

# Towards the Utilization of Date Palm (*Phoenix dactylifera*) Leaves as a Rich Source of Antioxidants

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**Abstract** Three varieties of date palm (*Phoenix dactylifera*) leaves (DPL) namely: Sagaie, Khalas, and Nabtat Alsaif were examined for their antioxidant activities. DPL extracts showed a total phenolic content ranged between  $1498.14 \pm 100.52$  -  $4374.76 \pm 657.64$  mg gallic acid equivalent (GAE)/100g dry weight (DW) and a total flavonoid ranged between  $134.70 \pm 16.24$  -  $224.62 \pm 22.35$  mg quercetin (QE)/100g (DW). The IC<sub>50</sub>s for scavenging ABTS radical were ranged between  $71.87 \pm 14.37$ -  $217.87 \pm 27.40$  μg Trolox equivalent, whereas that required for scavenging DPPH were ranged between  $197.86 \pm 17.60$ - $693.32 \pm 25.44$  μg ascorbic acid equivalent. DPL extracts potentially scavenged hydroxyl and superoxide anion radicals, IC<sub>50</sub>s values were ranged between  $208.04 \pm 12.76$ - $719.63 \pm 24.38$  and  $825.92 \pm 19.40$  - $1193.20 \pm 28.74$  μg GAE respectively. DPL extracts showed a good activity against ferric reducing antioxidant power (FRAP) ranged between  $7.69 \pm 0.47$ -  $22.13 \pm 1.36$  mmol ascorbic acid equivalent/100g. DPL extracts showed a considerable antioxidant activity, therefore, DPL could be effectively used as natural source of antioxidant in food and pharmaceutical industries.

**Keywords:** date palm leaves, total phenols, total flavonoid, free radicals, scavenging activity

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## 1. Introduction

Date palms, *Phoenix dactylifera*, are an important fruit crop in the Kingdom of Saudi Arabia. Annual production reached 1302859 tones [1]. There are more than 20 million fruit-producing date palm trees in the Kingdom which add substantial value to the national economy [2]. Thousands of tones of date palm leaves (DPL) and date palm seeds (DPS) are disposed off annually in Saudi Arabia; however, both are containing substantial secondary metabolites that include polyphenolic compounds such as flavonoids, antioxidants, and phenolic acids, that can be utilized as a good source for functional foods [3]. A number of phenolic compounds were detected in both DPL and DPS including catechin, epicatechin, rutin, ferulic acid, and proanthocyanidin [4]. DPL extracts have been reported to exhibit inhibitory effect against the growth of *Staphylococcus* (*S.*) *aureus* and *Bacillus* (*B.*) *subtilis* and resistance against *Pseudomonas* (*P.*) *aeruginosa* and *Escherichia* (*E.*) *coli*. [5]. A recent report conducted by Abuelgassim et al. [6] concluded that DPS extracts possess good antioxidant and antibacterial activity.

The anti-hyperglycemic effect of 70% ethanol extract of *Phoenix dactylifera* L leaves and its regulation of glucose absorption through the inhibition  $\alpha$ -amylase and

$\alpha$ -glucosidase has been reported [7]. The anti-diabetic effect of the methanol-water (4:1, v/v) extract of DPL on alloxan-induced diabetic rats had been also reported [8]. The aqueous extract of DPS has been demonstrated to possess potential protective effect against diabetic complications in liver and kidney tissues of streptozotocin-induced diabetic rats [9]. Another investigation also reported the antidiabetic effect of DPS extract on alloxan-induced diabetic rats [10].

Ajwa date polyphenol extract is reported to be a good source of phytochemical and micronutrients, thereafter, administration of Ajwa date polyphenol extract to hypercholesterolemic rats improved serum lipid profile and the antioxidant status in liver and plasma tissues [11]. Saryono et al. [12] reported that the levels of total cholesterol and LDL were significantly decreased in rats induced with high cholesterol diet, after their diet has been supplemented with PDS extract.

The protective effect of DPL methanolic extract against paracetamol- induced hepatotoxicity in albino rats has been reported [13]; the extract potentially reduced the elevated levels of catalase, superoxide dismutase, and glutathione peroxidase.

Here, we studied the antioxidant activity of three varieties of DPL namely: Sagaie, Khalas, and Nabtat Alsaif; the possibility of utilizing DPL in different industries was also a focus.

## 2. Materials and Methods

### 2.1. Chemicals

All chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA) unless stated otherwise. Trolox was obtained from Fluka Chemie AG (Buchs, Switzerland). Methanol and other chemicals were of analytical grade and obtained from BDH (Poole, UK).

### 2.2. Plant Material

Fresh and healthy green leaves (DPL) were collected from 16 years old date palm trees at Al-Karj region, Saudi Arabia in January 2015. Varieties include Sagaie, Khalas, and Nabtat Alsaif. DPL were allowed to dry at laboratory temperature (25°C) for 10 days, cut into small pieces by scissor, powdered by using a heavy duty miller.

### 2.3. Extraction Protocol for Antioxidant Determination

2g of DPL fine powder was extracted with 25mL solution of methanol: water (4:1v/v) and kept on a rotary shaker with gentle agitation for 24h. After 24h, each extract was centrifuged at 5000 rpm for 10 min. Supernatants were collected and residues were extracted with the same solution for 24h. Supernatants were pooled; solvent evaporation was carried out using rotary evaporator under vacuum (Buchi, Switzerland). After solvent evaporation, residues of each extract were dissolved in distilled water, final volumes were recorded and a serial dilution of 100, 500, and 2000 were performed.

### 2.4. Determination of Total Phenol Content

Total phenolic content was determined by Folin-Ciocalteu's reagent method of Mc Donald et al. [14]. 0.5 mL of each extract was mixed with 0.1 mL Folin-Ciocalteu's reagent and the mixture was incubated at room temperature for 15 min. Then 2.5 mL saturated sodium carbonate (7.5%) solution was added and further incubated for 30 min at room temperature and the absorbance was measured at 760 nm. Gallic acid (GA) in a range of 0-50µg was used as standard. Total phenol values were expressed as GA equivalent (GAE), mg GAE/100g dry weight.

### 2.5. Determination of Total Flavonoid Content

Total flavonoid content was determined by Aluminum chloride method of Chang et al. [15]. The reaction mixture of 3.0 mL consisted of 1.0 mL of each extract, 1.0 mL of ethanol, 0.5 mL of aluminum chloride (1.2%) and 0.5 mL potassium acetate (120 mM), solutions were incubated at room temperature for 30 min. Absorbance was measured at 415 nm. Quercetin in absolute ethanol and a range between 0-50µg was used as standard. Total flavonoid was expressed as quercetin equivalent (QE), mg QE/100g dry weight.

### 2.6. Radical Cation ABTS Scavenging Activity

The ABTS radical cation (ABTS<sup>+</sup>) solution was prepared through the reaction of 7 mM ABTS and 2.45 mM potassium persulphate, after incubation at 23°C in the dark for 16 h. The ABTS<sup>+</sup> solution was then diluted with 80% ethanol to obtain an absorbance of 0.7 at 734 nm. ABTS<sup>+</sup> solution (3.9 mL; absorbance of 0.7) was added to 0.1 mL of each extract and mixed vigorously. The reaction mixtures were allowed to stand at 23°C for 6 min and the absorbance at 734 nm was immediately recorded [16]. A standard curve was constructed by using Trolox standard in a range between 2-20µM in 80% ethanol. The absorbance of each extract was compared to that of the Trolox standard. The percentage of inhibition and the IC<sub>50s</sub> were calculated.

### 2.7. 2,2-Diphenyl-1-picryl-hydrazyl (DPPH) Free Radical Scavenging Activity

The activity of DPL extracts against DPPH was estimated using the method of McCune and Johns [17]. The reaction mixture composed 3.0 mL of 1.0 mL of DPPH in methanol (0.3 mM), 1.0 mL of each extract and 1.0 mL of methanol was incubated in dark for 10 min, the absorbance was read at 517 nm. Ascorbic acid in a range between 2-40µg was used as standard. The percentage of inhibition and the IC<sub>50s</sub> were calculated.

### 2.8. Hydroxyl Radical Scavenging Activity

The hydroxyl radical scavenging activity was measured by determining the competition between deoxyribose and extract for hydroxyl radicals generated by the Fe<sup>+3</sup> - ascorbic acid- EDTA-H<sub>2</sub>O<sub>2</sub> system, Fenton reaction [18]. The reaction mixture (1.0 mL), consisted of 100 µL of 2-deoxyribose (28 mM in 20 mM KH<sub>2</sub>PO<sub>4</sub>-KOH buffer, pH 7.4), 500 µL of each extract, 200 µL of EGTA (1.04 mM), 200 µM FeCl<sub>3</sub> (1:1 v/v), 100 µL of H<sub>2</sub>O<sub>2</sub> (1.0 mM) and 100 µL of ascorbic acid (1.0 mM). Reaction mixtures were incubated at 37°C for 1h. Thiobarbituric acid, TBA (1.0 mL of a 1% solution) and trichloroacetic acid, TCA (1 mL of a 2.8% solution) were added, and the reaction mixtures were incubated at 100°C for 20 min. After cooling, the absorbance was measured at 532 nm. GA in a range between 100-1000µg was used as standard. The percentage of inhibition and the IC<sub>50s</sub> were calculated.

### 2.9. Superoxide Anion Radical Scavenging Activity

Superoxide anion radical scavenging activity of extracts was estimated by the method of Robak and Gryglewski [19]. In the PMS/NADH-NBT system, the superoxide anion derived from dissolved oxygen from the PMS/NADH coupling reaction reduced nitroblue tetrazolium (NBT). The superoxide radical was generated in 3.0 mL of Tris-HCl buffer (16 mM, pH 8.0) containing 0.5 mL of NBT (0.3 mM), 0.5 mL of NADH (0.936 mM) solution, 1.0 mL of each extract and 0.5 mL of Tris-HCl buffer (16 mM, pH 8.0). The reaction was initiated by adding

0.5 mL of a phenazine methosulfate (PMS) solution (0.12 mM) to the reaction mixture followed by incubation at 25°C for 5 min. The absorbance was measured at 560 nm. GA in a range between 100-1000 $\mu$ g was used as standard. The percentage of inhibition and the IC<sub>50s</sub> were calculated.

### 2.10. Ferric Reducing Antioxidant Power (FRAP)

The reducing capacity was assessed by using the modified method of Athukorala et al. [20]. The reaction mixture (6.0 mL), consisted of 1 mL of each extract mixed with 2.5 mL of phosphate buffer (200 mM, pH 6.6) and 2.5 mL of potassium ferricyanide (30 mM). The reaction mixture was incubated at 50°C for 20 min. 2.5 mL of trichloroacetic acid (600 mM) was added followed by centrifugation at 3000 rpm for 10 min. The upper layer of the solution (2.5 mL) was mixed with distilled water (2.5 mL) and 0.5 mL FeCl<sub>3</sub> (6 mM), and the absorbance was measured at 700 nm. Ascorbic acid in a range between 20-200 $\mu$ g was used as standard.

### 2.11. Statistical Analysis

All experiments were carried out with three samples of DPL and each entire assay was carried out in triplicate. Mean and standard deviation values were calculated by using MS Excel 2010.

## 3. Results and Discussion

### 3.1. Total Phenolic and Total Flavonoid Content of DPL Extracts

The total phenol content of investigated DPL extracts was 4374.76  $\pm$  657.64, 1498.14 $\pm$ 100.52, and 1666.54 $\pm$ 64.95 (mg GAE/100g) for Sagaie, Khalas, and Nabtatai Saif respectively. The highest total phenol content was observed in Sagaie leaves (Table 1). Kriaa et al. [21] who studied the antioxidant activities of three varieties of DPL reported that the methanolic extracts showed total phenolics ranged between 69.06 $\pm$ 0.41- 146.46  $\pm$  2.61mg caffeic acid equivalent (CAE)/g. Another study conducted by Eddine et al. [22] reported that polyphenols content in three varieties of DPL was ranged from 156.46  $\pm$ 4.21 to 215.24  $\pm$ 9.25 mg GAE/g dry weight. The authors referred to that phenolic compounds content in DPL is influenced by several factors including water irrigation, soil, and environmental conditions.

Sagaie variety also showed the highest content of total flavonoid (224.62 $\pm$ 22.35mg QE/100g) compared to Khalas leaves (134.70 $\pm$ 16.24 mg QE/100g) and Nabtatai Saif leaves (152.13 $\pm$ 25.54 mg QE/100g) (Table 1). A report conducted by Saha et al. [23] on wild date palm fruit reported that when fruit was extracted with 70% acetone it showed the maximum total flavonoid content of 2121.77mg QE/100g whereas the lowest content was found in water extract (130.41mg QE/100g). Another report on palm date seed reported a total flavonoid content in the range of 300 - 325 mg catechin equivalent /100g when palm date seed was extracted with either 60% acetone or 60% ethanol respectively [24].

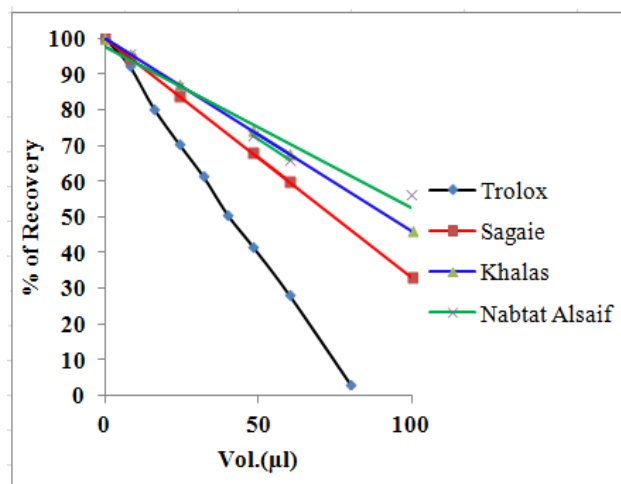
**Table 1. Total phenolic and total flavonoid content of Date Palm Leaf (DPL) extracts**

Variety	Total phenolic (mg/100g DW)	Total flavonoid (mg/100g DW)
Sagaie DPL	4374.76 $\pm$ 657.64	224.62 $\pm$ 22.35
Khalas DPL	1498.14 $\pm$ 100.52	134.70 $\pm$ 16.24
Nabtatai Saif DPL	1666.54 $\pm$ 64.95	152.13 $\pm$ 25.54

Data are expressed as mean  $\pm$ SD for three samples of each variety.

### 3.2. DPL Scavenging Activity against ABTS Radical

The three varieties of DPL extracts showed a significant scavenging activity against ABTS free radical as the calculated IC<sub>50</sub> concentrations were 71.87 $\pm$ 14.37, 217.87 $\pm$ 27.40, and 191.30 $\pm$ 22.8  $\mu$ g for Sagaie, Khalas, and Nabtatai Saif respectively. Kim et al. [25] documented that the decolorization assay using free blue-green ABTS radicals is a useful procedure for measuring the antioxidant activity. Trolox standard showed an IC<sub>50</sub> at a concentration of 11.29 $\mu$ M. Sagaie leaves showed the highest activity against scavenging ABTS radical as its recorded IC<sub>50</sub> was 2.66 times lower than that recorded by Nabtatai Saif and 3.03 times lower than IC<sub>50</sub> recorded by Khalas extract (Figure 1).

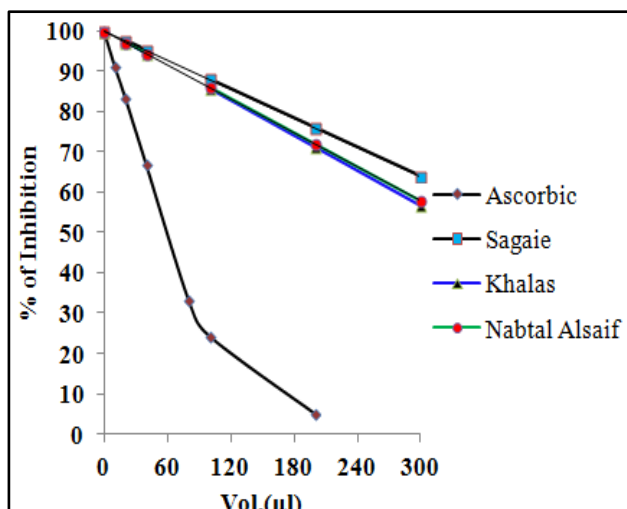


**Figure 1.** Date Palm Leaves extracts scavenging activity against ABTS radical

### 3.3. DPL Scavenging Activity against DPPH Radical

The potential of DPL extracts against DPPH is shown in (Figure 2). Sagaie leaves extract showed the most effective IC<sub>50</sub> of 197.86 $\pm$ 17.60 $\mu$ g, whereas Khalas and Nabtatai Saif leaves extracts showed IC<sub>50</sub> concentrations of 693.32  $\pm$ 25.44 and 594.25 $\pm$ 19.8 $\mu$ g respectively. However, the standard ascorbic acid recorded an IC<sub>50</sub> of 22.72 $\mu$ M (12 $\mu$ g). A report on date palm fruit conducted by El Abed et al. [26] stated that the ethanolic extract of the fruit showed strong activity against DPPH and showed an IC<sub>50</sub> of 0.15 $\pm$ 0.011mg/mL. On the other hand, the methanolic extracts of Deglet Nour, Medjhou, and Barhee date palm leaves showed an IC<sub>50</sub> of 2.08, 2.11, and 2.14 mg/mL respectively when Gallic acid was used as standard [21]. Recently, silver nanoparticles (Ag NPs) from the methanolic extract of *Phoenix dactylifera* reported to have

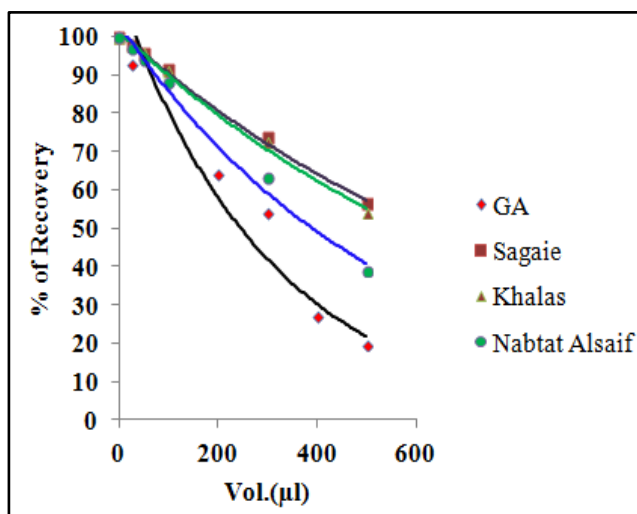
a potent activity against DPPH radical as a very low concentration of the extract (0.0064mg/mL) was shown to be capable to scavenge 100% of DPPH radicals [27].



**Figure 2.** Date Palm Leaves extracts scavenging activity against DPPH radical

### 3.4. DPL Scavenging Activity against Hydroxyl Radical

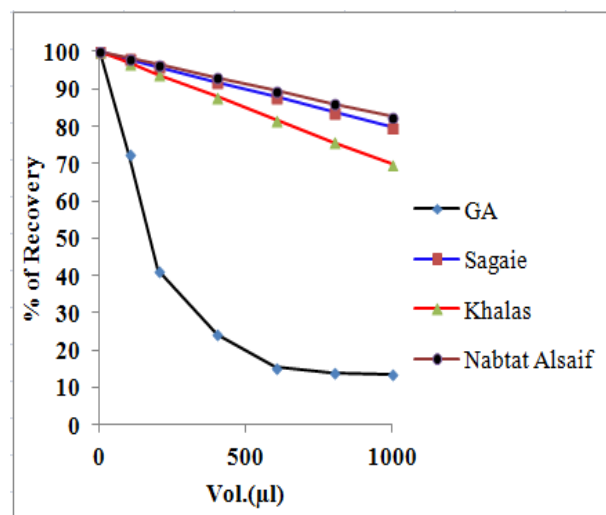
DPL extracts also showed a potential activity against hydroxyl radical as the calculated  $IC_{50}$  concentrations were  $208.04 \pm 12.76$ ,  $272.42 \pm 22.24$ , and  $719.63 \pm 24.3 \mu\text{g}$  for Nabtat Alsaif, Sagaie, and Khalas respectively (Figure 3). Standard GA showed an  $IC_{50}$  of  $337.38 \mu\text{g}$ , however, the calculated  $IC_{50}$  obtained by Nabtat Alsaif and Sagaie DPL extracts were 38.34% and 19.25% respectively lower than the  $IC_{50}$  recorded by the standard GA. Our finding regarding that DPL extracts showed a potential activity against hydroxyl radical is coincided with the report of Ouahida et al. [28] in that DPL extracted with ultra-sonic assisted procedure possess significant antioxidant activities.



**Figure 3.** Date Palm Leaves extracts scavenging activity against hydroxyl radical

### 3.5. DPL Scavenging Activity against Superoxide Anion Radical

DPL extracts also showed a good activity against superoxide anion when  $1000 \mu\text{l}$  of 2000 times diluted extracts were used they showed a percentage of inhibition of 30.27%, 20.24%, and 17.46% for Khalas, Sagaie, and Nabtat Alsaif respectively (Figure 4), and the calculated  $IC_{50}$ s were  $825.9 \pm 19.40$ ,  $1176.36 \pm 34.20$ , and  $1193.20 \pm 28.74 \mu\text{g}$  respectively. Standard GA showed an  $IC_{50}$  of  $170.42 \mu\text{g}$  against superoxide anion. Ghiaba et al. [29] studied five varieties of Algerian date palm fruit by cyclic voltammetric technique and reported that  $IC_{50}$  against the superoxide anion radical was ranged from 33.17 - 85.23 mg/L. Another study conducted by Algarni et al. [11] reported that oral administration of Ajwa date extract showed significant increase of superoxide dismutase (SOD) enzyme in hypercholesterolemic rats, however, the increase of SOD activity is in agreement with our finding that DPL extracts possess a good scavenging activity against superoxide anion.



**Figure 4.** Date Palm Leaves extracts scavenging activity against superoxide anion

### 3.6. DPL Extracts Reducing Power Activity (as FRAP)

Diluted extracts of DPL showed a powerful reducing power. The mean of calculated FRAP concentration was  $22.13 \pm 1.36$ ,  $21.46 \pm 2.04$ , and  $7.69 \pm 0.47 \text{ mmol ascorbic acid equivalent /100g}$  for Sagaie, Nabtat Alsaif, and Khalas respectively (Figure 5). A report conducted by Mohamed et al. [30] who studied six palm date fruit varieties grown in Sudan showed that FRAP activity ranged from 2.82-27.5 mmol/100g. Our findings regarding FRAP activity of DPL extracts were comparable with the report of Djouab et al. [31] who reported values of  $2527.60 \pm 327.03$ ,  $1994.73 \pm 28.44$ , and  $2228.49 \pm 53.32 \text{ mg ascorbic acid equivalent /100g}$  that correspond to 14.35; 11.33; and 12.65 mmol ascorbic acid equivalent /100g for flesh, peel, and brown tissue of *Phoenix canariensis* red date respectively.

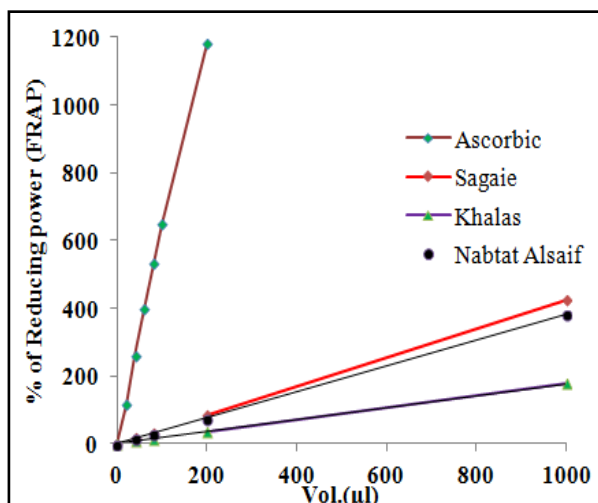


Figure 5. Date Palm Leaves extracts reducing power activity (as FRAP)

## 4. Conclusions

Our findings clearly showed that DPL extracts possess powerful antioxidant activities and potentially scavenged different sources of free radicals; therefore, our study suggests the possibility of effective use of DPL extract in food industry. Phenolic acids pattern and separation of DPL extracts are currently running in our laboratory targeting the possibility of using DPL extracts in pharmaceutical industry.

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## Conflicts of Interest

The author declares no conflict of interest.

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