IN VITRO ANTIOXIDANT ACTIVITY OF DIFFERENT EXTRACTS OF ALGERIAN *ALLIUM* PLANT (*Allium triquetrum* L.)

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Abstract To enrich the knowledge of new bioactive molecules and their natural resources, the present study aims to value an endemic plant growing in Algeria, named *Allium triquetrum* L., by evaluating the antioxidant activity of aqueous and methanolic extracts from the leaves (AEL, MEL) and the bulbs (AEB, MEB), by using DPPH scavenging and FRAP methods, with a search of main chemical groups and determination of total phenolic compounds. Phytochemical study revealed mainly, the presence of flavonoids, saponins and glycosides. Furthermore, AEL has presented the greatest levels of polyphenols (14.31 ± 0.36 mg GAE/g dw) and flavonoids (22.12 ± 0.33 mg QE/g dw). The aqueous extracts have a high antioxidant capacity comparing to the methanolic extracts (EC50: AEL = 0.97 mg/ml, AEB = 1.36 mg/ml). Our results indicate clearly that *Allium triquetrum* extracts show important antioxidant activity and could be used as a new source of natural antioxidants molecules
Keywords Antioxidant activity; *Allium triquetrum* L.; extracts; polyphenols; flavonoids.

ACTIVITÉ ANTIOXYDANTE DE DIFFÉRENTS EXTRAITS D'UNE PLANTE ALLIUM ALGÉRIENNE (*Allium triquetrum* L.) *IN VITRO*

Résumé: Afin d'enrichir les connaissances sur les nouvelles molécules bioactives et leurs ressources naturelles, cette présente étude a pour objectif de valoriser une plante endémique qui pousse en Algérie nommée *Allium triquetrum* L. par l'évaluation de l'activité antioxydante des extraits aqueux et méthanoliques des feuilles (AEL, MEL) et des bulbes (AEB, MEB) en utilisant la méthode du piégeage du radical libre DPPH et la méthode de FRAP, avec la recherche de principaux groupes chimiques et la détermination du taux de composés phénoliques. L'étude phytochimique a révélée principalement la présence de flavonoïdes, saponines et glycosides. En outre, AEL a présenté la plus grande teneur en polyphénols (14.31 ± 0.36 mg GAE/g dw) et flavonoïdes (22.12 ± 0.33 mg QE/g dw). Les extraits aqueux présentent une activité antioxydante élevée en comparaison avec les extraits méthanoliques (EC50: AEL = 0.97 mg/ml, AEB = 1.36 mg/ml). Nos résultats montrent clairement que les extraits d'*Allium triquetrum* présentent une activité antioxydante importante et pourrait être utilisé comme une nouvelle source de d'agents anti-oxydants naturels.
Mots clés Activité antioxydante, *Allium triquetrum* L.; extraits; polyphénols; flavonoïdes

Introduction

An antioxidant can be defined as any substance that when present at low concentrations compared to that of an oxidizable substrate, significantly delays or inhibits the oxidation of that substrate [3]. The body makes some of the antioxidants, it uses them to neutralize free radicals. These antioxidants are called endogenous antioxidants. However, the body relies on external (exogenous) sources, primarily the diet, to obtain the rest of the antioxidants it needs. These exogenous antioxidants are commonly called dietary antioxidants [4]. Dietary antioxidants compounds include flavonoids, phenolic acids, carotenoids and tocopherols that can inhibit Fe^{3+}/AA induced oxidation, scavenge free radicals, and act as reductants [5]. Medicinal and aromatic plants are still a most important part of complementary and alternative medicine in the developing countries. The use of medicinal herbs as antioxidant and anti-inflammatory is common in our

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country. For xample, garlic (*Allium sativum*) and shallots (*Allium ascalonicum*) have antioxidant and free radical-scavenging characteristics and identifiable odors at low concentrations. They contain two main classes of antioxidant compounds: flavonoids (flavones and quercetins) and sulfur-containing compounds (allyl-cysteine, diallyl sulfide and allyl trisulfide) [5].

Allium is the most representative genus of the *Liliaceae* family, which includes 700 species of widely distributed bulbous perennials and biennials. At present *Allium* spp. plants are considered the most important vegetables consumed fresh or in different cooked dishes in the world. These plants are used as common foods, and for the treatment of many diseases, due to their content of phytonutrients. In fact, the edible parts of *Allium* spp. plants are used for the treatment and prevention of a

In order to contribute to the search for new bioactive molecules and new naturals sources, this work aims to study a spontaneous endemic plant named *Allium triquetrum* L. (Algeria), by determining of its phytochemical components, and evaluating of *in vitro* antioxidant proprieties of aqueous and methanolic extracts of its two parts (leaves and bulbs) with their reducing capacity. Polyphenolics and flavonoids contents of different extracts have also been estimated

number of diseases: coronary heart disease, cancer, obesity, diabetes, disturbances of the gastrointestinal tract, hyper-cholesterolemia and inflammatory diseases [6].

Many researchers have studied the chemical composition, antioxidant activity and other biological properties of several species of *Allium* genus, especially cultured as garlic and onion, but few studies have been conducted on spontaneous species as *Allium triquetrum* L. This is a very early blooming species which grows vigorously in cultivations. It is characterized by green striped, white, pendulous flowers looking like small lilies. It possesses several vernacular names (e.g., triangle onion, triangular-stalked garlic, three-cornered leek), which refer to different taxa. The plant is used as a main ingredient in salads and soups, because of its mild taste similar to onion [7].

1- Material and methods

This work has been undertaken at the laboratory of physicochemical analyzes of Saidal group (Medea, West of Algeria).

Plant material

The harvest of *A. triquetrum* was carried out in the region of Mitidja (Blida, Algeria) during the flowering period (March-May, 2013). Its identification was performed at the laboratory of Plant Biology, Department of Biology and cellular physiology at the University of Blida 1 and the Department of Botany of the National College of Agronomy (ENSA El-Harrach, Algiers) (Fig. 1). The parts of the plant were separated, washed, cut into small pieces and then dried and protected from light at room temperature (25-28 °C). The plant material is finally reduced to powder.

Phytochemical tests

The chemical groups of the two parts of the plant (leaves and bulbs), were identified by precipitation and staining reactions according to the methods described in the literature [8,9,10].

Preparation of extracts

In order to determine the antioxidant efficacy of different parts of the plant, the aqueous and methanolic extracts were prepared from two parts (leaves and bulbs), using the protocol of Juvekar and Halade (2006) with slightly modification [11].

Aqueous extracts (AEL and AEB)

20 g of the powder of each part of the plant were placed in 500 ml of boiling distilled water (100 °C) under magnetic stirring, for 30 minutes. The aqueous extracts are then cooled, filtered by Whatman filter paper 4, evaporated using a vacuum rotary evaporator (Laborota 4001- efficient Heidolph 2, Schwabach, Germany) to obtain dry concretes and kept at 4 °C until further use [11].

Methanolic extracts (MEL and MEB)

These extracts were obtained by maceration of the plant powder (20 g) in 500 ml of methanol under magnetic stirring for 24 h (initially, the plant powder was defatted with petroleum ether to remove the lipids). Methanol enriched by polar substances was evaporated by a vacuum rotary evaporator at 40° C to obtain dry concrete. These dry concretes were conserved at 4° C, within 1 month, until further use [11].

Evaluation of antioxidant activity of the extracts

The antioxidant activity of the extracts was evaluated by two methods: DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging and ferric reducing antioxidant power assay (FRAP) [12-13-15].

DPPH radical scavenging capacity

The DPPH assay has many advantages comparing to other methods, such as good stability, credible sensitivity, simplicity and feasibility [12]. The protocol of Atoui and *al.* (2005) [13] with slightly modification was used to evaluate this capacity. A quantity of 2 ml of methanolic solution of DPPH (4%) was added to 1 ml of each extract at different concentrations. After, the mixtures were incubated at room temperature in the dark for 30 minutes and the decrease in the absorbance was determined at 515 nm using a UV-Visible spectrophotometer Lambda 25 (PerkinElmer, Waltham, Massachusetts,

USA). Methanol presents a blank and DPPH solution a negative control. A positive control was presented by gallic acid (GA). The percentage of DPPH scavenging activity was determined as follows:

$$DPPH\ radical\ scavenging\ activity\ (\%) = \frac{A_0 - A_1}{A_0} \times 100$$

A_0 is the absorbance of negative control and A_1 is the sample absorbance.

The efficient concentration EC50 (otherwise called the inhibitory concentration IC50) value was determined for each sample. This is defined as the concentration of substrate that causes 50% loss of the DPPH activity (color) [14].

Ferric Reducing Antioxidant Power FRAP assay

Reducing power was determined by the method prescribed by Yen and Chen [15]. The 1 ml of each sample at various concentrations was mixed with a phosphate buffer (2.5 ml, pH 6.6) and potassium ferricyanide $K_3[Fe(CN)_6]$ (2.5 ml, 1%), and the mixture was incubated at 50° C for 30 minutes. Next, 2.5 ml of trichloroaceticacid (10%) were added to the reaction mixture, which was then centrifuged at 3000 RPM (rotation per minute), for 10 min. The upper layer of the solution (2.5 ml) was mixed with distilled water (2.5 ml) and ferricchloride (0.5 ml, 1%), and the optical density (OD) was measured at 700 nm (UV-Visible spectrophotometer, Perkin-Elmer Lambda 25). A high absorbance will indicate an increase in reducing power. GA was used as a reference antioxidant substance [15].

Estimation of total polyphenols

The determination of total polyphenols is accomplished by the method described by Juntachote and *al*. [16]. To 0.5 ml of each sample, 5 ml of distilled water and 0.5 ml of Folin-Ciocalteu's were added. After 3 min, 0.5 ml of sodium carbonate at 20% were added and incubated for one hour at room temperature in the dark. The OD was read at 760 nm using a UV-Visible spectrophotometer (Perkin-Elmer Lambda 25). Standard solutions of GA with different concentrations were used to obtain a standard curve and the results were expressed as milligram gallic acid equivalents per gram of dry weight of extract (mg GAE/g dw) [16].

Estimation of total flavonoids

The method of aluminum trichloride $AlCl_3$ was adopted to quantify total flavonoids in different extracts. To 1 ml of each sample, 1 ml of 2% w/v $AlCl_3$ in methanol was added and incubated at room temperature for 30 minutes. Absorbance was read at 430 nm. Quercetin (Q) was used as a standard and the results were expressed in milligram of quercetin equivalents per gram of dry weight of extract (mg QE/g dw) [17].

Statistical analysis

The Microsoft Office Excel 2007 program was used to realize: linear regressions, histogram and curves of antioxidant activity, calibration curves, and means ± standard deviations.

Comparison between groups was conducted by one-way analysis of variance (ANOVA) followed by Tukey's post hoc multiple comparison test. Differences between groups with $P<0.05$ were considered statistically significant [18]. Statistical analysis was performed by probit analysis method using XLStats 2013 software (Pros statistical software, Addinsoft, Paris, France).

2- Results and discussion

Phytochemical screening

The results of the phytochemical study are shown in Table 1.

Table 1 Results of phytochemical screening of *Allium triquetrum* L.

Chemical compound	**Leaves**	**Bulbs**
Alkaloids	-	-
Anthocyanins	-	-
Anthracenosids (Combined forms)	-	+
Anthracenosids (Oxidative forms)	-	-
Anthraquinones	-	+
Cardiotonic heterosides	++	++
Coumarins	-	-
Flavonoids	+++	+++
Glycosides	++	+++
Polyphenols	++	+
Iridoids	++	+
Tannins	-	-
Mucilage	+	++
Phenolic derivatives	++	+
Quinones	-	-
Saponosids	++	+++
Terpenes	+	+
-: absent / +: slightly present / ++: moderately present / +++: highly present		

The phytochemical screening of leaves and bulbs of *A. triquetrum* showed the presence of polyphenols, flavonoids, phenolic derivatives, saponosides, cardiotonic heterosides, mucilage, glycosides and iridoids, trace amount of terpens and absence of alkaloids, tannins, quinones, anthraquinones, anthracenosids, coumarins and anthocyanins. Some combined forms of anthracenosids were present in the bulbs.

This work confirmed that *A. triquetrum* contains various chemical compounds as well as compared to the different species of *Allium* genus. Polyphenols, flavonoids, saponins and glycosides are the most important secondary metabolites in *Allium* spp., as garlic (*A. sativum*), onion (*A. cepa*) and leek (*A. porrum*). However, the species of this genus contains big amounts of other molecules such as coumarins, amine acids, tannins, sterols, etc [19,20,21]. The study of Najjaa *et al*. (2011) has shown the presence of a high amount of iridoids and coumarins with the absence of anthracenosids and anthraquinons in the leaves of *A. roseum* and *A. ampeloprasum* [8]. Another interesting study of Egyptian leek (*A. ampeloprasum* var. *kurrat*) showed the presence of alkaloids, tannins steroids, flavonoids, terpenoids and saponins [22].

The richness and diversity of secondary metabolites in the plants of the genus *Allium* explains their use for the treatment

and prevention of many diseases especially cancer diabetes and hypercholesterolemia.

Antioxidant activity

DPPH radical scavenging capacity

The linear regressions for antioxidant power of different extracts and control were used to calculate the EC50 values. The results of radical scavenging assays and EC50 values are given in Fig. 2 and Table 2, respectively.

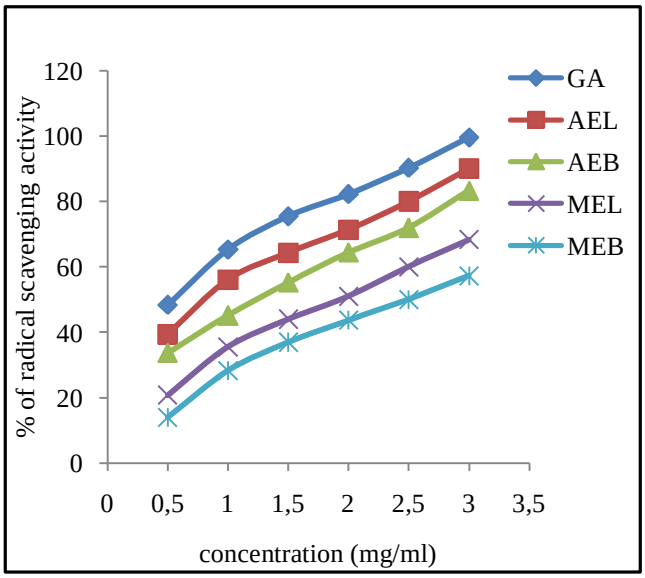

Fig. 2 Curves of DPPH radical scavenging activity (%) of control, aqueous and methanolic extracts of *Allium triquetrum* L. according to different concentrations

Table 2. EC50 values of control, aqueous and methanolic extracts of *Allium triquetrum* L.

Control/Extract	**EC50 Values (mg/ml)**
GA	0.62
AEL	0.97
AEB	1.36
MEL	1.98
MEB	2.45

The outcomes of this study showed that *A. triquetrum* is endowed with good antioxidant activity in comparing with the positive control GA which showed an inhibitory rate equal to 99.56% at 3 mg/ml with EC50 equal to 0.62 mg/ml. AEL has the highest radical scavenging activity (90%) followed by AEB (83.3%), their EC50 values are equal to 0.97 mg/ml and 1.36 mg/ml, respectively. While, the lowest activities are given by the methanolic extracts of bulbs (MEB 57.3%) and leaves (MEL 68.3 %) which EC50 are equal to 2.45 and 1.98 mg/ml, respectively. This study confirms the results obtained in previous researches [23,24]. Chang and *al.* (2013) showed that the DPPH free radical scavenging activity of *Allium* spp. ranged

from 67.34% to 90.03% for *A. fistulosum*, while it ranged from 63.63% to 88.33% for *A. sativum*. Furthermore, the leaf extracts had higher DPPH free radical scavenging activity than that of the stem, bulb and root. In contrast, Tugbobo and *al*. (2015) found that DPPH scavenging ability of the extracts was higher in aqueous extract than ethanolic extract as 41.08% was obtained for aqueous extract while 39.46% was obtained for ethanolic extract at same concentration.

The antioxidant activity of *Allium* spp. has been attributed mainly to a variety of sulphur containing compounds and their precursors. Scientific evidence shows that allicin, diallyl disulphide and diallyl trisulphide appeared to be the main antioxidative compounds. In addition, the antioxidant activity is also related to other bioactive compounds: dietary fibers, microelements (especially Se) and polyphenols [23,25,26]. Phenolic compounds are large groups of secondary metabolites that are able to neutralize or quench the free radicals. Flavonoids and their derivatives are the largest group of polyphenols found in plants that possess strong antioxidant activities due to the scavenging reactive oxygen species and inhibition of oxidative stress [27].

Ferric Reducing Power* FRAP *assay

The results are plotted in Fig. 3.

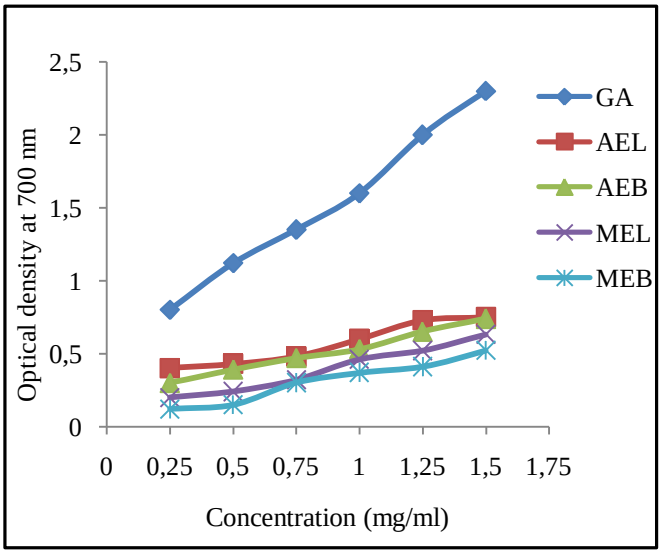

Fig. 3 Ferric reducing power of control, methanolic and aqueous extracts of *Allium triquetrum* L.

Similarly to DPPH radical scavenging capacity, FRAP assay also showed that the different extracts of *A. triquetrum* possess an antioxidant activity but which is less important compared to the control GA (OD at 3 mg/ml equal to 2.5). The higher reducing power is shown by the AEL with an OD equal to 1.22 followed by AEB (OD equal to 1.02), while the methanolic extracts have the lower reductive capacity (OD: MEL = 0.72, MEB = 0.4).

Also, the experiment of Tugbobo and *al*. (2015) has confirmed that aqueous extract of *Allium sativum* has higher reducing potential than ethanolic extract [24]. On the other hand, Nencini and *al*. (2007) has proved that the flowers of species growing wild showed the higher reducing power.

Interesting results were shown even by the leaves, while the antioxidant capacity of the bulbs was lower [28].

Reducing power is a novel antioxidation defence mechanism; the two mechanisms available to affect this property are by electron transfer and hydrogen atom transfer. This is because the ferric-to-ferrous ion reduction occurs rapidly with all reductants with half reaction reduction potentials above that of Fe^{3+}/Fe^{2+}, the values in the Ferric reducing antioxidant property (FRAP) assay will express the corresponding concentration of electron-donating antioxidants [29].

According to the literature [30], the antioxidant activity of plants depends on the type, quality, part (leaves, stalk, flower, seeds) of the plant, location of habitat, climatic conditions, soil characteristics, etc. Extraction method and solvent agent (water, alcohol, etc.) are also important factors, considerably affecting plant antioxidants capacity.

Total polyphenols and flavonoids content

The equations of calibration curves of quercetin ($y = 0.004 x + 0.013$; $R^2 = 0.996$) and gallic acid ($y = 0.049 x + 0.099$; $R^2 = 0.992$) were used for the estimation of total polyphenols and flavonoids respectively. The results are presented in Fig. 4 and Fig. 5.

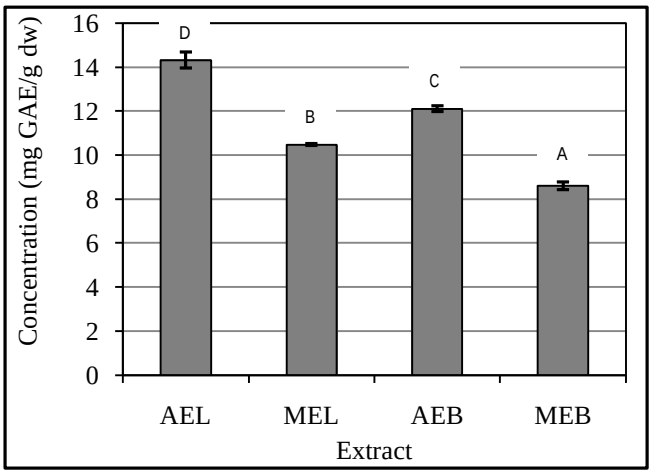

Fig. 4 Total polyphenols of various extracts of *Allium triquetrum* L expressed as means ± standard deviations (Means with different letter are significantly different (PB0.05) according to ANOVA oneway analysis followed by Tukey's post hoc multiple comparison test).

This study showed the presence of important levels of phenolic compounds in the different extracts with a significant differences between their means (P<0.05).

The largest concentration is presented by the AEL, the total of polyphenols and flavonoids which equal to 14.31 ± 0.36 mg.GAE/ g dw and 22.12 ± 0.33 mg QE/g dw, respectively, compared to 12.09 ± 0.13 mg GAE/g dw and 16.05 ± 0.13 mg QE/g dw for AEB. The methanol extracts showed somehow lower concentrations, MEL presents 10.46 ± 0.04 mg GAE/g dw of polyphenols and 14.27 ± 0.20 mg QE/g dw of flavonoids, however their amounts in MEB are equal to 8.59 ± 0.18 mg GAE/g dw and 12.79 ± 0.10 mg QE/g dw, respectively. On the other hand, we noticed that leaves contain higher levels comparing to the bulbs.

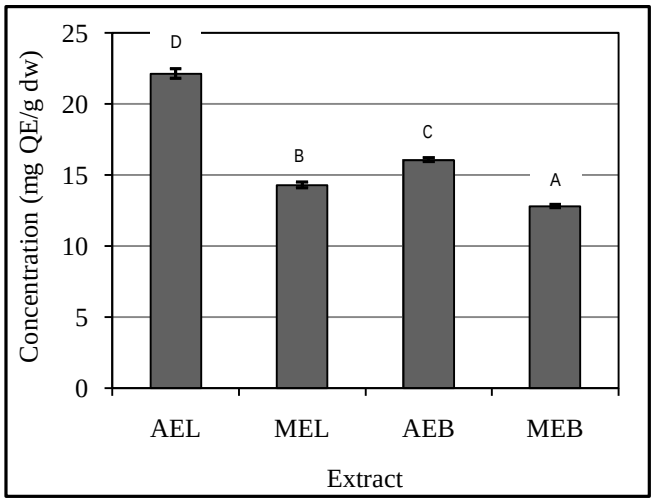

Fig. 5 Total flavonoids of various extracts of *Allium triquetrum* L expressed as means ± standard deviations. (Means with different letter are significantly different (PB0.05) according to ANOVA one way analysis followed by Tukey's post hoc multiple comparison test).

The content of phenolic acids and flavonoid varied in different solvents. Turkmen and *al*. (2006) reported that solvents with different polarity (ethanol and water) have significant effect on polyphenols content [31]. In addition, antioxidant activity is higher in more polar extraction solvents. Settharaksa and *al*. (2014) showed that water was the best solvent for phenol and flavonoid extraction [32].

These results justify the difference between potential of antioxidant activity for different extracts of *A. triquetrum*. Phenolics and flavonoids were the major contributors to antioxidant activity and their higher content was associated with higher antioxidant activity [33]. Since the antioxidant activity of phenolics is mainly due to their redox properties, this allows them to act as reducing agents, hydrogen donors and singlet oxygen quenchers In addition, to their physiological roles in plants as an important contributor to the survival of plant species they have benefits as antioxidants for human health [29].

Conclusion

This work showed that the Algerian plant *A. triquetrum* L. like the most *Allium* species contains various chemical compounds such us flavonoids, saponins and glycosides, and is endowed with good to moderate antioxidant activity. The aqueous extracts have a highest antioxidant capacity and showed the greatest levels of polyphenols and flavonoids comparing to the methanolic extracts. The difference between antioxidant capacities of its different extracts is related to phenolic content which is correlated to the plant part or portion and to the solvent and extraction method. *A. triquetrum* can be used as a new source of natural antioxidants molecules. However, these results require further research work, in order to identify and characterize the bioactive substances involved, in particular phenolic compounds, and to know their mechanisms of action.

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