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Research Article

Extraction of nutrients from *Rumex vesicarius*, a wild indigenous edible plant from United Arab Emirates

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Abstract

In the present study, an attempt has been done to explore the phytochemicals and proximate compositions from an edible plant *Rumex vesicarius*, which is found in the wild habitats in United Arab Emirates. Extracts were prepared from the dried powder of the areal parts of the plant using methanol as solvent in Soxhlet. The extract was tested for phytochemicals and also studied for the proximate composition. The antioxidant power of the extract was determined by using four different assays (ABTS, DPPH, superoxide anion and hydroxyl radical scavenging activity). The results revealed that the extracts contain phytochemicals, which can be used as effective radical scavengers. The antioxidant activities were highly significant in the extract, which shows that this plant has high potential to be used in traditional and alternative medical systems. The present information would be of helpful for the future isolation and pharmacologically active compound identification from this plant.

Keywords: antioxidants; edible wild plant; phytochemical analysis; proximate composition

Introduction

In spite of the issues with biodiversity loss and other criticism pertaining to medicinal plant usage, in developing countries, the majority of people still depends on medicinal plant material for their basic healthcare needs (Van Wyk and Prinsloo, 2018). Many of the plant scientists have the opinion that two-thirds of the world's plant species have medicinal value and specially antioxidant potential (Krishnaiah *et al*., 2011). In order to solve the issues of ailments due to the production of reactive oxygen species (ROS), presently many medicines are available, but most of them with various side effects (Xiu-Qin *et al*., 2009). An alternative method to overcome the ROS issues is the use of plant-based medicines, and that is the reason why plants are the basis in the preparation of many of the modern pharmaceutical medicine throughout the world (Lee *et al*., 2003). Also,

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natural antioxidants typically cost more than synthetic antioxidants and are less effective (Lourenço *et al*., 2019; Sakkir *et al*., 2012).

Medicinal plants are used to cure a wide range of diseases all around the world. In United Arab Emirates, there are many reports about usage of wild plants in the traditional medicinal system (Alsamri *et al*., 2021). Apart from some studies about the phytoactive compound isolations and antioxidant and other medicinal or clinical studies, the scientific basis of this traditional usage is not yet fully authenticated. Many of the plants used in the traditional system showed potent antioxidant activities (Cosme *et al*., 2020). In plant kingdom, the phytochemicals are naturally occurring compounds which show various bioactive properties against stress, diseases and other ailments (Maheshwari *et al*., 2022; Favela-González *et al*., 2020). There are many phytochemical constituents which showed potent antioxidant activities (Chang *et al*., 2019).

Rumex vesicarius L. is a perennial flowering plant in the family Polygonaceae growing in United Arab Emirates and popularly known as 'hummayd' among local peoples (Alfawaz, 2006). This is one of the important edible plants growing in UAE and is being used by people in UAE and Middle East countries in their food preparations as a salad crop. Apart from the edible properties, this plant is being used as a medicinal crop in traditional medicinal systems (Vasas *et al*., 2015; Khan *et al*., 2022). In traditional medicine, the decoction of the seeds has been used to cure leprosy, rheumatoid arthritis, fever, and venereal disease (Vasas *et al*., 2015). The plant has great potential in the future owing to its wide usage in traditional systems. Earlier research showed cardioprotective action of *Rumex vesicarius* Linn leaf extract (Khan *et al*., 2022). Recent studies showed the impact of its seed extracts on mice fertility (Alhimaidi *et al*., 2021), nanoparticle isolation and biological activities (Younes *et al*., 2021) and nephroprotective (Hasan *et al*., 2021) and anti-inflammatory properties (Tabaasum *et al*., 2021). Earlier studies characterized the phenolic fractions from *R. vesicarius* in Egypt, and they concluded the presence of many phytoactive compounds in the extracts (El-Hawary *et al*., 2011). This plant is widely used in the traditional medicinal system as well as in traditional foods as an edible salad crop. Keeping the above information in consideration, through this study an attempt was made to scientifically validate the basis of this traditional practice through individual phytochemical, proximate and antioxidant studies from the methanolic extract of this plant.

Materials and Methods

Plant collection

The completely developed *Rumex vesicarius* plants from the UAEU campus were uprooted and collected in April 2022 in labelled plant collection bags. The plant was identified and authenticated at UAEU.

Extract preparation

After collection, the plant samples were washed with tap water for removing the soil or any other possible impurities and shade dried. The dried samples were powdered with the help of a blender and kept in bottles at room temperature. The extraction was done with the help of 1.5 L of methanol (Sigma-Aldrich, USA) from 500 g samples in Soxhlet extraction. Filtered through Whatman filter paper No. 1 (Whatman Ltd., England), dried and stored at 4 °C for further use.

Tests for phytochemicals

The tests for finding phytochemicals were done according to the standard methods (Mostafa *et al*., 2011; Edeoga *et al*., 2005; Harborne, 1998) described earlier. The presence of compounds tested were flavonoids, carbohydrates, alkaloids, saponins, phenolics, tannin, phlobatannins, terpenoids, cardiac glycosides, proteins and volatile oils.

Total phenolics and flavonoids estimation

Quantification of total phenolic content in the methanolic plant extract was determined by the Folin-Ciocalteau reagent method (Harborne *et al*., 1999). The Methanolic plant extract powder will be dissolved in 25% ethanol (v/v) to obtain a concentration of 0.5% (w/v). The solution (0.5 mL) was added to 100 µL of Folin–Ciocalteu reagent (two-fold diluted with de-ionized water) and mixed thoroughly. After 3 min, 1.5 mL of 2% sodium carbonate solution was added. The reaction mixture was thoroughly mixed and placed in the dark for 40 minutes, and the absorbance was read at 760 nm (Cole-Parmer, USA).

Total flavonoids in the plant were determined using the method of Zhishen *et al*. (1999). Different concentrations of extracts (20, 40, 60, 80 µg/mL) were mixed with 0.075 mL of $NaNO_2$. After 6 minutes, 0.15 mL of aluminum chloride was added and allowed to stand for 5 minutes. After incubation, 0.5 mL NaOH was added and made up to 2.5 mL with distilled water. The solution was well mixed, and the absorbance was immediately measured at 510 nm using a spectrophotometer. Quercetin at a concentration ranging from (5-30 µg) was used as the standard. The total flavonoid content in the extract was expressed as quercetin equivalents.

Proximate analysis and elements estimation

The proximate analysis for dry matter, moisture, crude protein, fibre, fat, ash and carbohydrate content was done with the methods of the Association of Official Analytical Chemists (A.O.A.C) (Zhishen *et al*., 1999; AOAC, 1990). The element contents in the dried plant sample were done by the method explained earlier (AOAC, 1995).

%Ash = (weight of crucible + ash –weight of crucible)/weight of sample x 100/1

%dry matter = fresh weight-dried weight/weight of fresh sample x 100/1

%Protein = % Nitrogen × 6.25

% Carbohydrate 100 (100 – moisture + ash+ fibre+ fat+ protein)

% Crude fat = Weight of flask with fat - weight of empty flask x 100/ Weight of original sample

In vitro antioxidant analysis

Four different *in vitro* assays were done to estimate the antioxidant activity of the extracts. They are 2, 2 ’ -Azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS•+) radical cation decolorization assay (USEPA, 1994), and. ABTS•<+ was dissolved in water at a concentration of 7 mM. The stock solution was mixed with 2.5 mM potassium persulfate. The mixture was allowed to stand in the dark at room temperature for 12-16 h before use for the incomplete oxidation of ABTS•+. The incubation mixture, in a total volume of 5 mL, contained 0.54 mL of ABTS•+, 0.5 mL of phosphate buffer, and varying concentrations of plant extracts (20–100 mg GAE). The blank contained water in place of plant extract. The absorbance was read with a spectrophotometer at 734 nm and compared with that of standard gallic acid.

DPPH• radical scavenging assay (Wolfenden and Willson, 1982), the reaction mixture in a total volume of 3 mL, contained 1 mL of DPPH•, various concentrations of plant extracts (20–100 mg GAE) and made up to a volume of 3 mL with water. The tubes were incubated for 10 minutes at 37 °C. A blue colour chromophore was formed, the absorbance of which was measured at 517 nm. Gallic acid was used as the standard for the comparison.

Superoxide anion scavenging activity (Brand-Williams *et al*., 1995), the incubation mixture in a total volume of 1 ml contained 0.1 ml of buffer, varying concentrations of plant extracts (20- 100 µg GAE), 0.2 mL ferric chloride, 0.1 mL ascorbic acid, 0.1 mL EDTA, 0.1 mL H_2O_2 and 0.2 mL 2-deoxyribose. The contents were mixed thoroughly and incubated at room temperature for 60 minutes, and then 1 ml of TBA and 1 ml of TCA were added. All the tubes were kept in a boiling water bath for 30 minutes. Gallic acid was used as a positive control for comparison. The absorbance of the supernatant was read in a spectrophotometer at 535 nm with a reagent blank containing water in place of plant extract.

The hydroxyl radical scavenging activity (Nishimiki *et al*., 1972), 1 mL of NBT, 1 mL of NADH solution, and varying volumes of plant extracts (20-100 µg GAE) were added and mixed well. The reaction was started by the addition of 100 mL of PMS. The reaction mixture was incubated at 30 °C for 15 minutes. This forms a violet colour complex indicating the generation of superoxide anion, which was measured spectrophotometrically at 560 nm. Gallic acid was used as a reference for comparison.

The IC_{50} value was determined from the plotted graph of scavenging activity against the different concentrations of extracts, which is defined as the total antioxidant necessary to express the amount or concentration of extracts needed to scavenge 50% of the free radicals.

Statistical analysis

All the experiments were carried out in triplicate, and the results were expressed as mean ± SD. Statistical analysis was performed using SPSS 13.0 and Excel 2003. Results were expressed as mean ± standard deviation for six animals in each group. P values < 0.05 were considered statistically significant.

Results

Preliminary phytochemical screening

Table 1 shows the results of the phytochemical composition in aerial parts of *R. vesicarius*. The results show that the methanolic extract has a high concentration of phytochemicals, which will be useful in the future separation and purification of secondary metabolites from this plant. All the analysed compounds have high therapeutically significance. Mainly phenolics, saponins, tannins, flavonoids, terpenoids, phlobatannins and alkaloids were present in the methanolic extract. In our tests, cardiac glycoside was absent in the sample.

Table 1. Phytochemical analysis of methanolic extracts of *Rumex vesicarius* L.

Phytochemical constituents	Observation	Inference /results
Flavonoids	Yellow colour Persistent froth	+
Phenolics	A Blue color at the interface	+
Alkaloids	The formation of a reddish-brown precipitate	+
Carbohydrates	Green colour is a positive test for reducing sugars	+
Terpenoids	Reddish brown coloured solution	+
Saponins	Formation of stable emulsion	+
Proteins	White precipitate which turns red	+
Tannins	Brownish Green color	+
Phlobatannins	A red precipitate	+
Cardiac glycosides	No yellowish-brown ring interface	-
Volatile oils	White colour precipitate	+

+ = Presence - = Absence

Proximate analysis

In this study, the proximate compositions were significant in the dried samples (Table 2). The data were presented in percentages (%). Proximate composition shows the plant's innate contents of nutrients, both for animal and human consumption. In this plant, the fiber and ash content were high with appreciable amounts of carbohydrate (17.21) and fat contents (7.21). There was 92.85 dry matter with a crude protein value of 28.01 in the dried samples of *R. vesicarius*.

Table 2. Proximate composition of aerial parts of *Rumex vesicarius* L. (g/100 g) of dried sample

Parameter	Dry weight basis (%)
Dry matter	91.48
Moisture	8.52
Crude Protein	28.01
NDF	41.01
Fat (EE)	7.21
Ash	8.21
Carbohydrate	7.04

Microelements and macroelements

The results of various micro and macro elements are presented in Table 3, which shows that the plant extract contains significant amounts of these nutrients. In this table 3, each result is an average of at least three independent measurements with a precision of about ± 1%. Fe, Mo, P and Zn were presented in appreciable quantities and low concentrations of Co, Mn and Cu were observed in *R. vesicarius*.

Table 3. Mineral composition of *Rumex vesicarius* L.

Minerals	Microelements (ppm)
Zn	40.32
Cu	7.25
Cr	2.91423
Fe	288.5
Pb	<0.011
Mn	17.2
Ni	4.06
Cd	4.321
Co	0.181
	Macroelements (ppm)
Ca	1.600
Na	2.99
K	1.081
Mg	2.691
S	2.901
P	2.12

Total phenolic and flavonoid content

The results of total phenolic and flavonoid contents are given in Table 4. Total phenolic content was expressed in mg of gallic acid equivalent (mg /g) of dry extract. In the concentration of total phenolic content (TPC), *R. vesicarius* has a value of 18.48 in the aerial parts. Total flavonoid content was expressed in quercetin equivalent per mg plant dry extract (mg/g). The total flavonoid content in *R. vesicarius* was 10.24 in methanolic extracts.

Table 4. Total phenolic and flavonoid contents of methanolic extracts *Rumex vesicarius*

Solvent	Phenolics (mg of GAE/g of dry extract)	Flavonoids (mg of QE/g of dry extract)
Methanol	18.48	10.24

Antioxidant activities

Figure 1 shows the effectiveness of the methanolic extract of *R. vesicarius* in scavenging the ABTS radicals in comparison with the standard GAE. The percentage of inhibition was 51% for the plant extract and 45% for GAE respectively at 80 µg/mL concentration. In ABTS+ scavenging activity the values varied significantly ($P < 0.05$) and ranged from 20 to 100 µg GAE/mL extract. The inhibition percentage of *R. vesicarius* was higher than the GAE and IC_{50} values were 36.86 and 38.42 µg/mL, respectively. The ABTS cation scavenging activity results of the extracts and the positive control were not significant as they had IC_{50} variations.

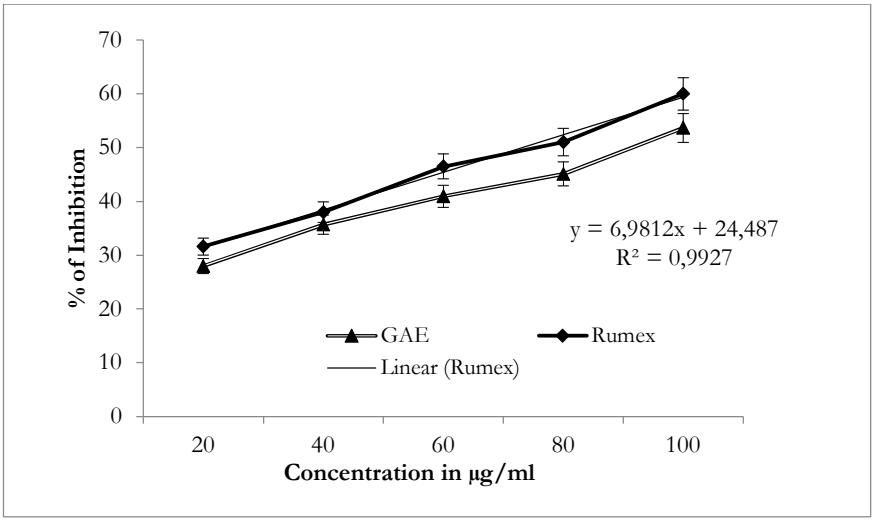

Figure 1. ABTS free radical scavenging activity of the methanol extracts of *R. vesicarius* in comparison with gallic acid

Values are the average of triplicate experiments and represented as mean±SD.

As in Figure 2, the methanolic extract of *R. vesicarius* showed a significant dose dependent inhibition of DPPH activity, with a 50% inhibition (IC_{50}) at a concentration of 54.1 µg/ml the IC_{50} value of the extract was found to be close to that of the standard; GAE (38.6 µg/ml). The percentage hydroxyl radical-scavenging activity of *R. vesicarius* and GA at various concentrations are presented in Figure 3. Both *R. vesicarius* and GAE showed significant inhibitory activity in a concentration-dependent manner. The superoxide scavenging activity of the aerial parts of the plant extracts and standard gallic acid is shown in Figure 4. The plant extracts exhibited concentration-dependent radical scavenging activity, that is, percentage inhibition increased with sample concentration. The increase in activity is due to increase in number of phenolic hydroxyl groups in the molecule.

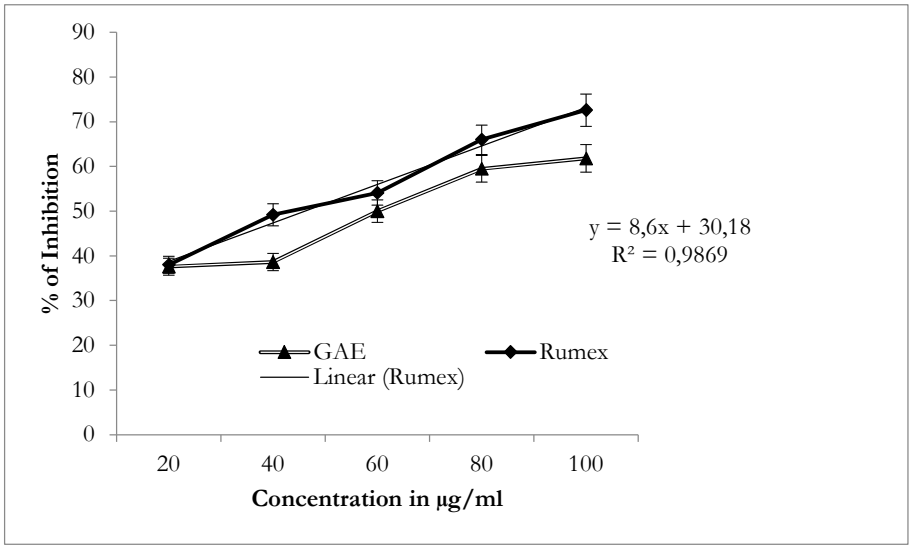

Figure 2. DPPH radical scavenging activity of the methanol extracts of *Rumex vesicarius* in comparison with gallic acid

Values are the average of triplicate experiments and represented as mean±standard deviation.

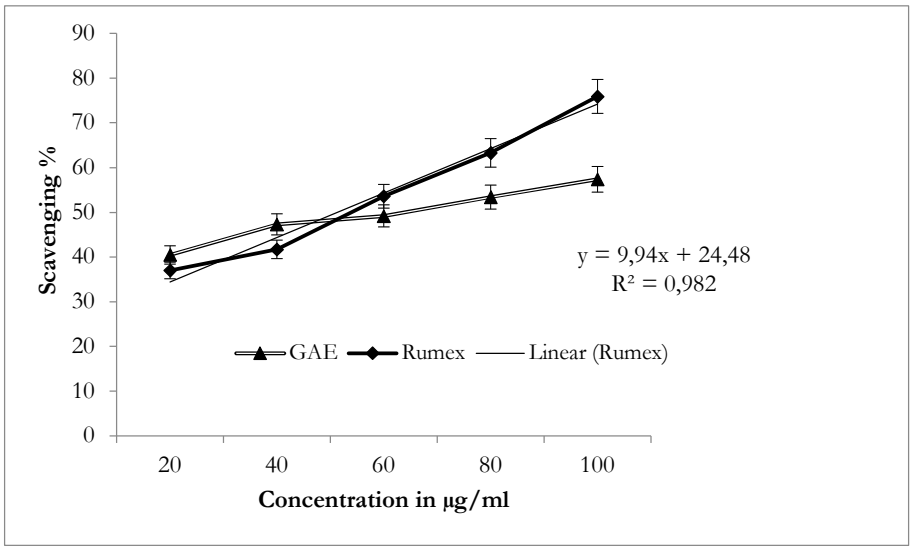

Figure 3. Hydroxyl radical scavenging action of methanol extract of *Rumex vesicarius* in comparison with gallic acid

Values are the average of triplicate experiments and represented as mean±standard deviation.

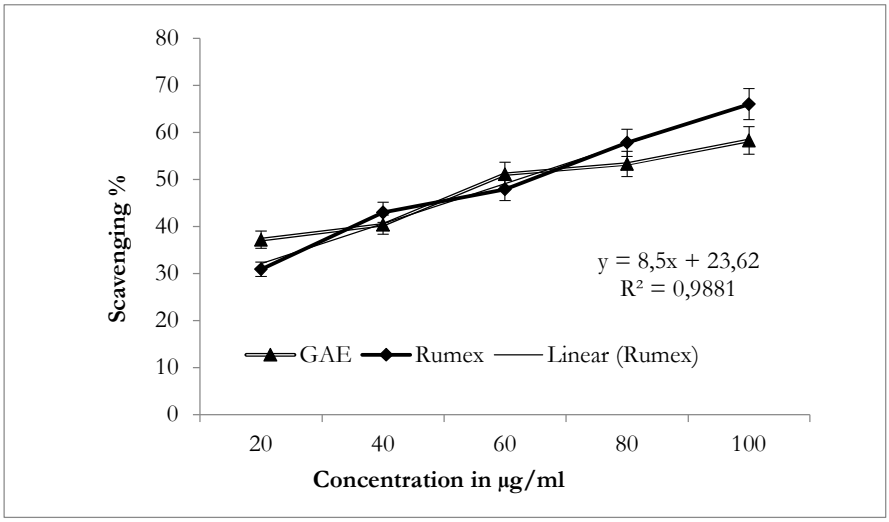

Figure 4. Superoxide radical scavenging action of methanol extract of *Rumex vesicarius* in comparison with gallic acid
Values are the average of triplicate experiments and represented as mean±standard deviation.

Discussion

In the present study, we reported presence of important secondary metabolites in the methanolic extracts of *R. vesicarius*. Earlier studies reported the phytochemicals in this plant, mainly from different extracts like chloroform, ethyl acetate etc., In an earlier work (Ammar *et al*., 2015), the different extracts of *R. vesicarius* showed the presence of phytochemicals, but the authors didn't study about methanolic fractions from this plant. In contrary to our results, an earlier study reported absence of alkaloids and saponins in this plant extract (Alfawaz, 2006). The presence or absence of phytochemicals depends on the environmental factors in which the plant is growing, and also the assay conditions (Ghasemzadeh *et al*., 2018).

In dried plant samples, the proximate composition showed high levels of nutrient contents. As this is an edible wild plant, the nutrient contents are highly significant, as the local populations consumes this plant in raw conditions. In an earlier report an effort was done to estimate the proximate composition and anti-nutrient factors from another species of the same genus (*Rumex crispus*) (Idris *et al*., 2019) and showed significant portions of proximate values, suggesting that this genus is highly nutritious in terms of human consumption. We reported 28.01 protein from the dried samples. This may be due to the seasonal and climatic variations in the habitat of the plant.

Micro and macro elements were present in significant amounts in the dried plant samples of *R. vesicarius*. Element contents were reported earlier from different species like *Rumex hastatus*, *Rumex dentatus* and *Rumex nepalensis* (Hameed and Dastagir, 2009; Edeoga *et al*., 2005). In another study, Calcium, copper, iron, magnesium, potassium, sodium and zinc were determined from the aerial parts of this plant (Salama *et al*., 2022), and reported significant amounts of these elements.

We reported the antioxidant activity of methanolic extracts from *R. vesicarius*. Earlier, the total antioxidant activities of different extracts like hexane, chloroform, ethyl acetate, n-butanol and water were reported and showed highly significant activities (Bhatt *et al*., 2022; Prasad and Ramakrishnan, 2011; Beddou *et al*., 2015; Tajdar *et al*., 2014).

Conclusions

From our results, it is clear that, the wild edible plant *R. vesicarius* has important secondary metabolites with potent antioxidant activities. The free radical scavenging activities were significant in all four assays. Also, the plant has excellent shelf life also, because of the low level of moisture content. Even though the plant has phytochemical contents and antioxidant activities, individual active compound isolation and characterization is needed in order to elucidate the structure of phyto-active principles compounds, which could be used for pharmaceutical use, which is the next step in our study.

Authors' Contributions

Conceptualization: A.J; Formal analysis: R.A.S.A., H.H.B.M.; Investigation: R.A.S.A., H.H.B.M., K.K.; Methodology: K.K.; Project administration: A.J.; Supervision: A.J.; Validation: A.J., Z.F.R.A.; Writing - original draft: R.A.S.A., H.H.B.M., K.K.; Writing - review & editing: Z.F.R.A., A.J. 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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