Evaluation of Wound Healing Effect of Aqueous Stem Extract of *Pterospermum Lanceiifolieum* on Wistar Rats

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Abstract

The present investigation has been undertaken to study the wound healing properties of aqueous extract of *Pterospermum Lanceiifolieum.* The plant *Pterospermum Lanceiifolieum has* a long history in herbal medicine in many countries. Experiments were conducted following standard procedures. The extracts were evaluated for their *in vitro* antioxidant, antimicrobial and total phenol and flavonoid content. The AQPA ointment were administered topically, for evaluating the wound healing potential in excision wound model for twenty one days. Povidone iodine ointment was used as a standard for wound healing in excision wound model. Extract treated group showed *in vitro* antioxidant, antimicrobial properties compared with standard and control. AQPA exhibited similar *in vivo* wound healing activity that of the standard but with lesser magnitude. The result may be attributed to the phytoconstituents such as flavonoids and phenolics present in it which may be due to their individual or cumulative effect that enhanced wound healing and provided scientific evidence to the ethnomedicinal futures of *Pterospermum Lanceiifolieum.* These findings could justify the inclusion of this plant in the management of wound healing.

Keywords: AQPA, Wound healing potential, Phytoconstituents.

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Introduction

Since the first chemicals were identified from plant material in the 1960s, a strategy to finding individual physiologically active molecules has been known as ethnopharmacology. It should be emphasised that using plants for purposes other than purely medical ones may lead to the development of new medications. As a result, compounds used as pesticides, poisons, in agriculture, as cosmetics, during fermentation processes, and for religious purposes may also include active ingredients that can be employed as leads for the development of new drugs. But due to public interest, which is larger than expected given that, according to the WHO, 80% of the world's population currently lives in developing nations, the article merely details traditional plants of Bangladesh and their application.

Accidental or surgical trauma, as well as a number of different medical problems, can result in wounds. This wound frequently results in pain, inflammation, and loss of function, which have an impact on the patient's quality of life and cost [1]. Acute wounds are one category of wounds.or persistent wounds. The intricate process of rebuilding tissue layers and damaged and malfunctioning cellular components is known as wound healing [2]. Acute wounds heal in stages, and after four weeks, the healing process is clearly visible. Chronic wounds do not heal normally over the course of four weeks and do not advance through the stages of healing. It can be argued that variables at the wound site, systemic mediators, the type of injury, or any underlying diseases affect how a wound heals [3]. Wound\treatment Here, we provide a review of natural substances (both from plants and animals) that are significant in the healing of wounds, as well as their healing methods and practical use restrictions. Based on how well those chemicals target bioactivities for wound healing, we categorised them. We detailed the most recent developments in the use of natural substances. We offered data tables so that readers could look up natural compounds according to their bioactivity, source, and phases of targeting in wound healing.

Material and Methods

PLANT COLLECTION AND AUTHENTICATION

The stem part of the plant *Pterospermum Lanceiifolieum* (Roxb) DC was collected from Palakkad District of Kerala and authenticated from Department of Botany, Saifia College, Bhopal. Soon after collection, the stems were cleaned and shade dried. After drying, these stems were crushed to a coarse powder, stored in air tight plastic container for further use.

EXTRACTION OF THE PLANT MATERIAL [40]

The coarsely powdered stem (200gm) were taken in a round bottom flask and with water for 48hours at room temperature. After extraction the extracts were evaporated or concentrated by using rotary evaporator and dried at room temperature. The obtained crude extracts were weighed and stored at 4^0C for the further analysis.

QUALITATIVE PHYTOCHEMICAL ANALYSIS OF AQPA [41][42][43][44]

Preparation of test sample

A small quantity of the extract was dissolved in 5ml of distilled water and filtered. The filtrate was tested to detect the presence of various phytochemical constituents in the sample.

INVITRO ANTIOXIDANT STUDY OF AQPA

DPPH Free Radical Scavenging Assay [47]

Principle

The DPPH [1, 1-diphenyl-2-picrylhydrazyl (α, α-diphenyl-β-picrylhydrazyl)] assay method is based on the reduction of DPPH, a stable free radical. The free radical DPPH with an odd electron gives a maximum absorption at 517nm (purple colour). When Antioxidants react with DPPH, which is a stable free radical becomes paired off in the presence of a hydrogen donor (e.g., a free radical scavenging antioxidant) and is

reduced to the DPPHH and as consequence the absorbance's decreased from the DPPH. DPPH radical is a stable radical by virtue of the delocalization of the spare electron over the molecule as a whole, so that the molecules do not dimerize, as would be the case with most other free radicals. The delocalization also gives rise to the deep violet colour, characterized by an absorption band in ethanol/methanol solution centred at about 520nm Radical to the DPPH-H form, results in decolorization (yellow colour) with respect to the number of electrons captured. More the decolorization more is the reducing ability. This test has been the most accepted model for evaluating the free radical scavenging activity of any new drug. When a solution of DPPH is mixed with that of a substance that can donate a hydrogen atom, then this gives rise to the reduced form (Diphenyl picryl hydrazine; non radical) with the loss of this violet colour (although there would be expected to be a residual pale yellow colour from the picryl group still present).

Procedure

The method adopted here is Blois method. Where by using the stable DPPH radical, the antioxidant capacity of the extract was measured in terms of hydrogen donating or radical scavenging ability. 1ml of 0.3mM solution of DPPH in ethanol was added to various concentrations of sample (10, 20, 40, 60, 80, 100 µg/ml) and the reference compound (5, 10, 15, 20, 25 and 30 µg/ml), shaken vigorously, and left to stand in the dark at room temperature. After 30 min absorbance was measured at 517nm against a blank. Quercetin was used as reference compound. A control reaction was also carried out without the test sample. All the tests were performed in triplicate in order to get the mean values. The percentage of inhibition was calculated by comparing the absorbance values of the control and test samples. Free radical scavenging activity was expressed as percentage inhibition (I%) and calculated using the following equation:

Percentage inhibition (I%) = (Abs control- Abs sample /Abs control) X 100

Different sample concentrations were used in order to obtain calibration curves and to calculate the IC_{50} values. (IC_{50} - concentration required to obtain a 50% radical.

ABTS Assay [48][49]

Principle

A method for the screening of antioxidant activity is reported as a decolorization assay applicable to both lipophilic and hydrophilic antioxidants, including flavonoids, hydroxyl cinnamates, carotenoids, and plasma antioxidants. The pre-formed radical monocation of 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS*+) is generated by oxidation of ABTS with potassium persulfate and is reduced in the presence of such hydrogen-donating antioxidants present in the sample. The influences of both the concentration of antioxidant and duration of reaction on the inhibition of the radical cation absorption are taken into account when determining the antioxidant activity. Chemistry involves the direct generation of the ABTS radical monocation with no involvement of an intermediary radical. It is a decolorization assay; thus the radical cation is pre-formed prior to addition of antioxidant test systems, rather than the generation of the radical taking place continually in the presence of the antioxidant. An antioxidant with an ability to donate a hydrogen atom will quench the stable free radical and inhibits the absorption of the radical cation which has characteristic long-wavelength absorption spectrum showing maxima at 660,734, and 820nm.The relatively stable ABTS radical has a green colour and is quantified spectrometrically at 734nm. It is applicable to both aqueous and lipophilic systems.

Procedure

ABTS radical scavenging activity of the extract was measured by Rice-Evans method. ABTS was dissolved in water to a 7mM concentration. ABTS radical cation (ABTS+) was produced by reacting ABTS stock solution with 2.45mM potassium persulfate and allowing the mixture to stand in the dark at room temperature for 12- 16hr before use. The radical was stable in this form for more than 2 days when stored in the dark at room temperature. For the study, ABTS solution was diluted with phosphate buffer saline pH 7.4 (PBS) to an absorbance of 0.70 (± 0.02) at 734nm and equilibrated at 30^0C. After addition of 1ml of diluted ABTS solution to various concentrations of sample or reference compound (Quercetin), the reaction mixture was incubated for 6min and then absorbance was measured at 734nm against a blank. A control reaction was carried out without the sample. All the tests were performed in triplicate in order to get

the mean values. The percentage inhibition of ABTS+ by the sample was calculated according to the formula:

Percentage inhibition (I %) = (Abs control- Abs sample /Abs control) X 100

Different sample concentrations were used in order to obtain calibration curves and to calculate the EC_{50} values. (EC_{50} - concentration required to obtain a 50% radical scavenging activity).

ANTI-MICROBIAL ACTIVITY OF AQPA [52]

Antibacterial study of AQPA Preparation of inoculums

The inoculums for the experiment were prepared in fresh nutrient broth from the preserved slant culture. The turbidity of the culture can be adjusted by the addition of broth or sterile saline (if it is excessive) or by further incubation to get the required turbidity, and the newly prepared inoculums were standardized by adjusting the turbidity of the culture to that of McFarland standards.

Preparation of sterile swabs

Cotton wool swab on wooden applicator or plastics were prepared and sterilized by autoclaving or by dry heat (only for the wooden swabs). It was sterilized by packing the swabs in culture tubes, papers or tins etc.

Sterilization of forceps

Forceps can be sterilized by dipping in alcohol and burning off the alcohol.

Experiment

The standardized inoculums is inoculated in the sterilized plates prepared earlier (aseptically) by dipping a sterile in the inoculums removing the excess of inoculums by passing and rotating the swab firmly against the side of the culture tube above the level of the liquid and finally streaking the swab all over the surface of the medium 3times rotating the plate through an angle of 60º after each application. Finally pass the swab round the edge of the agar surface. Leave the inoculums to dry at room temperature with the lid closed. The sterile discs are soaked overnight in sample solutions, AQPA.

Results

EXTRACTIVE YIELD

Percentage Yield:

Coarsely powdered *Pterospermum Lanceiifolieum* stem were extracted with aqueous using maceration technique.

The percentage yield of Aqueous stem extract of *Pterospermum Lanceiifolieum* (AQPA) was found to be 14 % w/w.

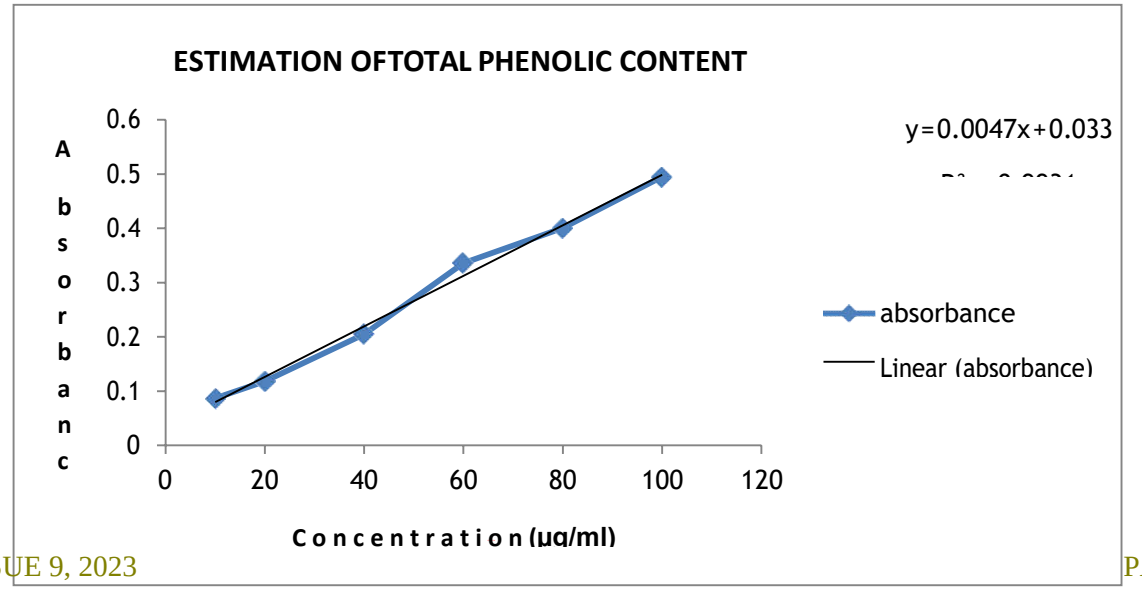
QUANTIFICATION OF TOTAL PHENOL AND FLAVONOIDS

ESTIMATION OF TOTAL PHENOL OF AQPA

Table 6: Estimation of total phenolic content of AQPA

Sample	Concentration (µg/ml)	Absorbance
Standard (Gallic acid) 1mg/ml	10	0.087
	20	0.118
	40	0.205
	60	0.336
	80	0.401
	100	0.495
AQPA	100	0.3185

Figure 2: Estimation of total phenolic content of AQPA

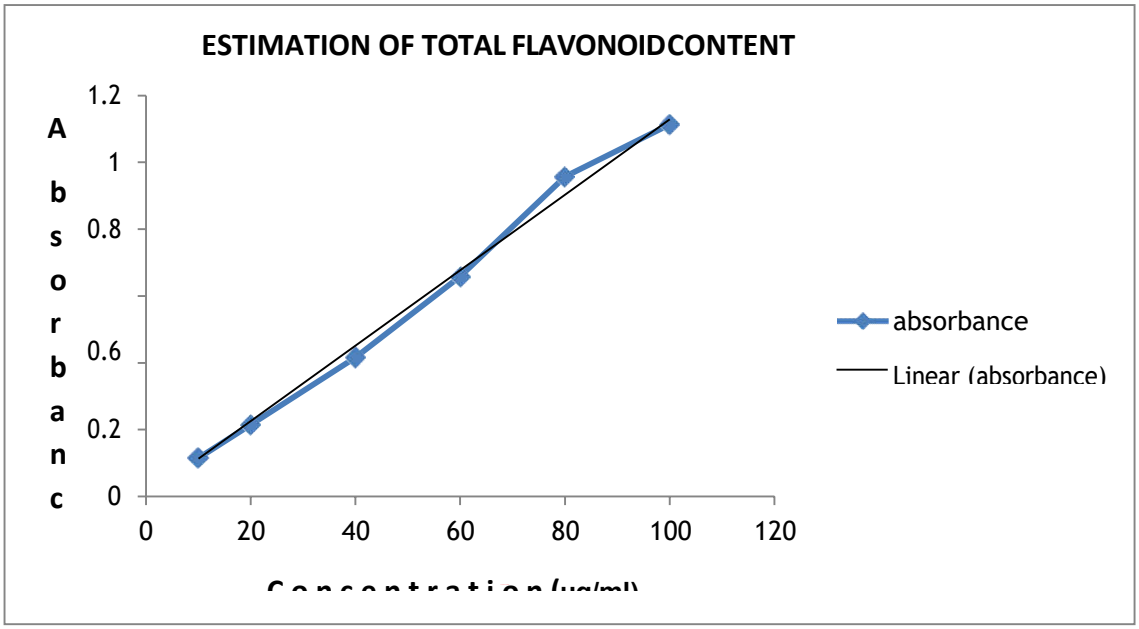


ESTIMATION OF TOTAL FLAVONOID CONTENT OF AQPA Table 7:

Estimation of total flavonoid content of AQPA

Sample	Concentration (µg/ml)	Absorbance
Standard (Quercetin) 1mg/ml	10	0.031
	20	0.085
	40	0.26
	60	0.5026
	80	0.776
	100	1.053
AQPA	100	0.7046

Figure 3: Estimation of total flavonoid content of AQPA



The total flavonoid content in AQPA was found to be 40.64 mg/g of extract calculated as Quercetin equivalent.

IN VITRO ANTIOXIDANT ACTIVITY OF AQPA

DPPH RADICAL SCAVENGING ACTIVITY

Table 8: Percentage inhibition and IC_{50} values of DPPH radical by Quercetin

Sl.No	Concentration (µg/ml)	Percentage inhibition	IC_{50}(µg/ml)
1	5	41.2	1.55
2	10	55.7	
3	15	69.4	
4	20	86.4	
5	25	96.5	
6	30	99.38	

Fig 4: DPPH radical scavenging activity of Quercetin

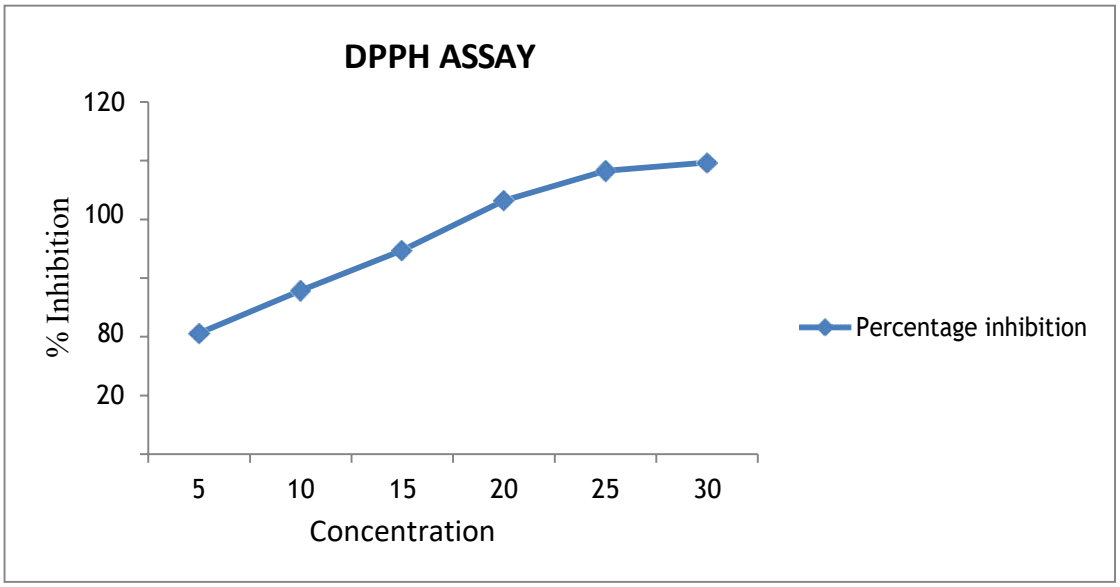
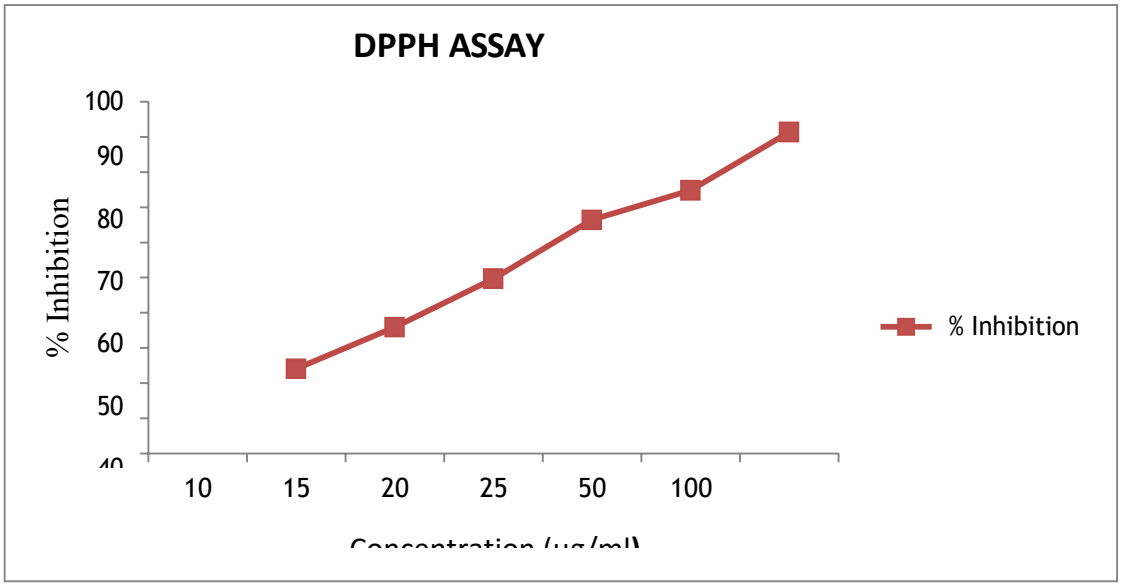


Table 9: Percentage inhibition and IC_{50} values of DPPH radical by AQPA

Sl.No	Concentration (µg/ml)	Percentage inhibition	IC $_{50}$(µg/ml)
1	10	26.69	17.77
2	15	43.89	
3	20	50.12	
4	25	59.67	
5	50	69.77	
6	100	91.36	

Fig 5: DPPH radical scavenging activity of AQPA

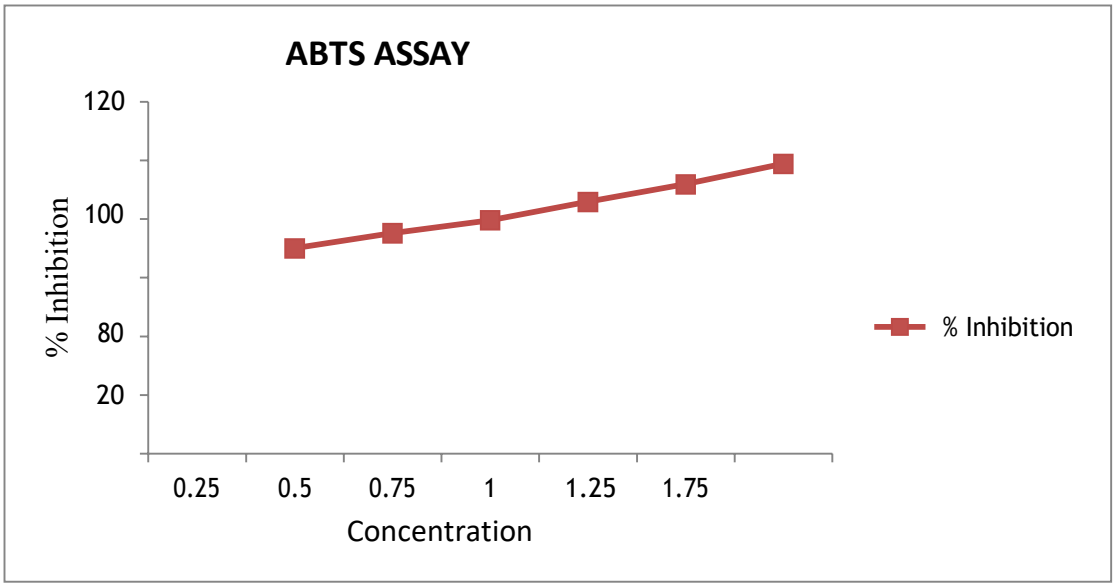


TOTAL ANTIOXIDANT ACTIVITY BY ABTS RADICAL CATION ASSAY

Table 10: Percentage inhibition and IC_{50} values of ABTS radical by Quercetin

Sl.No	Concentration (µg/ml)	Percentage inhibition	IC_{50}(µg/ml)
1	0.25	70.08	0.1142
2	0.5	75.22	
3	0.75	79.62	
4	1.0	85.88	
5	1.25	91.9	
6	1.75	98.85	

Fig 6: ABTS radical scavenging activity of Quercetin



ANTI MICROBIAL ACTIVITY OF AQPA

ANTI BACTERIAL ACTIVITY

Table 14: Zone of inhibition for Gram +ve organisms

Sino	Gram +ve organisms	Standard Ciprofloxacin(mm)	AQPA(mm)
1	*Bacillus subtilis*	25	10
2	*Staphylococcus aureus*	26	14

Table 15: Zone of inhibition for Gram –ve organisms

Sino	Gram –ve organisms	Standard Ciprofloxacin(mm)	AQPA(mm)
1	*E.coli*	26	9
2	*Pseudomonas auregenosa*	27	10

ANTIFUNGAL ACTIVITY OF AQPA

Table 16: Antifungal activity of AQPA

Sino	Organisms	Standard Fluconazole(mm)	AQPA(mm)
1	*Aspergillus niger*	21	12
2	*Monascus purpureus*	24	15

CONCLUSION

Wound healing is a complex and continuous process that begins immediately after injury, followed by homeostasis, blood clotting, inflammation, proliferation and remodeling phases. All these phases can promote or prolong healing by influencing external or internal factors including infection sex hormones and nutrition. Delay in healing process increases the possibility of getting infected, improper recovery, and formation of unpleasant scar. The study thus demonstrated the wound healing activity of aqueous stem extract of *Pterospermum Lanceiifolieum* (Roxb) DC and found to be effective in the functional recovery of the wound. The extracts promote wound contraction; increases hydroxyproline, hexosamine and uronic acid of excision wound as compared to control group. The result may be attributed to the phytoconstituents such as flavonoids and phenolics present in it which may be due to their individual or cumulative effect that enhanced wound healing and provided scientific evidence to the ethnomedicinal futures of

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