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POMEGRANATE (*Punica granatum* L.) PEEL EXTRACT- A STUDY ON POTENTIAL SOURCE OF PHARMACOLOGICAL ACTIVITIES

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ABSTRACT

Pomegranate (*Punica granatum* L.) has been used for ages in many civilizations for the prevention and treatment of a varied number of health maladies such as Cancer, diabetes, inflammation, dental plaque, dysentery, and to fight malarial parasites and intestinal infections. It is an important source of bioactive compounds such as Ellagitannins and the Punicalagin. In the present study, potential biological activities of aqueous peel extract of pomegranate were carried out. Antibacterial activity was studied against *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *and Salmonella typhii*; Antifungal activity against *Aspergillus niger and Aspergillus flavus*. Antioxidative study was evaluated by DPPH and FRAP assay. Antimicrobial study reveals all the tested bacterial and fungal strains were susceptible to aqueous peel extract as dose dependent manner. Maximum activity was observed in high concentration of extracts. DPPH and FRAP assay showed potential antioxidative activity due to the presence of various flavonoids.

KEYWORDS: **Pomegranates, Aqueous peel extract, antimicrobial, antioxidant.**

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INTRODUCTION

Pomegranate (*Punica granatum* L.); the common name is derived from the Latin words *ponus* and *granatus*, a granular apple which is a delectable fruit consumed worldwide. The fruit is a native shrub of western Asia and Mediterranean countries have a maximum content of health promoting compounds[1]. Pomegranate fruit extracts have shown many life preventing and attenuating activities against many life threatening ailments such as cancer[2-3], cardiovascular diseases, atherosclerosis[4-8] and type II diabetes[9]. Interestingly, the nutritional parameters mentioned above are not limited to the edible part of the fruit, the vital role are played by the non- edible fractions of fruit and tree i.e. leaves, barks, seeds, buds, flower and peel. Although, these parts are considered to be waste, they contain enormous amount of nutritional value and biological active compounds compared to the edible portion of the fruit[10]. Pomegranate peels are distinguished by an internal network of membranes encompassing almost 26–30% of total fruit weight and are characterized by considerable amounts of phenolic compounds, including flavonoids such as anthocyanins, catechins and other complex flavonoids and hydrolysable tannins (punicalagin, punicalin, pedunculagin, ellagic and gallic acid). These compounds are intense in pomegranate peel and juice, which depicts 92% of the antioxidant activity allied with the fruit[11-13].Punicalagin, Gallic acid and ellagic acid, in addition to their free radical-scavenging properties, also have antibacterial activities against intestinal flora, predominantly on enteric pathogens, i.e. *E.coli*, *Shigella* species, *Salmonella* species and *V.cholerae*[5, 14-16]. The therapeutic potential of Pomegranate peel has been broadly recognized by different ethos. In Egyptian ethos, numerous customary ailments such as intestinal worms, diarrhea, inflammation, infertility and cough have been treated by using pomegranate peel extract. The unique antioxidant potential and intense medicinal properties of Pomegranate peel steered the global scientific community to initiate demanding research in the last decade to further explore its role in human health. The technique of antimicrobial activity of pomegranate peel, phenolics encompasses precipitation of membrane Proteins ensuing in microbial cell lysis. The ethno pharmacological Profile of pomegranate Peel makes it a prized traditional asset due to its antimicrobial, antimutagenic and antioxidant properties. Additionally, the phytochemical concentration of Pomegranate peel is exalted enough to be effective without further augmentation with the extracts of any new fraction of the fruit[17].Several studies have stated the efficacy of extracts from different tree parts, such as bark, leaves and fruit to hinder the growth of Gram positive and Gram negative bacteria, which are foodborne and human pathogens[18-25]. Conversely, the chemical characteristics of these bioactive compounds that are responsible for antimicrobial activities were not elucidated in these studies. Therefore, the aim of the present study was to investigate the nature of these bioactive compounds which play a pivotal role in controlling the foodborne and human pathogens. Furthermore, the antioxidant activity of the pomegranate peel are analyzed; such knowledge about the peel's biological activity is indispensible for rising new uses for the agricultural waste of pomegranate juice industries and to produce natural biopesticides that may substitute the synthetic forms[1]. In the present study, the potential biological activities of aqueous peel extract of pomegranate were carried out.

MATERIALS AND METHODS

Preparation of Aqueous peel extract

Aqueous Extraction of Pomegranate Peels was carried by removing Pomegranate arils and the fruit peels separately. The peels were cut into 0.5−0.75cm^2 slices and shade dried until used. To prepare the peel aqueous extracts, 3-fold double-distilled (DDW) water (v/w) was added to the peel pieces, which were extracted at 50°C for 30minutes. The sample was centrifuged at 10000 rpm for 10minutes, and the supernatant was filtered through whatmann no 1 filter paper (Millipore). The filtered supernatant was collected into a clean screw cap vial and stored for further analysis.

Antibacterial activity

Preparation of inoculum

Stock cultures were maintained at 4°C on slant of nutrient agar. Active cultures for experiments were prepared by transferring a loop full of cells from the stock cultures to test tubes of nutrient broth for bacteria that were incubated at 24 hours at 37ºC. The Assay was performed by well diffusion method.

Well Diffusion Method of the aqueous peel extract

Antibacterial activity of sample was determined by disc diffusion method on Muller Hinton agar (MHA) medium. The Muller Hinton Agar medium was weighed as 3.8 grams and dissolved in 100mL of distilled water and add 1 gram of agar. Then the medium is kept for sterilization. After sterilization the media was poured in to sterile petriplates and were allowed to solidify for 1 hour. After the medium was solidified, the inoculums were spread on the solid plates with sterile swab moistened with the bacterial suspension. Wells were made on the solidified MHA and samples of different concentrations such as 50µL, 25µL, 10µL (500 µg, 250µg, 100µg) were loaded. Negative control 10µL of DMSO and positive control 10µL (10µg) streptomycin and placed on MHA plates. These plates were incubated for 24 hours at 37ºC.Then the microbial growth was determined by measuring the diameter of zone of inhibition.

Antifungal activity

Preparation of inoculum

Stock cultures were maintained at 4°C on slant of potato dextrose agar. Active cultures for experiments were prepared by transferring a loop full of cells from the stock cultures to test tubes of potato dextrose broth for fungi that were incubated at 24 hours at room temperature. The assay was performed by Food poisoning technique.

Food poisoning technique

Antifungal activity of sample was determined by food poisoning technique on potato dextrose agar (PDA) medium[26]. The potato dextrose agar medium was weighed as 3.9 grams and dissolved in 100mL of

distilled water and add 1 gram of agar. Then the medium is kept for sterilization. After sterilization the media was cooled, sample (100µL) was added to flask and poured in to sterile petriplates and were allowed to solidify for thirty minutes. After the medium was solidified, 10µL of fungal suspension was spotted on the solid plates. These plates were incubated for 24 hours at room temperature. Then the activity was determined by measuring the diameter of the growth.

Antioxidant activity
DPPH Assay

The percentage of antioxidant activity of the aqueous peel was assessed by DPPH free radical assay. The samples were reacted with the stable DPPH radical in a methanol solution. The reaction mixture consisted of 3.7mL of absolute methanol in all test tubes along with blank. The blank tube was added with 100µL of absolute methanol and 100µL of respective samples to all other tubes marked as tests[27]. Finally 200µL of DPPH reagent were added to all the test tubes including blank. The test tubes were incubated in dark condition for 30minutes to the reaction to take place. When DPPH reacts with an antioxidant compound, which can donate hydrogen, it is reduced. The changes in color (from deep violet to light yellow) were read (absorbance) at 517nm. The scavenging activity percentage was determined according;

$$\% \text{ Antioxidant activity} = \{(\text{absorbance at blank}) - (\text{absorbance at test}) / (\text{absorbance at blank})\} \times 100$$

FRAP Assay

Total antioxidant activity is measured by ferric reducing antioxidant power (FRAP) assay of Benzie and Strain (1996)[28]. FRAP assays uses antioxidants as reductant in a redox-linked colorimetric method, employing an easily reduced oxidant system present. At low pH, reduction of ferric tripyridyltriazine (Fe III TPTZ) complex to ferrous form (which has intense blue color) can be monitored by measuring the change in absorption at 593nm. The change in absorbance is therefore, directly related to the combined or total reducing power of the electron donating antioxidants present in the reaction mixture. Stock solution of aqueous peel extracts was prepared to the concentration of 1mg/mL. from the stock, Various concentrations of the samples (100 - 500µL) were aliquoted and made up to 1mL with distilled water and was mixed with 1.5mL of working FRAP reagent and incubated at 37°C for 4minutes. After incubation the absorbance was measured at 593nm. Ferrous Sulphate standard was processed in the same way and calibration curve was generated using various concentrations of Ferrous Sulphate (20 - 100µg/mL). Blank consist of all the reagents, except for the extract or standard solution and are substituted with water.

Determination of Total phenolics, flavonoids and antioxidants contents in aqueous peel extract

The content of total phenolic compounds in the extracts was determined according to the technique of Jayaprakasha, Singh, and Sakariah (2001)[29]. The extracts were dissolved in water. Aliquots of 0.5mL samples were mixed with 2.5mL of 10-fold-diluted Folin–Ciocalteu reagent and 2mL of 7.5% Sodium Carbonate. The mixture was allowed to incubate for 30minutes at room temperature before the absorbance was measured at 760nm**.** The total flavonoid content of crude extract was determined by the Aluminum chloride colorimetric method[30]. In brief, 50µL of aqueous peel extract (1mg/mL ethanol) were made up to 1mL with methanol, mixed with 4mL of distilled water and then 0.3mL of 5% Sodium Nitrite solution; 0.3mL of 10% Aluminum Chloride solution was added after 5minutes of incubation, and the mixture was allowed to stand for 6minutes. Then, 2mL of 1mol/L Sodium Hydroxide solution were added, and the final volume of the mixture was brought to 10mL with double distilled water. The mixture was allowed to incubate for 15minutes, and absorbance was measured at 510nm**.** The Total antioxidant activity was estimated by phosphomolybdenum assay[31]. Aqueous peel extract of pomegranate was added in different concentration ranging from 100µL to 500µL were added to each test tube individually containing 3mL of distilled water and 1mL of Molybdate reagent solution. These tubes were kept incubated at 95°C for 90minutes. After incubation, these tubes were normalized to room temperature for 20-30minutes and the absorbance of the reaction mixture was measured at 695 nm.

RESULTS AND DISCUSSION

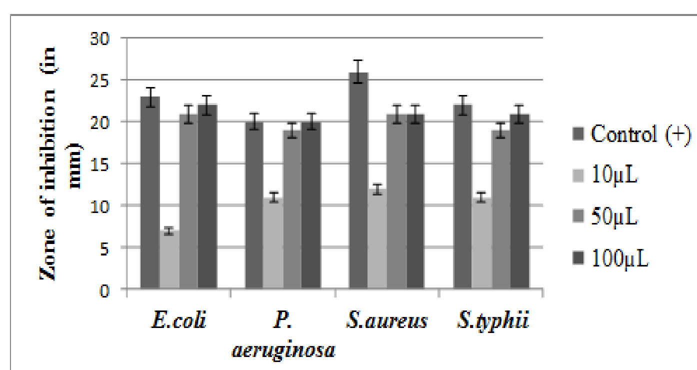
Pomegranate peel is rich in tannins, high-molecular-weight plant polyphenols, which can be categorized into two chemically and biologically separate groups: condensed hydrolysable tannin and tannin, the latter composed of glycosyl esters and phenolic acids. Hydrolyzable tannins are parted into gallotannins containing gallic acid and ellagitannins, containing ellagic acid[32-33].The fruit peel exhibits a high antioxidant potential. They have gained a wide acceptance for their pharmacological activities against serious maladies such as cancer, stomach ulcers, cardiovascular diseases and digestive disorders. The cytoprotective and inhibitory effects of peel demonstrates the potential to prevent some human carcinomas. As ethnopharmacological utilization of the peel extract is prevalent in a variety of cultures to cure common disorders without any consideration to its phytochemical profile and toxicological limit, safety verification and clinical trials are needed prior to its pharmacological exploitation by modern medicine. A more integrated approach is needed to use pomegranate peel for the treatment of diarrheal disorders, especially in the developing countries with poorer hygienic practices and unsanitary conditions. The prophylactic potential of the peel against viral epidemics and pandemics, specifically influenza, may open up new avenues for research in the nutritional and medical science domains[34].In the present study, anti-bacterial activity was studied against *E.Coli*, *Pseudomonas aeruginosa*, *S.aureus* and *S.typhii*. It can be seen that both the tested strains were susceptible to the aqueous peel extract as dose dependent manner (Figure 1). A steady increase in inhibitory zone was

recorded in high concentration. In the case of *P.aeruginosa*, maximum zone of inhibition was recorded at 100µL with 20mm followed by 50µL with 19mm; 10µL revealed 11mm of zone of inhibition whereas in the case of *S.aureus* and *S.typhii*, the zone of inhibition was around 21mm at the highest dosage level of 100µL. *E.coli* showed high sensitivity to high concentration of aqueous peel extract (100µL, 50µL, 10µL); 22, 21 and 7mm of zone of inhibition has been observed at the respective concentrations (Table 1) (Graph 1).

Table1
Antibacterial activity of aqueous peel extract by well diffusion method

S.No	Organism	Zone of inhibition in mm			
		Control (+)	10µL	50 µL	100 µL
1	*E.Coli*	23	7	21	22
2	*P. aeruginosa*	20	11	19	20
3	*S.aureus*	26	12	21	21
4	*S.typhii*	22	11	19	21

Graph 1
Zone of inhibition (mm) of aqueous peel extract at different concentration (µL) against pathogenic bacteria



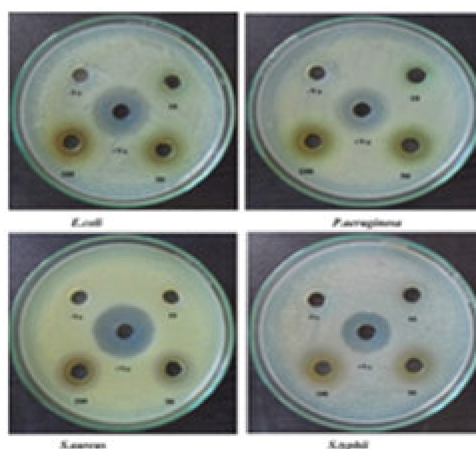


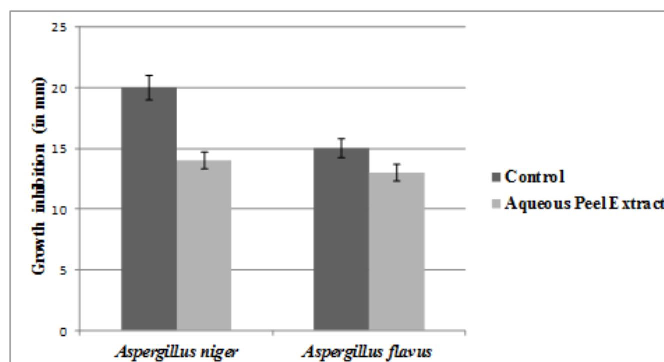
Figure 1
Antibacterial activity of aqueous peel extract by well diffusion method

In case of antifungal activity, Food poisoning technique was carried out to depict the growth inhibition of the *A.niger* and *A.flavus* against the aqueous peel extract (Figure 2). The maximum growth was seen in *A.niger* i.e.14mm compared to *A.flavus* i.e.13mm (Table 4) (Graph 2).The antibacterial and antifungal activity of the extract may be ascribed to the high content of flavonoids, which have been described to be involved in inhibition of metabolic processes and biosynthesis of nucleic acid[35].Flavonoids have also been stated to inhibit spore germination of plant pathogens[36]. Similarly, flavonoids are produced by plants in retort to microbial infection. Phenolic compounds with a C_3 side chain at a lessen level of oxidation and comprising no oxygen are often stated as antimicrobials[37].The method of the toxicity of polyphenols against microbes perchance related to inactivate microbial adhesins, inhibition of proteases, non-specific inter-actions with carbohydrates and cell envelope transport proteins[38].

Table 2
Antifungal activity of aqueous peel extract by Food poisoning technique

S.No	Microorganism	Control	Sample
1.	*Aspergillus niger*	20	14
2.	*Aspergillus flavus*	15	13

Graph 2
Growth inhibition (mm) of aqueous peel extract against Aspergillus niger and Aspergillus flavus



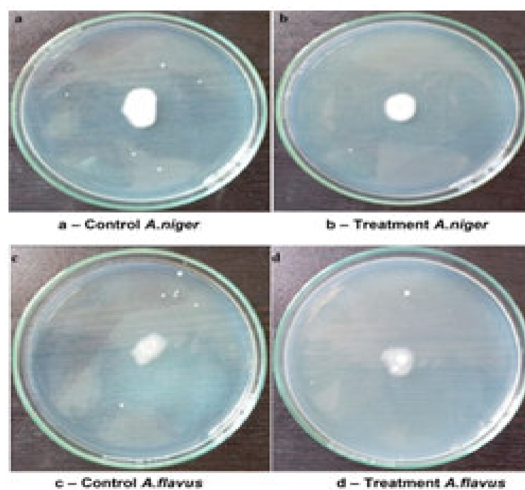


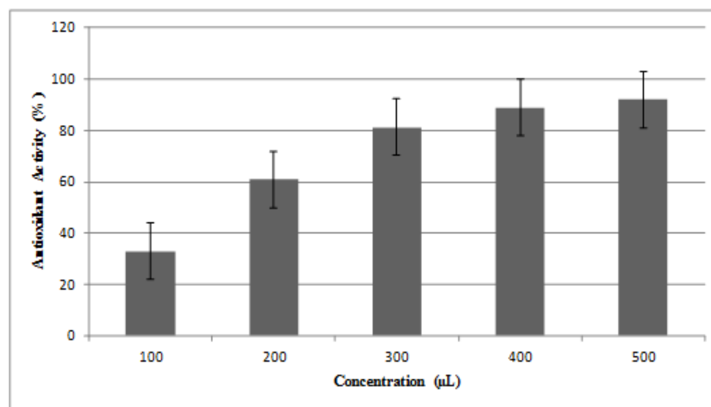
Figure 2
Antifungal activity of aqueous peel extract by Food poisoning technique

The DPPH· scavenging activity has been commonly used to detect antioxidant activity of different samples sources, due to its sensitivity to lower concentrations of active standards from natural sources. The steady radical, DPPH, has a maximum absorbance at 517nm and could swiftly undergo scavenging by antioxidants. Complex free radical scavenging activities of samples are indicated by lower absorbance at 517nm[27]. It was found that the highest concentration of aqueous extract at around 500µL had the highest percentage of antioxidant activity (Table 3) (Graph 3).

Table 3
Antioxidant activity of aqueous peel extract by DPPH assay

S.No	Concentration (µL)	% of antioxidant activity
1	100	32.8
2	200	60.9
3	300	81.2
4	400	89.0
5	500	92.1

Graph 3
Antioxidant activity of aqueous peel extract by DPPH assay

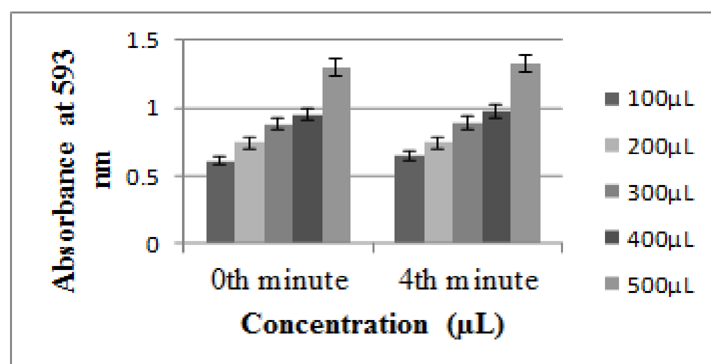


The FRAP assay considers the antioxidants contained in the samples as reductants in a redox linked colorimetric reaction and the value reflects the reducing power of the antioxidants. The procedure is comparatively simple to standardize. Based on FRAP value, the peel extract is stronger in reducing power in a dose-dependent manner depicting that peel extract has more potential antioxidant activity (Table 4) (Graph 4).

Table 4
Antioxidant activity of aqueous peel extract using Ferric reducing antioxidant assay

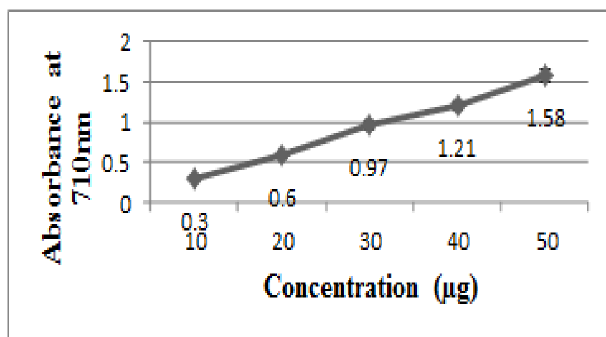
Concentration	100µl	200µl	300µl	400 µl	500 µl
Sample (0 min)	0.61	0.74	0.88	0.95	1.30
Sample (4 min)	0.65	0.74	0.89	0.97	1.33

Graph 4
Antioxidant activity of aqueous peel extract using Ferric reducing antioxidant assay

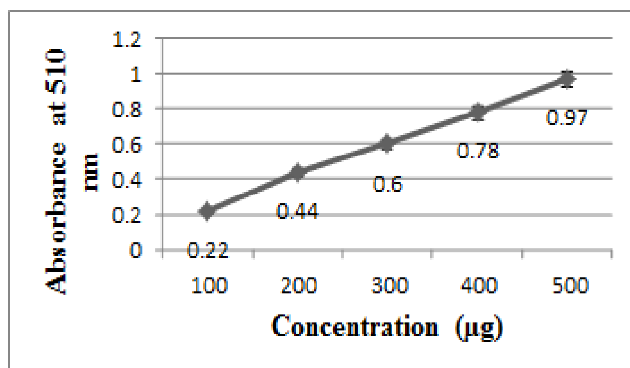


Plants are opulent in secondary metabolites, including phenolics, carotenoids and flavonoids due to their chemical structures and redox properties. As their free radical scavenging ability is enabled by their hydroxyl groups, the total phenolic concentration could be used as a base for quick assessment of antioxidant activity. Flavonoids, including flavanols, flavones, and tannins, are plant secondary metabolites, the activity of depends on the presence of free OH groups. Phosphomolybdenum is used to determine the total antioxidant content of the aqueous peel extract. It is a quantitative method to examine the reduction reaction rate among antioxidant, oxidant and molybdenum ligand. It encompasses the thermally generating auto-oxidation during prolonged incubation time at higher temperature. It gives us a complete estimation of reducing capacity of antioxidant. This particular study represented the higher percentage of phenolics, flavonoids and total antioxidants (Graph 5-7)[39].

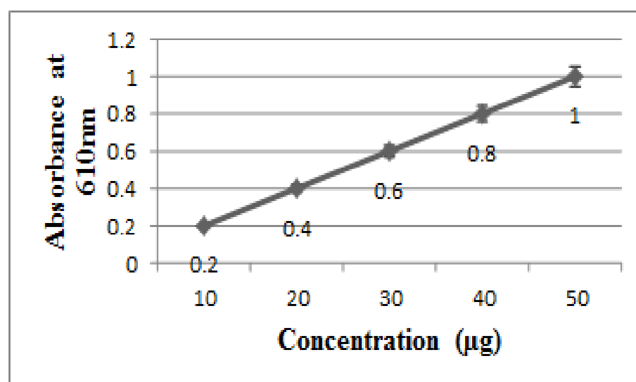
Graph 5
Total phenolic activity of aqueous pomegranate peel extract



Graph 6
Total flavonoid activity of aqueous pomegranate peel extract



Graph 7
Total antioxidant activity of aqueous pomegranate peel extract



CONCLUSION

Our results suggest that the aqueous peel extract of pomegranate is a potential source of antibacterial, antifungal and antioxidant agents and could be used as a natural antioxidant and preservative in food and non-food systems. Over viewing the reducing capacity, the use of these aqueous peel extract of pomegranate might contribute a certain level of health fortification against oxidative damages. With the established antioxidant activity of this extract, the specific isolation of the active components in the aqueous extract of pomegranate and characterization should be further examined. Further phytochemical analysis is entailed to isolate the elements of the peel that show a wide spectrum of pharmacological activity.

CONFLICTS OF INTEREST

Conflicts of interest declared as none.

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