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Title: **Antioxidant and Antimicrobial Activity of *Cuscuta reflexa* ROXB. and few Members of family *Convolvulaceae***

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Antioxidant and Antimicrobial Activity of *Cuscuta reflexa* ROXB. and Few Members of Family *Convolvulaceae*

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ABSTRACT

Antioxidant activity of 12 species, of which one was *Cuscuta reflexa* Roxb. which belongs to the family of *Cuscutaceae* as well as 11 of *Convolvulaceae*, collected from district Bhimber, Mirpur and Kotli were measured by using ABTS, DPPH and FRAP assay. The total phenol and flavonoid contents of the whole plant of all the selected species were investigated. *Ipomoea eriocarpa* showed a comparatively higher amount of total polyphenols (0.98 ± 0.073 mg/gdw). The maximum DPPH value was shown by *Ipomoea carnea* Jacq (5.6%) and the minimum value was shown by *Convolvulus arvensis* L. extracts (3.0 %). ABTS was the highest by *Convolvulus prostrastus* and the lowest by *Ipomoea carnea.* In FRAP assay, the highest value was 2.75 by *Ipomoea hederacia* and the lowest value was 0.31 by *I. arachnosperma* Welw. The antifungal and antibacterial activity of the methanolic extracts of the whole plant of all the selected species was carried out by using both bacterial and fungal strains. Two gram positive bacteria namely *Staphylococcus aureus* and *Bacillus subtilis,* two gram negative bacteria namely *E. coli* and *Pseudomonas aeruginosa,* two fungal strains of *Aspergillus niger* and *Aspergillus oryzae* were used to carry out antimicrobial activity. For the comparison of inhibition zones showed by plant extracts against bacterial and fungal strains. Erythromycin, tetracycline and cefoparazone were used as standard for bacterial strains. Moreover, fungivine and Terbinafine were used as standard antibiotics against fungal strains. Among fungal strains, the highest zone of inhibition was shown by *Cuscuta reflexa* Roxb. extract against *Aspergillus niger,* that is 5.55±0.3, while the lowest zone of inhibition, that is, 0.60±0.28mm was shown by *Evolvulus alsinoides* (L.) extract against *Aspergillus oryzae*. Plants from the *Convolvulaceae* family have had frequent traditional uses for different therapeutic purposes. Furthermore, all plants selected for this study showed strong antimicrobial, antifungal, and antioxidant activity.

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Their further detailed phytochemical studies can help to develop plant-based therapeutic agents

Keywords: ABTS, Antimicrobial, Antioxidant, Convolvulaceae, DPPH,

1. INTRODUCTION

Bioactive chemicals or phytochemicals are of plant origin. These chemicals are produced in every part of a plant including its leaves, roots, stem, fruit, seeds and bark. Hence all the plants are studied for phytochemicals. Convolvulaceae family is a distinctive and well recognized plant family. It includes large number of significant plants which are used in preparing the cures of different diseases, such as constipation, headache, diabetes, skin infection and rheumatism [1].

Flavonoids are found in all plants including in *Convolvulacea* [2]. These have several natural effects, including antiviral, antibacterial, anti-inflammation, antithrombotic, vasodilatory, and anti-allergy effects [3]. Bio flavonoid and phenolic compound found in herbal plants have considerable antioxidant properties [4]. Phenolic compounds show significant anti-inflammatory activity and are effective in treatment of kidney and stomach diseases [5]. Within plant kingdom phytochemicals are diverse structurally and their distribution is limited in few species. On the bases of their characteristics these chemicals are used as bio diagnostic markers in chemotaxonomic investigations [6].

In recent years, the role of flavonoids and phenolic acid as an antioxidant used to control diseases has been identified. Moreover, they are an excellent source of natural diet [7]. As antioxidants, these chemicals care biological systems and reduce the threat of cancer, have the ability to scaveng free superoxide radicals. In human diet and plants, the most important group of compounds are flavonoids. More than 6000 naturally occurring flavonoids have been identified in various plants and their number continues to increase [8]. Food stuffs having flavonoids show excellent antioxidant activity. The current study of C. reflexa and other species from Convolvulaceae was undertaken to identify the antioxidant potential in these species. Due to the ever-increasing use of antibiotics and growing bacterial resistance to them, it has become imperative to discover new drugs in the pharmaceutical industry. It has been observed that *Cuscuta reflexa* extracts show antibacterial activity against various microorganisms. *Cuscuta reflexa* and other members belonging to the *Convolvulaceae* family

has bioactive molecules with substantial antibacterial and antioxidant activity [9].

2. MATERIAL AND METHOD

2.1. Extraction of plant material

The maceration technique was used for the extraction of plant material. About 250g of the powder of the whole plant was soaked in 500ml aqueous methanol in a flask. The flask was then sealed with aluminium foil and left for 7 days with infrequent shaking at room temperature. The process was confirmed three times by using a similar quantity of methanol. Then, the extracts were cleaned by using Whatman no.1 filter paper and evaporated in a rotary evaporator. The thick gummy mass thus obtained was stored at -4^{o} C for further investigation. The colour, texture, appearance and odour of the different extracts were analysed. The dried extracts were then dissolved in methanol at the concentration of 100mg/ml to make the dilutions. By using the following formula, the percentage yield of methanolic extract of all the species was measured.

$$\%\text{extraction yield} = \frac{weight\ of\ the\ plant\ extract}{weight\ of\ initial\ plant\ sample} \times 100.$$

2.2. Antioxidant activity

Antioxidant activity was determined through already published procedures i.e., ABTS assay, FRAP assay, and DPPH assay. ABTS assay was prepared by adding manganese dioxide (80 mg) to a 5 mM stock solution of ABTS (20mL using a 75mM Na/K buffer of pH 7), Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid). The construction of the standard calibration curve was carried out for Trolox at 0, 50, 100, 150, 200, 250, 300, and 350 µM concentrations. According to the antioxidant activity the dilution of sample was geared up in Na/K buffer pH,7. With the diluted sample, 200µL of ABTS+ radical cation solution was mixed and absorbance was inspected (at 750nm) after 5 mins. Furthermore, TEAC values can be calculated from the Trolox standard curve and expressed as Trolox equivalents (in mM) [9]. 100µL of diluted sample and 3mL of the prepared FRAP reagent were mixed and absorbance at 593nm was scored [10]. To calculate the antioxidant potential through free radical scavenging by using the test sample, the change in optical density of DPPH radicle was measured [11].

2.3. Evaluation of antimicrobial activity

In order to test antifungal activities, 2 fungal strains were selected. One was black fungus known as *Aspergillus niger* and the other was white fungus known as *Aspergillus oryzae.* These species were taken from the fungal section of the Department of Botany MUST Mirpur. For antimicrobial activities, 4 bacterial strains were selected. Out of four bacterial strains, two strains were of gram positive bacteria namely *Staphylococcus aureus* and *Bacillus subtilus*. The other two strains were of gram negative bacteria, namely *Escherichia coli* and *Pseudomonas aeruginosa*. The antibacterial and antifungal activity was performed by using minimum inhibitory concentration (MIC), measurement of the zone of inhibition, and agar well diffusion method [12]. By adopting the agar well diffusion method all the zones of inhibition were shown by methanolic extract [13].

3. RESULTS

3.1. Antioxidant activity

The whole plants of all the twelve selected species were screened for antioxidant activity. By using the maceration process, the respective extract was prepared. The percentage yield of the extracts was calculated before further analysis was carried out. The percentage yield of the extract is actually the measure of their efficiency, as shown in Table 1.

Table 1. Antioxidant Activity by Different Species

Plant specie	Percentage yield	TEAC (mg/g dry weight)			Total phenolic	Total flavonoid
		DPPH	ABTS	FRAP		
Ipomoea carnea	5.60%	1.50±0.09	1.11±0.08	1.73±0.13	0.93±0.38	0.90±0.07
Cuscuta reflexa	3.90%	1.44±0.12	0.65±0.05	2.69±0.11	1.52±0.07	0.61±0.35
Ipomoea hederacea	3.00%	1.47±0.07	1.70±0.31	2.75±0.04	1.62±0.12	0.71±0.06
Ipomoea eriocarpa	4.30%	0.76±0.06	1.99±0.31	1.00±0.07	0.43±0.04	0.87±0.09
Ipomoea carica	3.90%	0.45±0.03	1.82±0.07	0.73±0.05	0.23±0.04	0.98±0.73
Ipomoea pes-tigridis	5	1.40±0.04	1.78±0.09	1.28±0.41	0.52±0.04	0.63±0.42
Ipomoea arachnosperma	5.5	0.20±0.02	3.34±0.14	0.31±0.05	1.14±0.07	0.76±0.62
Ipomoea quamoclit	5.60%	0.21±0.02	1.83±0.08	0.36±0.02	3.07±0.15	0.67±0.22

Plant specie	Percentage yield	TEAC (mg/g dry weight)			Total phenolic	Total flavonoid
		DPPH	ABTS	FRAP		
Merremia aegyptia	3.90%	1.44±0.10	2.53±0.12	0.81±0.05	1.88±0.54	0.87±0.17
Convolvulus arvensis	3.00%	0.94±0.07	1.86±0.11	0.43±0.03	0.23±0.04	1.67±0.22
Convolvulus prostrates	4.30%	0.81±0.06	3.37±0.29	0.88±0.05	0.75±0.06	1.12±0.23
Evolvulus alsinoides	3.90%	0.93±0.07	1.43±0.09	1.9±0.39	0.58±0.04	0.88±0.58

The DPPH (2,2-diphenyl-1-picrylhydrazyl.), ABTS (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) and FRAP (Ferric Reducing Antioxidant Power Assay) methods were used to investigate the antioxidant potential of all species. The maximum DPPH value was shown by *Ipomoea carnea* Jacq, that is, 5.6% and the minimum value was shown by *Convolvulus arvensis* L. extracts that is, 3.0%. The highest ABTS value was shown by *Convolvulus prostrastus* and the lowest was shown by *Ipomoea carnea.* In FRAP assay, the highest value of 2.75 was shown by *Ipomoea hederacia* and the lowest value was shown by *I. arachnosperma* Welw, as shown in Table 1 and Figure 1.

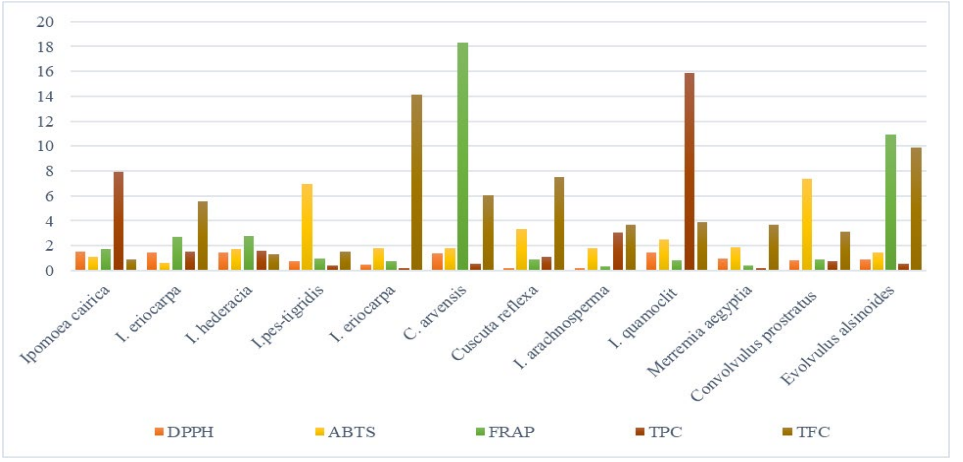

Figure 1. Antioxidant Activity of Different Species of *Convolvulacea* and *Cuscutaceae*

The maximum DPPH value was shown by *Ipomoea carnea* and the minimum value was shown by *Convolvulus arvensis* L. The highest ABTS value was shown by *Convolvulus prostrastus* and the lowest by *Ipomoea*

carnea. In FRAP assay, *Ipomoea hederacia* showed the maximum value and *I. arachnosperma* Welw showed the mininmum value.

3.2. Antimicrobial activity:

The antifungal and antibacterial activity of the methanolic extracts of whole plants of the selected species was carried out by using both bacterial and fungal strains. Two gram positive bacteria namely *Staphylococcus aureus* and *Bacillus subtilis,* two gram negative bacteria namely *E. coli* and *Pseudomonas aeruginosa,* and two fungal strains of *Aspergillus niger* and *Aspergillus oryzae* were used to carry out the antimicrobial activity. Erythromycin, tetracycline and cefoparazone were used as standard treatment for bacterial strains and fungivine and Terbinafine were used as standard antibiotics against fungal strains.

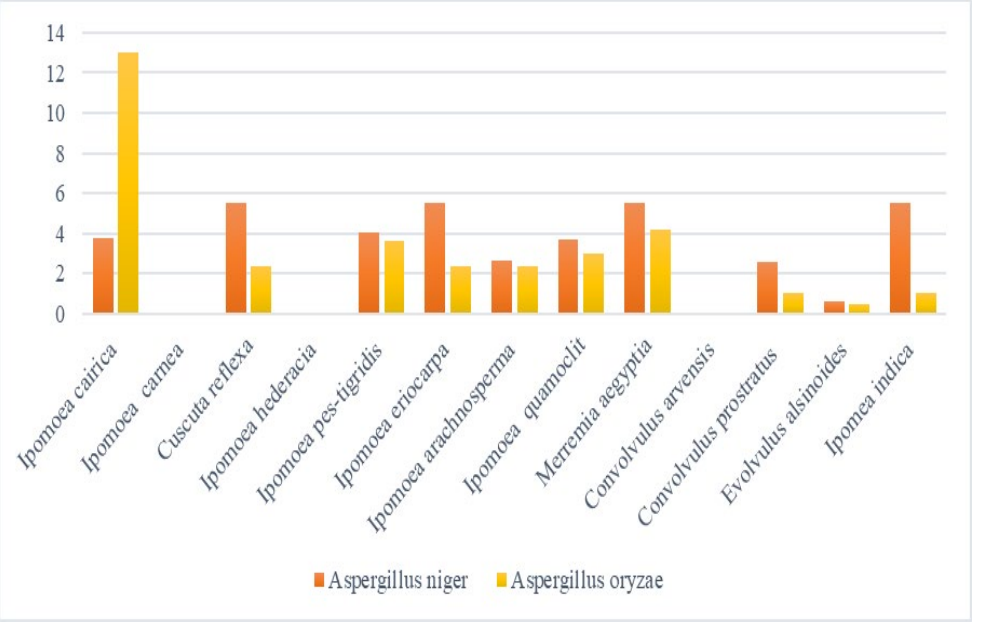

Figure 2. Antifungal Activity of Different Species of *Convolvulaceae* and *Cuscutaceae*

Among the fungal strains, the highest zone of inhibition was shown by *Cuscuta reflexa* Roxb. extract against *Aspergillus niger,* while the lowest zone of inhibition was shown by *Evolvulus alsinoides* (L.) extract against *Aspergillus oryzae*.

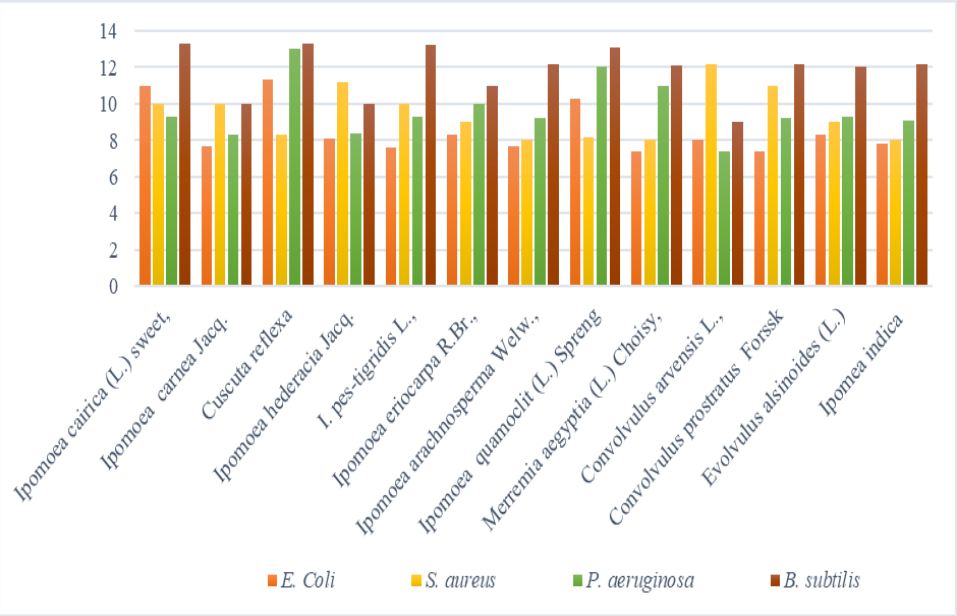

Figure 3. Antibacterial Activity of Different Species of *Convolvulaceae* and *Cuscutaceae*

The highest zone of inhibition was shown by *Ipomoea cairica* (L) sweet against *Bacillus subtilis* while the lowest zone of inhibition was shown by *I. pes-tigridis L.,* against *E. coli***.**

Among fungal strains the highest zone of inhibition was shown by *Cuscuta reflexa* Roxb. extract against *Aspergillus niger,* that is, 5.55±0.3 whereas the lowest zone of inhibition, that is, 0.60±0.28mm was shown by *Evolvulus alsinoides* (L.) extract against *Aspergillus oryzae,* as shown in tables 3 and 4 and figures 2 and 3.

Table 2. Anti-Fungal Activity by Methanolic Extracts of Different Species

Species	Plant parts	Zones of inhibition (mm)	
		Aspergillus niger	*Aspergillus oryzae*
Ipomoea cairica	Whole plant	11±1.15	13±0.58
Ipomoea carnea	Whole plant	-	-
Cuscuta reflexa	Whole plant	10±0.38	9±0.58
Ipomoea hederacia	Whole plant	-	-

Species	Plant parts	Zones of inhibition (mm)	
		Aspergillus niger	*Aspergillus oryzae*
I. pes-tigridis	Whole plant	12±0.58	8±0.00
Ipomoea eriocarpa	Whole plant	7±0.38	13.3±0.28
Ipomoea arachnosperma	Whole plant	12.1±0.00	11.1±0.58
Ipomoea quamoclit	Whole plant	10.5±0.08	9.5±0.01
Merremia aegyptia	Whole plant	5.55±0.38	6.20±0.18
Convolvulus arvensis	Whole plant	-	-
Convolvulus prostrates	Whole plant	8.60±0.18	9.01±0.36
Evolvulus alsinoides	Whole plant	7.60±0.28	6.50±0.26

Table 3. Anti-Bacterial Activity by Different Species of *Convolvulaceae* and *Cuscutaceae*

Species	Plant parts	Zones of inhibition (mm)			
		E. coli	*S. aureus*	*P. aeruginosa*	*B. subtilis*
Ipomoea cairica	Whole plant	11±0.58	10±0.58	9.3±0.33	13.3±0.88
Ipomoea carnea	Whole plant	7.7±0.9	10±0.6	8.3±0.9	10.0±0.6
Cuscuta reflexa	Whole plant	11.3±0.3	8.3±0.3	13.0±0.6	13.3±0.9
Ipomoea hederacia	Whole plant	8.1±0.4	11.2±0.3	8.4±0.9	10.0±0.8
I. pes-tigridis	Whole plant	7.6±0.9	10±0.56	9.3±0.36	13.2±0.88
Ipomoea eriocarpa	Whole plant	8.3±0.4	9.0±0.54	10±0.7	11±0.54
Ipomoea arachnosperma	Whole plant	7.7±0.8	8.0±0.4	9.2±0.34	12.2±0.86
Ipomoea quamoclit	Whole plant	10.3±0.3	8.2±0.3	12.0±0.6	13.1±0.9
Merremia aegyptia	Whole plant	7.4±0.8	8.0±0.4	11.0±0.5	12.1±0.84
Convolvulus arvensis	Whole plant	8.0±0.4	12.2±0.3	7.4±0.9	9.0±0.8

Species	Plant parts	Zones of inhibition (mm)			
		E. coli	*S. aureus*	*P. aeruginosa*	*B. subtilis*
Convolvulus prostratus	Whole plant	7.4±0.9	11±0.56	9.2±0.36	12.2±0.88
Evolvulus alsinoides	Whole plant	8.3±0.4	9.0±0.54	9.3±0.7	12±0.54

4. DISCUSSION

The current study was carried out to estimate the, antimicrobial and antioxidant activities of all the twelve plant species. The extract of the selected plant parts was obtained through the process of maceration. Methanol was used as a solvent for the purpose of maceration [14].

Cuscuta species has been reported as a rich source of phytochemicals used in traditional medicine for the treatment of itching and bilious disorders, as well as purgatives, diaphoretics, anthelmintics, diuretics, and tonics [15]. Al-Rifai et al. in 2017 reported the presence of coumarins, steroids, flavonoids, saponins, tannins, triterpenoids, carbohydrates, amino acids, and proteins in the plant extract analysis of different *Convolvulacea* species. They described these secondary metabolites as biologically active ingredients which are directly responsible for different activities, such as antifungal, antimicrobial, antioxidant, and anticancer activities [16].

All the zones of inhibition showed by bacterial strains varied from 7.7±0.9 to 13.0±0.6. The methanolic extract of whole plant maximum zone of inhibition of 13.0±0.6 mm against P. aeruginosa. Among the fungal strains, the highest zone of inhibition was shown by leaves extract against *Aspergillus niger* while the lowest zone of inhibition was shown by seed extract against *Aspergillus oryzae.* All of the above results are compatible with the results given by [17]. Well diffusion method was used to check the antimicrobial activity against *E. coli.* The highest zone of inhibition was noted in aqueous extract and the highest antifungal zone of inhibition was found against *Aspergillus niger* and *Aspergillus oryzae,* respectively [18]. Recently, *Convolvulus arvensis* was reported as a source of carvacrol and thymol. These are potentially active compounds with antioxidant and antibacterial properties and their extracts create a pow erful antioxidant effect by reducing lipid oxidation [19, 20].

Significant bacterial activity was observed against gram positive and gram negative bacteria by using well diffusion method [21].

Three methods including total phenolic content, total flavonoid content, and DPPH were used to determine the antioxidant potential of the selected parts of *Ipomoea hederacea*. In the total phenolic content method, the highest value 0.30±0.00 GAE (µg/ml) was shown by the methanolic extract of the whole plant at the concentration of 500 µg/ml. The antioxidant prospective of plants varied across species. Within similar species, differences were noted on the bases of solvent extraction, the physical form of the plant material (fresh or dried), and environmental factors. Excellent correlation exists for plants antioxidant activity [22].

Antioxidants were investigated by using DPPH, FRAP and TAEC methods. *Ipomoea eriocarpa* showed a higher amount of total polyphenols (0.98 ± 0.073 mg/g dry weight). Methnolic extract of *Ipomoea* species contain flowers ethanol extract contains highest amount of polyphenols which was in agreement with the current results [23]. Phytochemical screening of *Ipomoea* species also revealed the presence of tannins, saponins, flavonoids, triterpenes, phenols and alkaloids. Antimicrobial sensitivity results showed that methanolic extract had promising antibacterial and fungal activity at the highest concentration of 500 mg/ml [24].

Free radical is a molecule with an unpaired electron. It is involved in parasitic and bacterial infections, inflammation, and also has anti-bacterial and antimicrobial properties [25]. The results demonstrated that the methanolic extracts of the selected species showed free radical scavenging activity like DPPH, ABTS, and FRAP. DPPH, FRAP, and FTC assay were used to determine the antioxidant potential of plant species. It was found that methyl extract showed the highest DDPH scavenging activity [26]. Antioxidant activity was assessed by using various methods including FRAP, BHT, DPPH, and TPC. The highest antioxidant value was obtained by using FRAP assay. Current investigation showed that the use of phytocompounds in combination with antibiotic therapy to reduce the incidence allergic reactions and drug resistance is promising.

4.1. Conclusion

The current study was carried out to show the antioxidant and antimicrobial activity of 12 plant species that is *Cuscuta reflexa* Roxb

belonging to family of Cuscutaceae and eleven species from Convolvulaceae against microorganisms. The study concluded that all the species have antioxidant potential and are a significant source of natural antioxidant; however, it is recommended that future research should be carried out to explore potent antioxidant agents.

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