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Phytochemical Screening, Pharmacological Profiling and Identification of Potential Therapeutic Applications of *Rosa multiflora*

Jehan Zada[1,5*], Sami Ullah[2], Sumaira Shah[1], Shahana[3], Ali Hazrat[4], Imtiaz Ahmad[1], Haroon Raza[1], Muhammad Khaliq[5]

[1]*Department of Botany, Bacha Khan University Charsadda, Pakistan.* (24420)
*jehanzada777@gmail.com
sunehra23@gmail.com
imtiazbkuc@gmail.com
haroontermizi@gmail.com
[2]*Department of Botany, University of Peshawar, Peshawar, Pakistan.* (25000)
sami_jan69@yahoo.com
[3]*Department of Botany, Islamia College Peshawar, Pakistan.* (25000)
shahana36910@gmail.com
[4]*Department of Botany, University of Malakand, Dir, Pakistan.* (18800)
aliuom@gmail.com
[5]*Department of Botany, Govt; Degree College Lal Qilla, Dir Lower, Pakistan. (*18300).
mkhaliq1988@gmail.com

Correspondence
Jehan Zada
jehanzada777@gmail.com

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Abstract

The pharmacological screening of plant products gained significant attention against disease causing microbes and oxidative damage of free radicals. The current attempt was aimed to screen out phytochemicals, pharmacological and antioxidant potential of the *Rosa multiflora*. Phytochemical screenings of leaves revealed the presence of carbohydrates, proteins, phenols, flavonoids, tannins, and saponins. FTIR analysis of leaf powder revealed the existence of aldehyde/ketone functional groups, evident from the C=O stretch at 1719 cm^{-1} indicating the presence of aldehyde compounds. Spectral analysis confirmed the presence of major functional groups of aldehyde/ketone(C=O), phenol (OH) and polysaccharides. The ethanolic crude extract of leaf was tested for antioxidant, antibacterial, and antifungal potential. The extract was found effective against *Enterobacter aerogenes* (13.76±0.51mm), followed by *Pseudomonas aureus* (12.37±0.55mm), *Streptococcus aureus* (11.06±1.05mm), *Bacillus subtilis* (10.96±0.45mm), *Escherichia coli* (9.1±0.66mm) and *Salmonella typhi* (8.7±0.52mm) at dose of 20mg/ml. The extract was found effective against bacteria, specifically at a concentration of 20mg/ml. The antifungal activities show that crude extract of leaf was effective against *Aspergillus nigar* (7.33±1.35mm) followed by *Aspergillus fumigatus* (6.86±1.8mm) and *Aspergillus flavus* (6.16±1.26mm), indicating that leaf extract effectively inhibits the growth of these fungal strains. The antioxidant potential of the plant leaf extract was evaluated using a DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical scavenging assay. The extract exhibited significant activity at a concentration of 62.5mg/ml with 76.53% inhibition. In comparison, Vitamin-C (ascorbic acid) demonstrated 100% inhibition at the same concentration. The selected plant potential of could prove ground-breaking for pharmaceutical industry, researchers and scientific community for further research in this area.

KEYWORDS; Antioxidant, DPPH, antibacterial, Antifungal, Extract, Rosa.

1.0 INTRODUCTION

Plants are the most common, accessible and richest sources of potentially active drugs for combating various diseases. Natural products are more potent and safe for combating and treating diseases as compare to synthetic one. Therefore, it is important to find out pharmacological features of medicinal plants for the development of new drugs. Oxidation produce new free radical which causes damage of DNA and cell, loosen tissue, genetic disorder and cancer cell formation etc. Various artificial antioxidants are used such as propyl gallate, butylated hydroxyanisole, butylated and hydroxytoluene for the protection of cell from damages in human body however recent research discover that all of these synthetic antioxidant is dangerous for health and cause damage to liver cells [1]. So the application of natural antioxidant is vital to protect human body from free radical and control heart diseases, cancer, and arthritis process [2]. Secondary metabolites such as flavonoids, glycosides alkaloids, phenolic compounds, saponins, tannins, volatile oils are mostly chemical constituents of medicinal plants [3, 4]. Alkaloids, flavonoids,

tannins, saponins, terpenoids and phenolic compounds are the most crucial bioactive constituents of plants [5]. Bacteria cause different diseases in humans, plants and animals. Bacterial pneumonia, cholera, whooping cough, Leprosy, tetanus and Lyme are some bacterial disease of humans. Antibacterial activities of *Rosa indica* has been confirmed against various microorganism viz., *Staphylococcus aureus, Proteus mirabilis, Escherichia coli, Streptococcus pneumonia, Shigella flexneri, Pneumonia auregenosa, Salmonella typhimuruim, Vibia cholea* and showed excellent effect [6]. The antimicrobial activities of *R. rugosa* petals are effective against the pathogenic and intestinal bacteria, gram positive and gram negative bacteria and the selective ability was similar with the capacity of prebiotics [7]. The increasing bacterial and fungal infections, the difficulties faced in their treatment and the increase in resistance to antibacterial and antifungal agents have motivated the search for therapeutic substitutes [8, 9]. Medicinal plants are the main source of antifungal and antibacterial compounds and used in many countries as source and effective drugs [10]. Bahl *et al.,* [11] recorded that different parts of rose plant, including leaves, stem and petals possess antibacterial and antifungal properties. The petal extract of rose plant shows antifungal activity against *C. albicans* [11]. Various researchers also reported that the effectiveness of plants extracts against microbes is due to the existence of various secondary metabolites [12]. *Rosa multiflora* Thunb. is deciduous scrambling shrub climbing over other plants up-to a height of 7 m. Stem have curved prickles. Leaflets are ovate to obviate, obtuse, acute or acuminate at apex, with serrated margins, glabrous or sparsely pubescent above and beneath. Stipules with long fringes at base of petiole. The flowers are white or rarely pink, 1.5- 2 cm in diameter and bloom in clusters. The fruit is a small, globose and 6-7 mm in diameter and become red on maturation.

2.0 Materials and Methods

2.1 Collection and Preservation of Plant Material

Rosa multiflora leaves in fresh and mature form were collected from Maidan valley, Dir, Khyber Pakhtunkhwa, Pakistan. The collected leaves were washed, cleaned with tap water, separated and dried in shade. The dried leaves were grinded to fine powdered by electric grinder.

2.2 Phytochemical screening tests

The qualitative phytochemical screening tests were carried out for leaf extract of *R. multiflora* for the detection for certain secondary and primary metabolites.

2.2.1 Detection of carbohydrates

Benedict's Test: The existence of reducing sugar was confirmed by the formation of reddish-brown precipitate after boiling the extract with addition of few drops of Benedict's solution in a water bath [13].

***Fehling's Test*:** The reducing sugar were determined by the formation of brick-red precipitate after boiling the extract with equal volume of Fehling's A and B solutions to the extract solution [14].

2.2.2 Detection of protein and amino acids

Millon's test: The presence of amino acids or proteins was identified by white precipitate that turned red upon gentle heating of the solution [14].

Ninhydrin test: 10ml of the sample solution was mixed with 0.2% Ninhydrin solution and boiled; the formation of violet color indicated the presence of proteins [15].

2.2.3 Detection of alkaloids

Hager's test: The existence of alkaloids in the extract solution was confirmed by the formation of a yellow precipitate upon the addition of a few drops of Hager's reagent [16].

Wagner's Test: Extract solution was treated with a few drops of Wagner's reagent. The appearance of reddish- brown precipitate indicates the presence of alkaloids [16].

2.2.4 Detection of phenol

Ferric chloride test: Equal volumes (2ml each) of Ferric Chloride and sample extract were mixed; a deep bluish-green colour indicated the presence of phenols [17].

2.2.5 Detection of flavonoids

Alkali reagent test: The appearance of yellow to red precipitate upon treatment with NaOH solution indicates the presence of flavonoids [18].

2.2.6 Detection of tannins

Ferric chloride test: The formation of a yellow to red precipitate upon the addition of $FeCl_3$ confirms the presence of tannins in the extract solution [18].

Alkali reagent test: The existence of tannins was identified by the rapid formation of a yellow to red precipitate upon the addition of NaOH [18].

2.2.7 Detection of saponins

Frothing test: The presence of saponins was indicated by froth formation on the surface of solution after vigorous shaking which persisted for some time [19].

2.3 FT-IR SPECTROSCOPY

FT-IR spectroscopy was performed using a PerkinElmer spectrometer (FT-IR SPECTRUM ONE) in the range of 4000-400 cm^{-1} with a resolution of 4 cm^{-1}. The leaf sample in powder form was mixed with Potassium Bromide (KBr) acquired from Merck chemicals. The mixture was then pressed into a thin pellet using Hydraulic Pellet Press and analysed using FT-IR spectroscopy. This analysis was carried out in Chemistry Department, Bacha Khan University Charsadda, Pakistan.

2.4 PREPARATION OF CRUDE EXTRACT

Leaf powder of *R. multiflora* (500g) was macerated in 3 L of ethanol for 15days with intermittent shaking on a magnetic stirrer. The mixture was filtered and the ethanol was evaporated under reduced pressure through rotary evaporator. The resulting crude extract was collected in airtight bottle and used for subsequent pharmacological screening.

2.5 Pharmacological activities

The Antioxidant activity was carried out by following the method of Chang *et al.* [20].

Antibacterial screening was conducted for ethanolic leaf extract of *Rosa multiflora* following Carron *et al*. [21] methodology.

The antibacterial activities were carried out against *Bacillus subtilis, Enterobacter aerogenes, Escherichia coli, Pseudomonas aureus, Streptococcus aureus* and *Salonella typhi*.

Antifungal potential of ethanolic leaf extract of *Rosa multiflora* were conducted using agar tube dilution method of Atta-Ur-Rahman *et al.* [22] with minor modification.

The extract was tested against *Aspergillus flavus, Aspergillus nigar, Aspergillus fumigatus* in a controlled environment.

2.6 Statistical Analysis

All the experiments were carried out in triplicate. The results obtained was analysed and values are expressed as mean±SD.

3.0 Results and Discussion

3.1 Phytochemical screening

The qualitative phytochemical screening shows that *R. multiflora* is a rich source of both primary and secondary metabolites as indicated in table (1). The analysis revealed the existence of carbohydrates proteins, flavonoids, phenols, saponins and tannins in the ethanolic extract of leaves but lack alkaloids. The similar findings were reported by Saeed *et al.*, [6] for leaf ethanolic extracts of *R. indica.* The Phytochemical screening of methanolic extract of *R. moschata* fruits indicated the presence of a variety of active compounds like saponins, proteins, tannins, carbohydrates, phenols and terpenoids [23]. The synthetic antibiotics along with treatment, cause's adverse side effects and some uncommon serious infection have been reported, which enforced scientist to search new source of natural's drugs which have the potential to combat microbes. In this respect, plants with common curative usage are being screened more capably to be considered as a substitution or as a better substitute for chemical based food preservatives [24].

Table 1. Phytochemical Screening of Leaf of R. multiflora Thunb.

S. No.	Constituents	Test Name	Ethanolic Extract
1	Carbohydrates	Fehling Test	+
		Benedict's Test	+
2	Proteins	Ninhydrine Test	+
		Millon's Test	+
3	Alkaloids	Wagner's Test	-
		Hagner's Test	-
4	Tannins	Ferric chloride Test	+
		Alkali Test	+
5	Flavonoids	Alkali Reagent Test	+
6	Phenols	Ferric chloride Test	+
7	Saponins	Frothing Test	+

3.2 Fourier Transform Infrared Spectroscopy of Leaf Extract of *R. multiflora*

FTIR analysis was conducted for the identification of potential biomolecules in leaf extract of *R. multiflora.* The FTIR spectra of leaf extract as displayed in (Fig. 1) shows the absorption bands at 3293, 2918, 2843, 1719, 1606, 1356, 1244 and 1031 cm^{-1}. The strongest absorption peak was observed at 1719 cm^{-1} reflects the existence of aldehyde/ketone functional group

(C=O) which indicate the presence of flavonoid and terpenoids compounds in leaf powder. The other functional groups for recorded absorption band i.e. 3293, 2918, 1244 and 1031 cm^{-1}) are OH stretching, C-H stretching, Collagen I & IV, C-C skeleton coupled with C-O bending respectively. Spectral analysis revealed that *R. multiflora* contains the major functional groups of aldehyde/ketone(C=O), phenol (OH) and polysaccharides (Fig. 1). The FTIR spectroscopy was carried out by different scientist to investigate the different functional group in the powder of various plants. Mak *et al.* [24] carried out (FTIR) spectral analysis for the powdered flower petals of hibiscus and Cassia and reported polysaccharides, suberin, and lipid and also the OH group of the phenolic compounds. Similarly, Jamuna *et al.,* [25] also conducted (FTIR) spectroscopy for essential oil of *Cymbopogon citratus* and concluded CH3, C=O, C-O and functional groups. The finding of these workers supported the present findings.

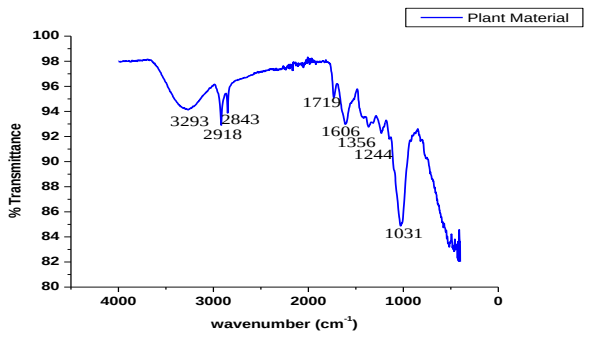


Fig. 1. FT-IR Spectroscopy of *R. multiflora* Leaf Extract

3.3 Pharmacological Profiling

3.3.1 Antioxidant Activity

The antioxidant activity of leaf extract was conducted in comparison with Ascorbic acid. The reducing power of leaf extract of *R. multiflora* is spectroscopically determined by changes in its colour from purple to yellow. Percent inhibition of leaf extract are 76.53% at concentration of 62.5 mg/ml respectively, while ascorbic acid showed 100% inhibition at the same concentration as shown in (Fig. 2). The comparative analysis of test sample and standard ascorbic acid indicated that ascorbic acid possess more reducing power which is then followed by leaf extract. Free radicals are those molecules which have one or more unpaired electron with no specific location on molecules. A stable compound like DPPH accepts electron or hydrogen and gets reduced. Free radicals are neutralized by antioxidants through giving their own single electron, stop the electron stealing reaction. The antioxidant agents do not become free radical by giving an electron due stability in either form. The phenolic compounds in plants are one of the main groups of compounds which possess antioxidant property. Various studies have shown that polyphenols and tea catechins are effective scavengers of free radicals/reactive oxygen species (ROS) produced due to various oxidative stresses [26, 27]. Phenolic compounds are essential plant components due to their free radical scavenging capacity aided by their hydroxyl groups [28]. They possess the ability to provide electron and scavenge reactive oxygen species (ROS) [29]. The present study revealed that leaf extract act as strong reducing agent by fast electron transfer from phenoxide anion to DPPH. The heart disease occurs due to the

oxidation of low density lipoproteins in blood. The main sources of phytochemicals are plants, fruits and vegetables which reduce the oxidation of compound and decrease the risk of several diseases like heart disease, cancer and some neurological disease. Some synthetic antioxidants have been used to control lipid oxidation but natural antioxidants are more useful and safe. Lu & Wang, [8] investigated the antioxidant abilities of *R. rugosa* and reported that the antioxidant activity is not only in vitro, but also in cell level and in vivo. So the results indicated that medicinal plants including *R. multiflora* have antioxidant efficiency that make it therapeutically important.

Table 2. Percent inhibition of leaf Extract of *R. multiflora.*

Concentration mg/ml	Ascorbic acid	Plant extract (DPPH)
1000mg/ml	100	31.09
500mg/ml	100	31.62
250mg/ml	100	42.01
125mg/ml	100	45.85
62.5mg/ml	100	76.53

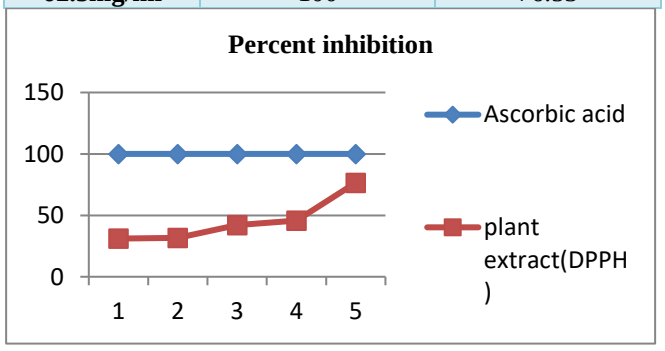


Fig. 2. Antioxidant activity of *Rosa Multiflora* leaf

3.3.2 Antibacterial Assays

Ethanolic extracts of *Rosa multiflora* leaf was tested against various bacterial strain i.e. *Bacillus subtilis, Enterobacter aerogenes, Escherichia coli, Pseudomonas aureus, Streptococcus aureus* and *Salmonella typhi* as shown in Table (3). The bactericide potential of leaf extracts was evaluated in comparison to negative control as DMSO and Cefixime as positive control. It was detected that all the extract has inhibitory effect against all the tested bacterial strains. The zone of inhibitions was measured in millimetre for various bacterial strains are summarized in Table (3). *R. multiflora* leaf extract was found effective against *Enterobacter aerogenes* (13.76±0.51mm), followed by *Pseudomonas aureus* (12.37±0.55mm), *Streptococcus aureus* (11.06±1.05mm), *Bacillus subtilis* (10.96±0.45mm), *Escherichia coli* (9.1±0.66mm) and *Salmonella typhi* (8.7±0.52mm) at dose of 20mg/ml. As the results shown in the table (3) the zones of inhibition increase with the increases in dose concentration. The ethanolic leaf extract of *R. multiflora* was found more active against *Bacillus subtilis* (19.23±0.75mm), followed by *Escherichia coli* (19.2±0.92mm), *Pseudomonas aureus* (19.06±0.21mm), *Enterobacter aerogenes* (18.9±0.66mm), *Streptococcus aureus* (18.43±0.75mm), and *Salmonella typhi* (16.6±0.53mm) at dose of 80mg/ml. The ethanolic extract of *Rosa multiflora* showed weak activity against all bacterial species as compared to standard drug however with increasing dose the inhibition was found significant. All the test bacterial species shows significant sensitivities but *Bacillus subtilis* (19.23±0.75mm) show higher sensitivity at dose of 80mg/ml among all tested bacterial species. Other investigator found similar antibacterial investigation on plants. The practice of natural plant products as

antimicrobials can be extremely effective in decreasing the dependence on antibiotics, reduce the chances of antibiotic resistance in food borne pathogenic organisms as well as help in preventing cross-contaminations by food borne pathogens [30]. The various extract of Cassia inhibited the growth of *Bacillus cereus* and *Klebsiella pneumonia* [24]. The extract of *Cassia fistula* was found to be more effective against gram positive bacterial strains i.e. *Staphylococcus aureus, Streptococcus pyogens;* two strains of gram negative bacteria i.e. *Escherichia coli, Pseudomonas aeruginosa* [31]. The extract and fraction of aerial parts of *Heliotropium bacciferum* are active against *Salmonella typhi, Escherichia coli, Pseudomonas areginosa, Staphylococcus aureus, Erwinia carotovora, Kelbsiella pneumonia, bacillus subtilis and Bacillus atrophaeusa* [32]. The ethanolic extract of rose petal demonstrates considerable antibacterial activity against gram positive bacteria [11]. The essential oils samples from *Rosa alba* demonstrated antimicrobial activity against all tested microorganisms except bacteria belonging to genera *Pseudomonas* [33].

Table 3. Antibacterial activities of leaves ethanolic extract of R. multiflora Thunb.

Concentration	Cefixime (Positive)	20 mg/ml	40 mg/ml	60 mg/ml	80 mg/ml
Bacillus subtilis	30	10.96±0.45	13.96±0.45	17.1±0.79	19.23±0.75
Enterobacter aerogenes	32	13.76±0.51	15.76±0.85	17.7±0.64	18.9±0.66
Escherichia coli	28	9.1±0.66	14.16±0.91	16.16±1.04	19.2±0.92
Pseudomonas aureus	36	12.37±0.55	14.7±0.36	16.7±0.61	19.06±0.21
Streptococcus aureus	24	11.06±1.05	13.03±0.29	15.6±0.79	18.43±0.75
Salmonella typhi	32	8.7±0.52	11.8±0.72	13.96±0.06	16.6±0.53

3.3.3 Antifungal activities

The ethanolic extract of *R. multiflora* leaf was evaluated for antifungal potential against few fungal strains like *Aspergillus nigar, Aspergillus flavus* and *Aspergillus fumigatus.* The fungicide potential of leaf extracts was estimated in comparison to negative control as DMSO and Fluconazole as positive control. The results are presented in table (4) shows that a general trend of dose dependency was detected i.e. effect became more distinct with increasing concentration on various tested samples. The ethanolic extract of *R. multiflora* leaf was found effective against *Aspergillus nigar* (7.33±1.35mm) followed by *Aspergillus fumigatus* (6.86±1.8mm) and *Aspergillus flavus* (6.16±1.26mm) at dose of 20 mg/ml. The results presented in table (4) demonstrate a positive correlation and zone of inhibition, where the zone of inhibition increases as the concentration increases. All the fungal strain shows higher zone of inhibition with the increases in the dose (80 mg/ml) i.e. *Aspergillus nigar* (15.2±1.47mm) followed by *Aspergillus fumigatus* (14.76±1.1mm) and *Aspergillus flavus* (14.43±0.6mm) at dose of 80

mg/ml. The present investigation on *R. multiflora* has also indicated very encouraging antibacterial potential. The ethanolic extract of *Rosa multiflora* showed significant activity against all fungal strains i.e. *Aspergillus nigar, Aspergillus flavus* and *Aspergillus fumigatus* as compared to standard drug however with the increasing dose the inhibition was found significant (Table 4). Plants are being used as medicines for the treatment of different diseases throughout the world from prehistoric time [34]. Other researcher found similar findings of antifungal activities on plants to find a substitute of synthetic medicines. Medicinal plants are used in making of different drugs singly or in combination [35]. Plants are used as the main source of raw materials for preparation of other medicines [36]. Different varieties of fungus have been reported to cause several types of complications in human beings. Higher plant extracts have the potential to decrease or completely stop the growth of fungi [37]. These extracts can demonstrate to be active and safe antifungal agents as compared to synthetic fungicides [38, 39]. Similar investigation was carried by other investigators on various plants; *Rosa rugosa* Thunb. bioactive compounds [40], flower extracts of *Hibiscus rosa-sinensis* L. and *Senna bicasularis* L. [24], essential oil of *Cymbopogon schoenanthus* [41]. All these studies suggested that plants including *R. multiflora* have antifungal potentials.

Table 4. Antifungal Activities of Ethanolic extract of Rosa multiflora leaves

Species	20mg/ml	40mg/ml	60mg/ml	80mg/ml	S.D*
Aspergillus flavus	7.33±1.35	9.76±0.25	12.3±1.28	15.2±1.47	18.8
Aspergillus nigar	6.166±1.26	9.2±0.36	11.43±1.1	14.43±0.6	17.9
Aspergillus fumigatus	6.86±1.8	9.2±0.8	12.03±0.49	14.76±1.1	18.3

S.D*=Fluconazole

4.0 Conclusion

The findings in present investigation substantiate the medicinal efficacy of *R. multiflora* leaf extract for its antioxidant, antibacterial and antifungal properties. Qualitative preliminary phytochemical screening indicated the existence of carbohydrates, proteins, flavonoids, alkaloids, tannin and saponins in the leaf extract of the tested plants. FT-IR analysis is useful for detection of biochemical by assessing their functional groups in extract. Spectral analysis revealed that leaf extract contains the major functional groups of aldehyde/ketone(C=O), phenol (OH) and polysaccharides. Antibacterial bioassays of ethanolic extracts of leaf of *R. multiflora* showed that the extract were effective against the various tested bacterial strains. A dose dependent antifungal activity was recorded for tested fungal strains. Antioxidant potential of the plant leaf was evaluated and higher activity was recorded at lower concentration. These results reflect *R. multiflora* as a natural source of antioxidants with potential therapeutic benefits against free radicals and microbes. However, further

study is warranted to determine and isolate the active metabolites for application.

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Conflict of Interest
The authors have no potential conflict of interest to declare.

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5.0. References

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