Alpha-glucosidase and alpha-amylase inhibitor activities of extracts (IC_{50}, µg/mL)

Extracts and standart*	*α*-amylase inhibitor activity	*α*-glucosidase inhibitor activity
CCC	164.30 ± 3.89^{b}	833.30 ± 3.96^{c}
CCA	596.30 ± 4.95^{f}	-
CKC	228.10 ± 0.99^{e}	1107.00 ± 24.75^{d}
CKA	230.80 ± 2.12^{e}	-
CSSC	846.70 ± 4.17^{h}	463.70 ± 0.00^{b}
CSSA	658.10 ± 3.82^{g}	-
CSC	305.60 ± 2.12^{e}	-
CSA	253.00 ± 3.61^{d}	-
Acarbose	16.61 ± 0.33^{a}	113.70 ± 0.64^{a}

* The capitula and aerial parts except capitula of *Centaurea cuneifolia*, *C. kilaea*, *C. solstitialis* subsp. *solstitialis* and *C. stenolepis* are abbreviated as CCC, CCA, CKC, CKA, CSSC, CSSA, CSC, CSA.
** Each value in the table is the mean of three replicates and was represented as mean ± SD.
*** Different and same letter superscripts in the same column show statistical significance ($p < 0.05$) and non-significance ($p > 0.05$), respectively.
-: IC_{50} values could not be calculated since the extracts did not show any activity against *α*-glucosidase enzyme at all determined concentrations.

tivity was found in CSSC with an IC_{50} value of 463.70 µg/mL, while the activities of all extracts were lower than acarbose (113.70 µg/mL) used as standard (Table 1). CSSC (26.27%) exhibited the strongest inhibition against α-glucosidase enzyme at a concentration of 250 µg/mL, followed by CCC (14.38%) and CKC (10.78), respectively. Other extracts did not exhibit any inhibition against the α-glucosidase enzyme (Figure 2).

4. Discussion

This study aimed to comparatively and scientifically examine the antidiabetic effects of methanol extracts obtained from different parts of *C. cuneifolia, C. kilaea, C. solstitialis* subsp. *solstitialis* and *C. stenolepis* via inhibition of alpha amylase and alpha glucosidase enzymes.

When the extracts were compared in terms of their antidiabetic activities, it was found that CCC and CSSC exhibited the best activity against α-amylase and α-glucosidase enzymes, respectively.

In addition to the fact that the extracts were generally effective against alpha amylase enzyme, it was also found remarkable that only the extracts obtained from the capitulum of *Centaurea* species were active against alpha glucosidase enzyme.

In this study, α-amylase and α-glucosidase inhibitory activity tests on other *Centaurea* species (*C. cuneifolia*, *C. solstitialis* subsp. *solstitialis* and *C. stenolepis*) except *C. kilaea* were performed for the first time. In the literature, only one study like the current study was found on *C. kilaea*. In the mentioned study, Kısa et al. reported that methanol–chloroform (4:1) extract from *Centaurea kilaea* exhibited antidiabetic activity against α-amylase and α-glucosidase enzymes with IC_{50} values of 121.1 and 110.5 µg/mL [15]. These values are better than the results of our current study.

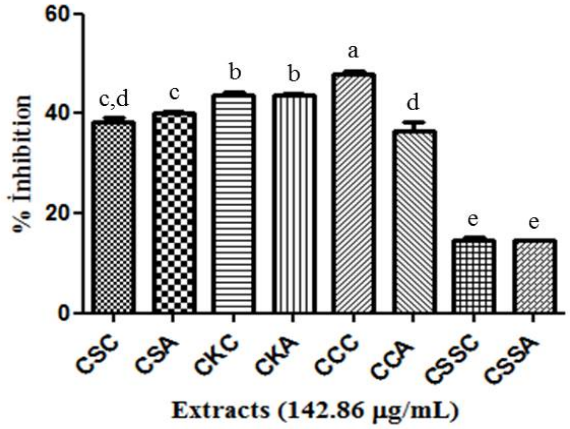

Figure 1 Inhibitory effects of extracts on α-amylase enzyme (The capitula and aerial parts except capitula of *Centaurea cuneifolia, C. kilaea, C. solstitialis* subsp. *solstitialis* and *C. stenolepis* are abbreviated as CCC, CCA, CKC, CKA, CSSC, CSSA, CSC, CSA. Different and same letter superscripts in the same column show statistical significance ($p < 0.05$) and non-significance ($p > 0.05$), respectively.)

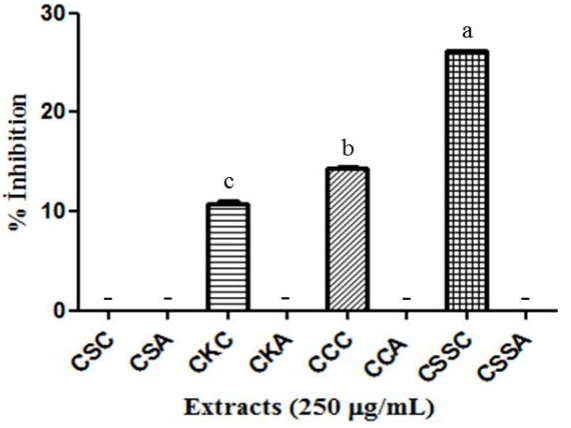

Figure 2 Inhibitory effects of extracts on α-glucosidase enzyme (The capitula and aerial parts except capitula of *Centaurea cuneifolia, C. kilaea, C. solstitialis* subsp. *solstitialis* and *C. stenolepis* are abbreviated as CCC, CCA, CKC, CKA, CSSC, CSSA, CSC, CSA. -: no inhibition. Different and same letter superscripts in the same column show statistical significance ($p < 0.05$) and non-significance ($p > 0.05$), respectively.)

The reason for this may be that less polar compounds contributed to the activity because less polar extract was used.

Studies on alpha amylase and alpha glucosidase activities of different *Centaurea* species have been found in the literature. In one of these studies, Zengin et al. evaluated the activities of chloroform and ethyl acetate extracts obtained from the aerial parts of 8 *Centaurea* species (*C. depressa*, *C. drabifolia* subsp. *detonsa*, *C. kotschyi* var. *persica*, *C. patula*, *C. pulchella*, *C. tchihatcheffi*, *C. triumfettii*, *C. urvillei* subsp. *hayekiana*) on the inhibition of diabetic enzymes and observed that at 2 mg/mL concentration, *C. pulchella* chloroform extract inhibited the activity of alpha amylase enzyme by 59.54%, while *C. triumfettii* ethyl acetate extract inhibited the activity of alpha glucosidase enzyme by 69.88% [16]. In the present study, CCC (47.86% inhibition at 142.86 µg/mL for α-amylase) and CSSC (26.27% inhibition at 250 µg/mL for α-glucosidase) exhibited better antidiabetic activity at much lower concentrations. This may be due to the difference in species (thus the difference in chemical composition) and the difference in extracts obtained.

In another study, methanol extract obtained from the aerial parts of *Centaurea cadmea* subsp. *pontica* was found to have IC_{50} values of 69.15 and 82.48 µg/mL against alpha glucosidase and alpha amylase enzymes, respectively [17]. The results were found to be better compared to the current study. This can be explained by the thought that the phytochemical contents of the related species may also be different due to species differences.

Corforti et al. (2008) evaluated the antidiabetic effects of methanol extract prepared from the roots of *C. centaurium* and its fraction against alpha amylase enzyme and revealed that methanol extract inhibited the enzyme by 32.51% (IC_{50}: >1000 µg/mL) at a concentration of 1000 µg/mL and hexane fraction inhibited the enzyme by 82.34% (IC_{50}: 158 µg/mL) at a concentration of 500 µg/mL [18]. In our current study, CCC (47.86%), CKC (43.79%), CKA (43.74%), CSA (39.96%), CSC (38.44%), CCA (36.38%) exhibited better anti-alpha-amylase activity than *C. centaurium* methanol extract even at about 7 times lower extract concentration (142.86 µg/mL). This may be due to the difference in chemical content due to species differences. The *C. centaurium* hexane fraction exhibited slightly better activity than CCC (164.30 µg/mL) in the current study when compared in terms of IC_{50} values. This can be explained by the fact that lipophilic compounds also contributed to the activity.

Yırtıcı et al. (2002) revealed that *C. sivasica* aerial part methanol extract has antidiabetic effects against α-amylase and α-glucosidase enzymes with IC_{50} values of 279.40 and 252.60 µg/mL, respectively [19]. When the results of this study were compared with the results of the current study in terms of alpha amylase inhibitory activity, CCC (164.30 µg/mL), CKC (228.10 µg/mL), CKA (230.80 µg/mL) and CSA (253.00 µg/mL) showed better activity, while *C. sivasica* aerial part methanol extract showed better activity in terms of alpha glucosidase inhibitory activity. The reason for this situation may be due to the change in chemical composition due to species difference.

In a study by Dalar et al., the inhibitory effects of hydrophilic (80% ethanol, 19% H_2O and 1% of 0.1% trifluoroacetic acid, v/v/v) extracts prepared from roots, stems, leaves and flowers of *C. karduchorum* were investigated against alpha amylase and alpha glucosidase enzymes. According to the results of this study, it was observed that the extracts prepared from roots, stems, leaves and flowers of *C. karduchorum* exhibited IC_{50} values of 5.35, 1.42, 0.63 and 1.51 mg/mL against alpha glucosidase enzyme, respectively. It was reported that only the extract prepared from the leaves of *C. karduchorum* was active against alpha amylase enzyme and showed an IC_{50} value of 14.63 mg/mL [20]. When the results of this study were compared with the current study results, it showed lower activity than all extracts according to alpha amylase inhibitor test results and lower activity than CSSC according to alpha glucosidase inhibitor test results. The reason for this situation may be due to the change in chemical composition due to species difference.

Nobarirezaeyeh et al. (2024) found that methanol extracts prepared from dried fruit aerial parts of *C. amanicola* and *C. antitauri* inhibited the activity of α-amylase enzyme by 21.11% and 42.91% at 5000 µg/mL concentration, respectively, while they did not have any activity against α-glucosidase enzyme [21]. In the current study, CCC (47.86%), CKC (43.79%) and CKA (43.74%) at about 35 times lower concentration (142.86 µg/mL) against α-amylase enzyme; CSSC (26.27%), CCC (14.38%) and CKC (10.78%) at about 20 times lower concentration (250 µg/mL) against α-glucosidase enzyme showed a better inhibitory effect. The difference in phytochemical composition due to species differences may be responsible for this difference in results.

However, although estimating the antidiabetic effect of the extract on the enzymes used in this study is important for a preliminary evaluation, there are some

limitations to the study. The relevant extracts were applied directly to the enzyme *in vitro*, that is, in a controlled environment outside the body. In a living organism, when these extracts are applied, they will be metabolized in the gastrointestinal system and then enter the systemic circulation and show their effects. Therefore, *in vivo* studies will be important at this stage in evaluating the pharmacokinetics of the extracts.

These results indicated that *C. cuneifolia* capitula (CCC) and *C. solstitialis* subsp. *solstitialis* capitula (CSSC) methanol extracts have antidiabetic effects against α-amylase and α-glucosidase enzymes, respectively and support the use of *Centaurea* species in traditional folk medicine. However, *in vivo* experiments (e.g. streptozotocin-induced diabetes model in rats) are needed to definitively reveal the antidiabetic effects of the extracts. It may also be important to conduct further studies such as bioactivity-directed fractionation and isolation studies to reveal the compounds responsible for the activity of the extracts.

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