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Research Article

Phytochemical studies and *in vitro* evaluation of the antioxidant activity of some medicinal and aromatic plants from Morocco

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Abstract

The present work was carried out to evaluate the phenolic compounds and the antioxidant activities of some solvent extract (methanol, hydroethanol and aqueous) of several Moroccan medicinal plants known for their high antioxidant properties. The extracts were obtained by sonication, then, the total phenolics and flavonoids compounds were determined using Folin-Ciocalteu and Aluminium chloride. Afterwards, the Total Antioxidant Capacity and DPPH scavenging methods were performed. Results of phytochemical analysis showed that the total phenolics content were the highest in the hydroethanolic extract of *Arbutus unedo* with 160.76 mg GAE g^{-1}DM, and the flavonoids content were the highest for the hydroethanolic extracts of *Inula viscosa* with 489.77 mg QE g^{-1} DM. Also, it can be noted that *Arbutus unedo*, *Argania spinosa*, and *Myrtus communis* exhibited the most potent antioxidant activity respectively with 0.026; 0.043; 0.036 mg ml^{-1}.

Keywords: antioxidant activity; medicinal plants; Morocco; oxidative stress; phenolic compounds; radical scavenging

Introduction

Oxidative stress is a phenomenon that reflects an imbalance between the production of reactive oxygen species (ROS) called oxidants, and their elimination by protective mechanisms called antioxidant systems, which can detoxify the reactive intermediates, or repair the resulting damage, causing toxic effects through the production of peroxides and free radicals (Pizzino *et al.*, 2017). In addition, some reactive oxidant species act as cellular messengers in redox signaling that can cause disruptions in normal cell signaling mechanisms (Milkovic *et al.*, 2019).

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Oxidative stress is thought to be involved in the development of atherosclerosis, neurodegenerative diseases such as Alzheimer's and Parkinson's, cancer, diabetes mellitus, and inflammatory diseases, as well as psychological diseases or aging processes (Forman and Zhang, 2021).

The participation of oxidative stress, which is connected to the formation of reactive oxygen and nitrogen species by all aerobic organisms, including free radicals, is a common factor in the pathogenesis of the majority of chronic diseases (Reza *et al.*, 2010). These reactive molecular species have a major impact on intra- and extra cellular signaling, and can start harmful metabolic processes. A sophisticated antioxidant defense has been established to counteract this damage, and dietary antioxidants play an important role in this defense (Chib *et al.*, 2020).

Antioxidants prevent oxidative stress in biological systems. Because of the unpaired electron in their structure, antioxidants are able to neutralize free radicals or radical ions. They have the effect to remove the radical ions produced as a result of oxidation from the system without damaging the biological system (Çalişkan and Cengiz Çalişkan, 2021).

Medicinal and aromatic plants (MAP) are inexhaustible natural sources of bioactive compounds, including antioxidants (Lourenço *et al.*, 2019). In light of its geographical location, Morocco is said to have a large variety of MAP, with over 4,200 species and subspecies, of which 22% are endemic (Rankou *et al.*, 2013).

The aim of our work is to exploit the potential of Moroccan medicinal plants in order to highlight antioxidant activity which is closely related to the content of phenolic compounds (Aryal *et al.*, 2019) and demonstrate their possible use as alternative therapies against oxidative diseases.

Materials and Methods

Plant material

The plant material used for the present study consists of seven medicinal and aromatic plants that were selected according to their known medicinal effects, their uses in traditional medicine, and the endemic character for some plants. The data related to these plants are represented in Table 1. The leaves of these MAPs were sorted and dried for 72 h at 35 °C. Thereafter, they were crushed and sieved to 300 µm to obtain a fine homogeneous powder, that will be used for extracts.

Table 1. Medicinal and aromatic plants studied

Species	Family	GPS data
Marrubium vulgare L.	Lamiaceae	Douar Sahel Boutaher (34°30.4718'N, 4°47.8572'O)
Inula viscosa L.	Asteraceae	Douar Sahel Boutaher (34°30.1870'N, 4°46.9117'O)
Retama monosperma L.	Fabaceae	Settat (33°0.4556'N, 7°34.9844'O)
Myrtus communis L.	Myrtaceae	ANPMA (34°29.8993'N, 4°48.1756'O)
Arbutus unedo L.	Ericaceae	Tamesnit Ratba (34°44.6810'N, 4°53.3705'O)
Argania spinosa L.	Sapotaceae	Agadir (30°26.1546'N, 9°27.3926'O)
Cannabis sativa	Cannabinaceae	Douar Rkaiba (34°43.9322'N, 4°52.0309'O)

Preparation of the extract

Three extracts were prepared for each plant: a methanol, hydroethanol (20/80) and aqueous extract. To do this, 40 mg of dry matter was added to 10 ml of solvent, the extraction was made by ultrasound at 35 Khz, the whole was then centrifuged for 30 min at 3000 rpm. The supernatant was recovered and stored at 4 °C.

Total Phenolic Content (TPC)

The Folin-Ciocalteu technique was used to determine the total phenolic content of these MAPs (Cheng and Li, 2004). Indeed, 200 µL of the extract was mixed with 1.5 mL of 10% Folin-Ciocalteu reagent. After 5 minutes, 1.5 mL of 5% sodium carbonate was added. The absorbance was measured at 725 nm after 2 hours of incubation. The concentration of total polyphenols was calculated based on a previous calibration curve performed with standard gallic acid. Results are expressed as mg gallic acid equivalents per gram dry matter (mg GAE g^{-1} DM).

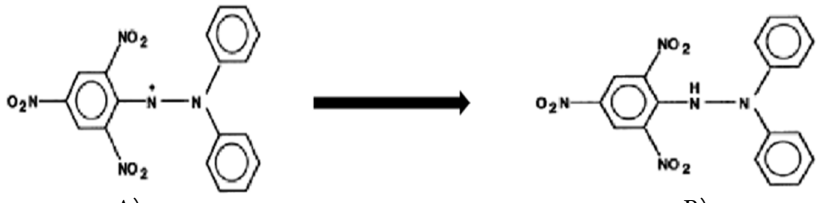
Total Flavonoids Contents (TFC)

The flavonoid contents were assessed in accordance with the method used Barros *et al.*, 2011. Therefore, 0.3 mL of 5% $NaNO_2$ were added to 1 mL of extract. After 5 minutes, 0.3 mL of 10% $AlCl_3$ were added. Then, 2 mL of NaOH 1 M were added, and the mixture's volume was subsequently raised to 10 mL using distilled water. The absorbance was measured at 510 nm. Total flavonoids were calculated from a standard curve made with quercetin. Results are expressed as milligrams of quercetin equivalents per gram of dry matter (mg QE g^{-1} DM)

Evaluation of the antioxidant activity

DPPH assay

The purpose of this method was to determine which of the prepared extracts had the greatest antioxidant activity against DPPH (2,2'-diphenyl-1-picrylhydrazyl). It is important to note that DPPH is a stable free radical, and in the presence of antioxidants, the characteristic purple colour of DPPH changes to yellow, and the absorbance is measured at 517 nm.



A) B)

Figure 1. Reaction of an antioxidant with DPPH (Molyneux, 2004); (A) Diphenylpicrylhydrazyl + Antioxidant-OH (Purple color); (B) Diphenylpicrylhydrazyl + Antioxidant-O (Yellow color)

The antioxidant activity was tested according to the method described by Brand-Williams *et al.* (1995). This procedure consists in preparing dilutions of the order of 1/2, 1/4, 1/8 and 1/16 from a stock solution of 4 mg ml^{-1} of the studied extract. Then, 1 ml of each dilution was added to 1 ml of DPPH (0.004%). Ascorbic acid was used as a reference molecule. The absorbance was measured at 517 nm by spectrophotometer, and the antioxidant activity was calculated according to the following formula:

$$\text{Antioxidant activity (\%)} = \frac{\text{Abs DPPH} - \textit{Abs of the extract}}{\text{Abs DPPH}} \times 100$$

- Abs DPPH: Absorbance of the solution of DPPH
- Abs of the extract: value of the absorbance after the addition of the extract

The regression curve of this activity allowed to determine the concentration that corresponds to 50% inhibition (The half maximal inhibitory concentration IC_{50}: Concentration of the tested sample or ascorbic acid necessary to reduce 50% of the DPPH radical). A low IC_{50} value indicates a high capacity of the extract to act as a DPPH scavenger (Cheng and Li, 2004).

Total antioxidant capacity (TAC)

The total antioxidant capacity was measured using the protocol described by Prieto (Prieto *et al.*, 1999). In practice, 200 µL of extract were mixed with 3 mL of reagent solution (6M sulfuric acid, 280 mM sodium phosphate and 40 mM aluminum molybdate). The incubation was done at 95 °C for 90 min. After cooling, the absorbance was measured at 695 nm. The total antioxidant capacity was expressed as milligram ascorbic acid equivalent / gram dry matter (mg AAE g^{-1} DM).

Statistical analysis

The data obtained were subjected to analysis of variance (ANOVA). Statistical analysis was based on Two-way ANOVA followed by Tukey's Honestly Significant Difference Test. The data were processed with the software "SYS-TAT 12". A test of comparison of the means was done each time there was a significant effect of factor studied by the ANOVA

Results

Total phenolic content

Figure 2 displays the results of the total phenolic contents. They ranged from 7.90 to 160.76 mg GAE g^{-1} DM. The highest concentration of phenols was observed in the hydroethanol extract for *A. unedo*. On the other hand, the aqueous extract of *M. vulgare* had the lowest content.

The analysis of variance for total phenol content showed a highly significant difference between both the species and the solvents used in the present experimentation ($df = 12$, $F\text{-}ratio = 10.843$, $p < 0.001$).

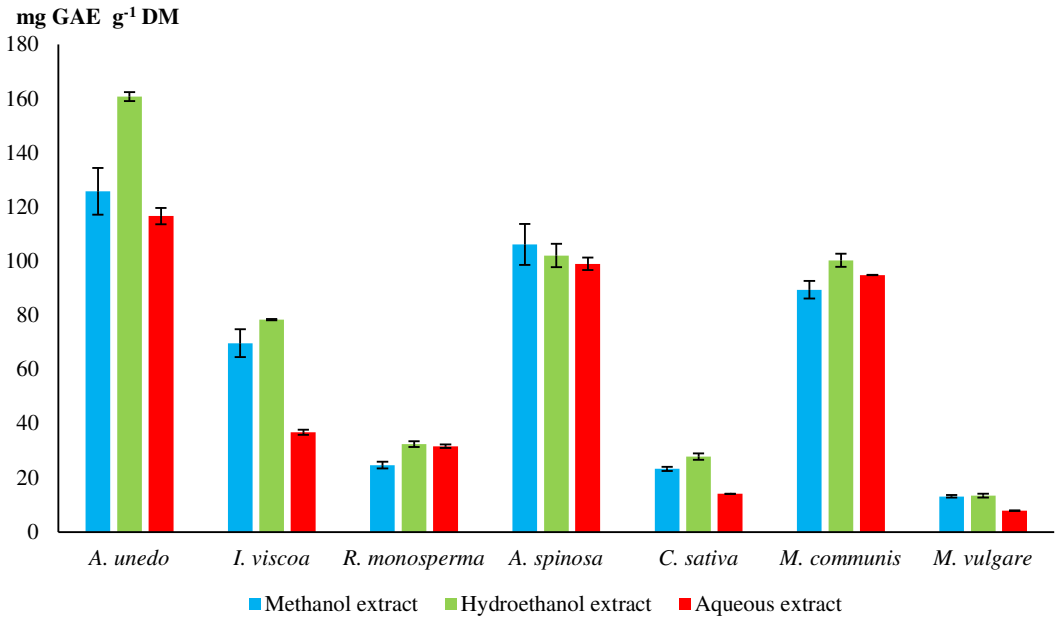


Figure 2. Total phenolic content of the extracts

Total flavonoids contents

The results of flavonoid contents were showed in Figure 3. Therefore, the concentration of flavonoids in the extract ranged from 36.01 to 489.77 mg QE g^{-1} DM. Analysis of the results allowed us to highlight that the highest content was the ethanol extract of *I. viscosa*. However, the aqueous extract of *R. monosperma*, had the lowest concentration.

Statistically a highly significant difference was observed between the different species studied and the three extraction solvents used ($dl = 12$, *F-ratio* = 23.251, $p < 0.001$).

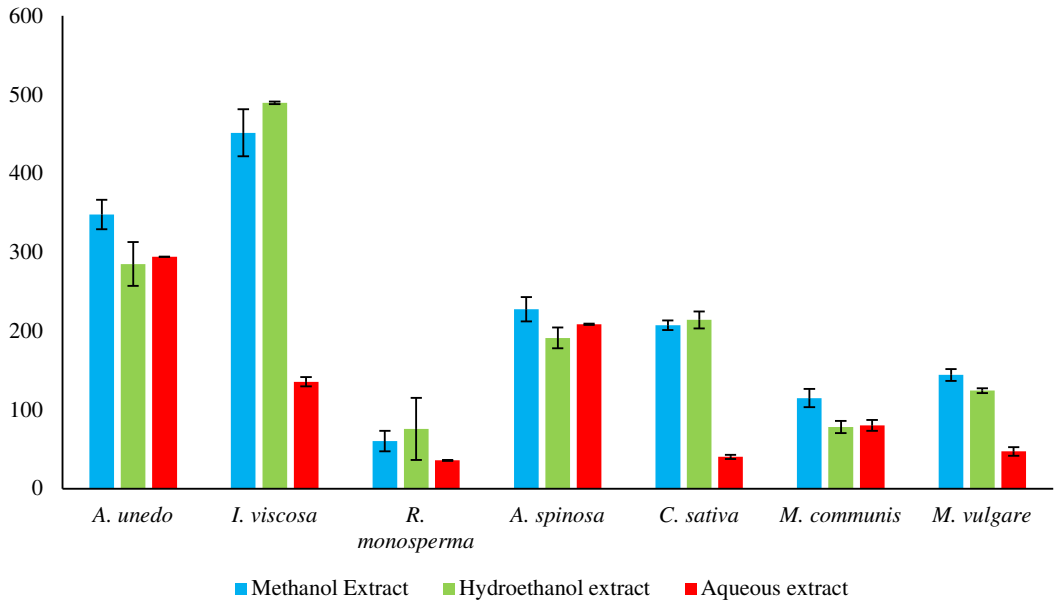


Figure 3. Total flavonoid content of the extracts

Evaluation of antioxidant activity

DPPH method

From the results, we found that all the extracts were able to reduce DPPH free radicals. Table 2 summarizes the IC_{50} values of the examined extracts and ascorbic acid. *A. unedo* recorded the lowest IC_{50} values for the three extraction solvents (methanol, hydroethanol, and aqueous extracts) with respectively 0.033; 0.026; and 0.034 mg ml^{-1}, reflecting its potent antioxidant activity approaching that of ascorbic acid (IC_{50} = 0.01 mg ml^{-1}). On the other hand, *R. monosperma* had the lowest anti-radical activity (IC_{50} = 0.957; 0.873 and 0.860 mg ml^{-1}).

The analysis of variance for IC_{50} showed a highly significant difference between the extracts and solvents used ($dl = 12$, *F-ration* = 101.246, $p < 0.001$).

Table 2. IC_{50} values (mg ml^{-1}) of tested extracts and ascorbic acid

MAPs / Extract	IC50 (mg ml^{-1})		
	Methanol	Hydroethanol	Aqueous
A. unedo	0.033 ± 0.0004	0.026 ± 0.0006	0.034 ± 0.0007
I. viscosa	0.065 ± 0.0001	0.057 ± 0.0004	0.094 ± 0.0007
R. monosperma	0.957 ± 0.0101	0.873 ± 0.0045	0.86 ± 0.0495
A. spinosa	0.048 ± 0.0001	0.043 ± 0.0012	0.046 ± 0.0011
C. sativa	0.666 ± 0.0175	0.467 ± 0.0040	1.032 ± 0.0175
M. communis	0.037 ± 0.0002	0.036 ± 0.0002	0.038 ± 0.0001
M. vulgare	0.346 ± 0.0017	0.329 ± 0.0009	0.635 ± 0.0081
Ascorbic acid	0.01 ± 0.0001		

Total antioxidant capacity

The results of the total antioxidant capacity are showed in Figure 4. The values obtained are expressed in terms of mg equivalent of ascorbic acid per gram of dry matter. According to these results, the evaluation of the total antioxidant capacity of the extracts showed a variability relative to the solvents. We found that the hydroethanol extracts present the highest antioxidant capacity, with 267.37 and 269.32 mg AAE g^{-1} DM respectively for *A. unedo* and *A. spinosa*. In contrast, the lowest concentration belongs to *R. monosperma* for the aqueous extract, with a value of 29.37 mg AAE g^{-1} DM.

The statistical analysis of the total antioxidant capacity shows a highly significant difference between the studied plants and the different solvents used (dl = 12, *F-ratio* = 15.293, p <0.001).

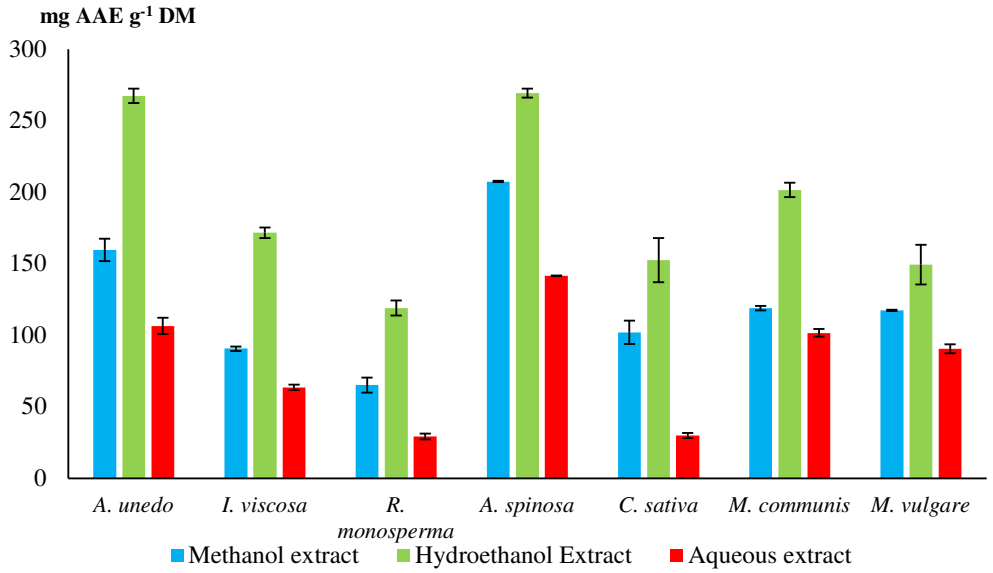


Figure 4. Total antioxidant capacity of the extracts

Discussion

Organic solvent extraction is the most commonly used method for the preparation of plant extracts (Zhang *et al.*, 2018), due to the modification of the solvent influencing the yield/composition of the isolated molecules, it also offers a selective method for the extraction of bioactive compounds (Kapadia *et al*., 2022).

In terms of antioxidant activity, our results indicated that hydroethanol extracts have the strongest antioxidant power, a finding consistent with the study conducted by Özbek *et al*. (2020).

For the phenolic contents of Arbutus unedo, our results ranged from 116.67 to 160.76 mg GAE g^{-1} DM, which are higher compared to those reported by Ait lhaj *et al*. (2022) with a highest result of 107.67 mg GAE g^{-1} DM and Habachi *et al*. (2022) with a highest result of 86 mg GAE g^{-1} DM, who used different extraction methods and solvents for the leaves. The best IC_{50} value in our study for the antioxidant activity was 0.026 mg ml^{-1} obtained in the hydroethanol extract, which is higher than the value reported by Bebek Markovinović *et al*. (2022) of 0.076 mg ml^{-1}, who used the same solvent.

In the case of *I. viscosa*, the phenolic contents were found to range from 36.82 to 78.41 mg GAE g^{-1} DM, consistent with the results of Aydar *et al*. (2022) who obtained a value of 54.39 mg GAE g^{-1} DM using a combination of ultrasonic and microwave extraction. Our best IC_{50} value was 0.057 mg ml^{-1} obtained in the hydroethanol extract, which is lower than the result of Yıldırım *et al*. (2022) who obtained 0.014 mg ml^{-1}, while using chloroform as solvent.

For *R. monosperma*, the phenolic contents were found to range from 24.7 to 32.44 mg GAE g^{-1} DM, with an IC_{50} value of 0.86 mg ml^{-1}. These results are lower compared to those reported by Selaimia *et al*. (2020) of 155.61 mg GAE g^{-1} DM and 0.118 mg ml^{-1}, they used a diethyl ether solvent and a maceration method.

For *A. spinosa*, we obtained for phenolic contents values between 99.02-106.21 mg GAE g^{-1} DM, which is higher than what was reported by Afrokh *et al*. (2023) with 47.75 mg GAE g^{-1} DM. On the other side, our IC_{50} value of 0.043 mg ml^{-1} obtained in the hydroethanol extract is consistent with the IC_{50} obtained in the same study of 0.048 mg ml^{-1}.

M. vulgare, resulted in values between 7.9-13.43 mg GAE g^{-1} DM for the phenolic contents, which is lower than what reported Afrokh *et al*. (2023) with a value of 29.25 mg GAE g^{-1} DM, whereas our IC_{50} of 0.329 mg ml^{-1} is higher than their result of 2.42 mg ml^{-1}.

For *C. sativa*, the phenolic contents were found to range from 14.07 to 27.88 mg GAE g^{-1} DM, which is in agreement with the findings of Aazza (2021) who reported a value of 19.07 mg GAE g^{-1} DM. The best IC_{50} value obtained was 0.467 mg ml^{-1}, which is higher compared to the results reported by Benkirane *et al*. (2023) at 1.83 mg ml^{-1}.

Finally, for the phenolic contents of *M. Communis* the results were between 89.47-100.3 mg GAE g^{-1} DM, which is higher compared to the results reported by Hazrati *et al*. (2022) at 66.52 mg GAE g^{-1} DM using a 24-hour hydro-ethanol maceration extraction method. The best IC_{50} value in our study was 0.036 mg ml^{-1} (hydroethanol extract), which is consistent with the findings of Yangui *et al*. (2021) of 0.038 mg ml^{-1} where they used a hydromethanol solvent.

The difference in phenol contents and antioxidant activity can be explained by the polarity of the solvent used in the extraction, knowing that the high solubility of phenols in polar solvents facilitates a better yield in extracts obtained from them (Alara *et al.*, 2021). This difference may also be due to several factors such as climate, soil, harvest period, storage condition, environmental factors (temperature, pH), or the extraction method (Ben Ahmed *et al.*, 2017; Sun *et al.*, 2019; Ngoune Liliane and Shelton Charles, 2020; Zeroual *et al.*, 2021; Zhang *et al.*, 2022).

Furthermore, it has been established that antioxidant activity is positively correlated with the structure of phenols (Osman *et al.*, 2020). Generally, phenols with a high number of hydroxyl groups present the highest antioxidant activity (Heim *et al.*, 2002) and that is due to their power to give more atoms to stabilize the free

radicals (Torres de Pinedo *et al.*, 2007). Thus, the antioxidant effect is not only dose-dependent but also structure-dependent (Rodríguez-Bernaldo de Quirós *et al.*, 2010).

Therefore, a mixture of these plants could be considered and could accentuate even more their antioxidant effects, allowing them to be considered as a possible alternative to remedy oxidative diseases.

Conclusions

During this work, we were able to highlight the medical potential of these plants. The quantitative analysis of the methanol, ethanol and aqueous extracts showed the presence of flavonoid and polyphenols. Furthermore, we were able to show that these plants have a good antioxidant activity especially *A. unedo*, *A. spinosa* and *M. communis*. Additionally, these plants compounds have significant therapeutic effects with few side effects, their natural antioxidants may offer an alternative to conventional treatments for oxidative stress and could be considerate strong candidates for the prevention of free radical diseases like cancer, aging process, cardiovascular disease, and diabetes. It would be important to extend the range of this study parameters as well as the isolation, characterization, and identification of active compounds to valorise more these plants.

Authors' Contributions

Data curation: AEM, MK; Methodology: AEM, CS, CR; Supervision: MK, CR, AN, EC, FB; Writing - original draft: AEM; Writing - review and editing: AEM, CS.

All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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