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Antibacterial activity of seed extracts of *Swietenia mahagoni* Jacq

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ABSTRACT

Swietenia mahagoni Jacq. (Meliaceae) seeds and bark were used traditionally for the treatment of hypertension, diabetes, malaria, and in epilepsy. In the present study the phytochemical investigations involve the extraction of plant material obtained by following cold maceration with ethanol and the percentage yield of the extract is 12.86% and identification of the phytoconstituents. Then the solvent extraction process was processed by the following order of polarity as petroleum ether, benzene, ethyl acetate and ethanol. These extractions were evaluated for anti microbial activity by Cup Plate method, a commercial sample ofloxacin is used as standard antibiotic. Sterile Petri dishes were filled to a depth of 4.5 mm with sterile nutrient agar medium that had previously been inoculated with a suitable inoculum of test organism of P.aeruginosa-MCC2080, B.licheniformis-MCC2297, P.mirabilis-, S.aureus-MCC2408 and E.Coli-MCC2246 individually at 45°C to 50°C. 0.1ml of the standard drug and test extract were poured into bored wells by means of 1ml sterile syringe and incubated at 37 °c for 24 hours. The ethanolic extract and ethyl acetate extract of S.mahagoni Jacq. seed at different dose (40,60,80mg/ml) level showing significant anti-microbial activity. The plant can be considered as low cost, potent, herbal medicine for good anti-microbial activity.

Keywords: *Swietenia mahagoni* Jacq. Anti microbial activity, extracts
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INTRODUCTION

Swietenia mahagoni Jacq. (Meliaceae) is a large, deciduous, and economically important timber tree native to the Central America and is commonly known as "Mahogany". Generally called as Spanish, Cuban, Puerto Rico or Jamaica Mahagony tree [1]. It is a valuable species closely related to the African genus Khaya and the source of one of the most popular traditional medicines in Africa [2].

In India, traditionally it is used for several medicinal purposes. The seeds and bark are used for the treatment of hypertension, diabetes, malaria, and in epilepsy as a folk medicine in Indonesia and India [3-4]. Traditionally the bark decoction is used orally to increase appetite, to restore strength in cases of tuberculosis, to treat anaemia, diarrhoea(as decoction), dysentery, fever and toothache [5-6].

The local people of East Medinipur (West Bengal), Balasore (Orissa) traditionally use the aqueous extract of its seed and bark for curing psoriasis and also used as an antiseptic in cuts and wounds [7]. The leaf decoction is used against nerve disorders, the seed infusion against Chest pain and a leaf or root poultice against bleeding [8]. Mahogany seeds have also been reported to have medicinal value for treatment of cancer, amoebiasis, coughs and intestinal parasitism [9]. Decoction of seeds used as abortifacient, Used by Ifugao migrants for malaria, cough and miscarriages [10].

This paper presents a preliminary phytochemical investigation and study the Anti-microbial activity of the seeds of *Swietenia mahagoni Jacq.* The phytochemical investigations of seed of plant are carried from the following different

extractions. The solvent extraction process was processed by the following order of polarity as Petroleum ether, Benzene, Ethyl acetate and Ethanol. These extractions were evaluated for anti microbial activity as a part of pharmacological screening of *Swietenia mahagoni* Jacq.

MATERIALS AND METHODS

2.1 Plant collection and authentication [11]
The seeds of the plant of *Swietenia mahagoni Jacq* .were collected during the month of October 2013 in the premises of Ainapur Estate from Shimoga district of Karnataka and authenticated by Prof. Ajith Kumar, Department of Botany, Government College, Kasaragod, Kerala.

2.2 Phytochemical Investigations of *Swietenia mahagoni* Jacq.
2.2.1 Plant drying & size reduction [12]
The seeds of *Swietenia mahagoni* Jacq. were cut into small pieces of 2 cm and shade dried (20-25 ^{0} C) at room temperature. The plant material was stored in PVC bag and stored in dark & clean place in airtight container. The phytochemical investigations of a plant may involve the extraction of plant material and Identification of the phytoconstituents.

2.2.2 Extraction method [13-14]
About 500gm of the shade dried powder of seeds of *S.mahagoni* Jacq. was extracted successively with 2 litres of solvents viz., petroleum ether, benzene, ethyl acetate and methanol of increasing polarity using Soxhlet extractor.
All the above extracts were concentrated in vacuum using rotary-evaporator under reduced pressure. The residues were subjected for the antibacterial screening:

2.2.3 Identification of plant constituents by preliminary phytochemical tests [15-19]
The various extracts of the plant *Swietenia mahagoni Jacq***.** were subjected to phytochemical tests for identification of its active constituents.

Test for Alkaloids - A small portion of solvent free extracts were stirred separately with few drops of dilute hydrochloric acid and filtered and tested carefully with various alkaloidal reagents. **(**Mayer's reagent -Cream precipitate; Dragendorff's reagent-Orange brown precipitate; Hager's reagent-Yellow precipitate; Wagner's reagent-Reddish brown precipitate).

Test for Carbohydrates and Glycosides - The minimum amount of extracts were dissolved in 5ml of distilled water and filtered. The filtrate was subjected to test for carbohydrates and glycosides.

Molish test - The filtrate was treated with 2-3 drops of 1% alcoholic alpha naphthol and 2 ml concentrated sulphuric acid was added along the sides of test tube. Violet ring was observed at the junction of 2 layers which showed the presence of carbohydrate.

Legal's test - The hydrolysed solution (1ml), sodium nitroprusside solution (1 ml) was mixed and then it was made alkaline with sodium nitroprusside. Pink colour was observed which indicated of the presence of glycosides.

Borntrager's test – 1 ml Hydrolysate was treated with chloroform and the chloroform layer was separated. To this equal quantity of dilute ammonia solution was added. No colour change in ammoniacal layer was observed.

Test for Flavonoids – a) With aqueous sodium hydroxide solution - blue to violet colour (Anthocyanins); yellow colour (Flavones); yellow to orange colour (Flavonones), b) With concentrated sulphuric acid – yellowish orange colour (Anthocyanins); yellow to orange colour (Flavones); orange to crimson (Flavonones) c).

Shinoda's test : Test extracts were dissolved in alcohol, and then pieces of magnesium turnings followed by concentrated hydrochloric acid were added drop by drop and heated. Appearance of magenta colour showed the presence of flavonoids. d) **Test for Phytosterol**: 1 g of different extracts was dissolved in few drops of dilute acetic acid; 3ml of acetic anhydride was added followed by few drops of concentrated sulphuric acid. Appearance of bluish green colour showed the presence of phytosterol.

Test for Fixed oil & Fats – a) Small quantities of various extracts were separately pressed between two filter papers. No oil stain on the paper indicated the absence of fixed oil, b) Few drops of 0.5N alcoholic potassium hydroxide were added to small quantity of various extracts along with a drop of phenolphthalein. Mixture was

heated on water bath for 1-2 hours. No soap formation, neutralization of alkali indicated the absence of fixed oil and fats.

Test for Saponins- The extracts were diluted with 20 ml of distilled water and it was agitated on graduated cylinder for 15 minutes. The presence of saponins was indicated by formation of 1cm layer of foam.

Test for Tannins and Phenolic Compounds - Small quantities of various extracts were taken separately in water and tested for presence of phenolic compounds and treated with dilute ferric chloride solution (5%) gives violet colour, with1% sodium gelatin containing 10% sodium chloride gives white precipitate and with 10% lead acetate solution – white precipitate

Test for Lignin - With alcoholic solution of phloroglucinol and hydrochloric acid the appearance of red colour showed the presence of lignin.

Test for Proteins and Free Amino Acids - Small quantities of various extracts were dissolved in few ml of water and treated with **Millon's reagent** gives red colour showed the presence of proteins and free amino acids With **Ninhydrin reagent** gives Purple colour showed the presence of free amino acids. With **Biuret test** gives equal volume of 40% NaoH solution and 5% copper sulphate solutions were added. Appearance of violet colour showed the presence of proteins and free amino acids.

Test for Gums and Mucilage - Powdered drug was treated with ruthenium red solution. No characteristic colour change was obtained indicating the absence of gums and mucilage.

2.3 Screening of anti-microbial activity

Antimicrobial susceptibility test is done in order to find out the effectiveness of our plant seed extracts. As most of the pathogen develops drug resistance we need to find effective dose to kill or inhibit the infectious agents. In order to evaluate the antimicrobial activity we perform the study by Cup - Plate method based on CLSI 2011 Guideline procedure by Kirby bauer manual.

A. Preparation of standardized test organism

The clinical sample of the following strains were obtained from the national collection of industrial micro-organism at Pune and this organism was used for our studies. The microbial cultures were aseptically inoculated into nutrient broth medium and incubated under aerobic condition at 37°C for 24 hours. The sub culture was standardized by pour plate technique to have 1000cells per ml of culture. (*Staphylococcus aureus- MCC2408, Escherichia coli- MCC2246, Pseudomonas aeruginosa- MCC2080, Bacillus licheniformis- MCC2297*).

B. Preparation of standard antibiotic solution

The standard anti biotic used in this evaluation was ofloxacin and the standard drug solution was prepared at a concentration of 100 µg/ml. All the solutions were prepared by dissolving the drug in Dimethyl sulfoxide.

C. Preparation of test drug solution

All the newly prepared solvent extracts of *Swietenia mahagoni Jacq.* were dissolved in Dimethyl sulfoxide to prepare the different concentration (40mg/l, 60mg/l and 80mg/l) drug solution.

D. Media used for the evaluation of microbial activity

Constituents and Preparation of Mueller Hinton Nutrient Agar Media

Beef extract: 30gm, Casein hydrolysate: 1.75gm, Starch: 0.15gm, Agar: 1.7gm, pH: Neutral at 25^0 C, Distilled water: 100ml were weighed, placed in 100ml of water, adjusted to the pH 7.4 and distributed 30 ml each in different test tube closed with cotton then autoclaved at 121°C at 15 1b for 20 minutes.

Constituents of nutrient broth medium

Meat extract: 3gm, Peptone : 5gm, Sodium chloride: 8gm, Distilled water: 1000ml were weigh placed in 1000 ml of water, adjusted to the pH 7.4 and autoclaved at 121°C at 151b pressure for 15 minutes and used for subculture of all test organisms.

E. Procedure for cup plate method

For screening of antimicrobial activity by Cup Plate method, a commercial sample of Ofloxacin for both gram +ve and gram -ve organism were used as standard antibiotic [20-21].

Sterile petri dishes were filled to a depth of 4.5 mm with sterile nutrient agar medium that had previously been inoculated with a suitable inoculum of test organism of *P.aeruginosa-MCC2080, B.licheniformis-MCC2297,*

P.mirabilis-, S.aureus-MCC2408 and E.Coli-MCC2246 individually. When it was inoculated, the temperature of the Mueller Hinton agar medium was maintained at 45°C to 50°C. The petridishes were allowed to cool for 1 hour before to make cavities. With sterile borer of uniform size (0.5mm dia) aluminium tube, four wells were made in each petri plate (1 for standard and three for test). In similar manner, 0.1ml of the standard drug and test extract were poured into wells by means of 1ml sterile syringe. Almost care was taken to avoid spilling of drug on surface of medium and the zone of inhibition was measured after incubation at 37 °C for 24 hours. The experiment repeated for three times.

RESULTS AND DISCUSSION

3.1 Extractive value and percentage yield

About 500gm of the shade dried powder of seeds of *S.mahagoni* Jacq. was extracted successively with 2 liters of solvents viz., petroleum ether, benzene, ethyl acetate and methanol of increasing polarity using Soxhlet extractor and concentrated in vacuum using rotary-evaporator under reduced pressure.(Table-1)

Table 1: Successive Solvent Extraction of Swietenia *mahagoni* Jacq

S. No.	Extracts	Colour	Percentage yield of extracts of *Swietenia mahagoni Jacq.* (w/w)
1.	Petroleum ether	brown colour	1.45
2.	Benzene	pale brown colour	1.68
3	Ethyl actate	dark brown colour	10.24
4.	Ethanol	yellowish brown colour	12.86

Table 2: Data showing the preliminary phytochemical screening of *Swietenia mahagoni* Jacq. *seed* extracts

Phytoconstituents	Petroleum ether extract	Benzene extract	Ethyl acetate extract	Ethanol extracts
Alkaloids	(+)	(+)	(+)	(+)
Carbohydrates	(+)	(+)	(+)	(+)
Glycosides	(-)	(-)	(+)	(+)
Flavonoids	(+)	(+)	(+)	(+)
Phytosterols	(+)	(-)	(+)	(+)
Fixed oils and Fats	(-)	(-)	(-)	(+)
Saponins	(+)	(-)	(+)	(+)
Phenolic compounds and Tannins	(+)	(+)	(+)	(+)
Lignins	(+)	(+)	(+)	(+)
Proteins and free Amino acids	(+)	(+)	(+)	(+)
Gums and Mucilage	(+)	(+)	(+)	(+)

(+) Presence (-) Absent

3.2 Observation of antimicrobial screening of different extracts

Table-3 Antimicrobial activity of p*etroleum Ether Extract of Swietenia mahagoni Jacq*

Concentration	Zone of inhibition *(mm)			
	S. aureus	*B.licheniformis*	*E.Coli*	*P.aerugino*
STD Ofloxacin (100µg)	24 ± 0.707	32.83 ± 1.47	31.33 ± 1.08	32 ± 0.707
40mg (C_1)	18 ± 0.707	06 ± 0.707	10.33 ± 1.08	09 ± 0.707
60mg(C_2)	19.66±1.08	09 ± 0.707	09 ± 0.707	19 ± 1.77
80mg(C_3)	20.66±1.47	06 ± 0.707	07 ± 0.707	07 ±0.707

**Mean ±SEM ,n=3*

Table-4 Antimicrobial activity of benzene extract of Swietenia *mahagoni* Jacq

Concentration	Zone of inhibition * (mm)			
	S. aureus	*B.licheniformis*	*E.Coli*	*P.aerugino*
STD Ofloxacin(100µg)	33 ± 0.577	36 ± 0.577	37 ± 0.577	36.33 ± 0.882
40mg (C_1)	13 ± 0.577	12 ± 0.882	19.66 ± 0.882	14 ± 0.577
60mg (C_2)	14± 0.882	15 ± 0.577	15 ± 1.202	16.66 ± 0.882
80mg (C_3)	15 ± 1.453	14 ± 1.528	11 ± 0.577	19 ± 0.577

**Mean ± SEM, n=3*

Table-5 Antimicrobial activity of ethyl acetate extract of *Swietenia mahagoni* Jacq

Concentration	*Zone of inhibition** (mm)			
	S. aureus	*B.licheniformis*	*E.Coli*	*P.aerugino*
STD Ofloxacin(100µg)	40.66 ± 1.202	33 ± 0.577	28 ± 0.577	30 ± 0.577
40mg(C_1)	30 ± 0.577	25.3 ± 1.202	33.6 ± 0.882	27 ± 0.577
60mg(C_2)	29 ± 0.577	24 ± 0.577	32 ± 0.577	27.6 ± 0.882
80mg(C_3)	21.66 ± 0.882	34.6 ± 1.202	31 ± 0.577	31 ± 0.882

**Mean ± SEM, n=3*

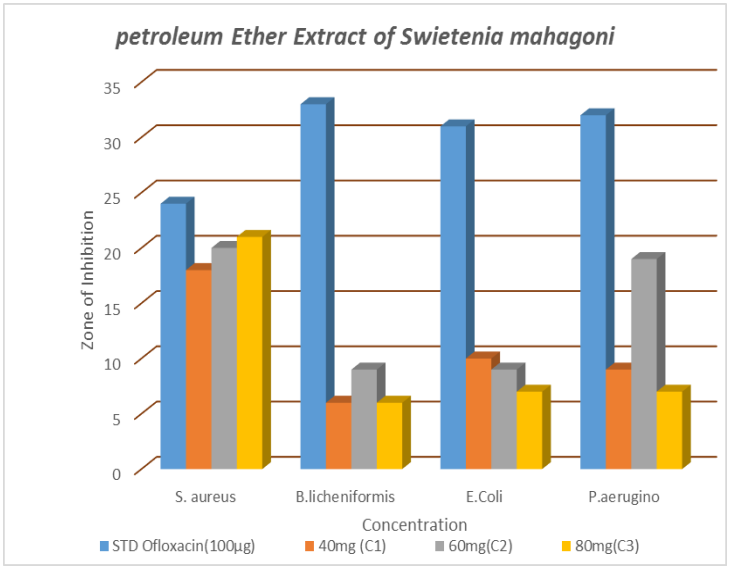

Fig. 1 Comparison of antimicrobial activity of *petroleum Ether Extract of Swietenia mahagoni Jacq*

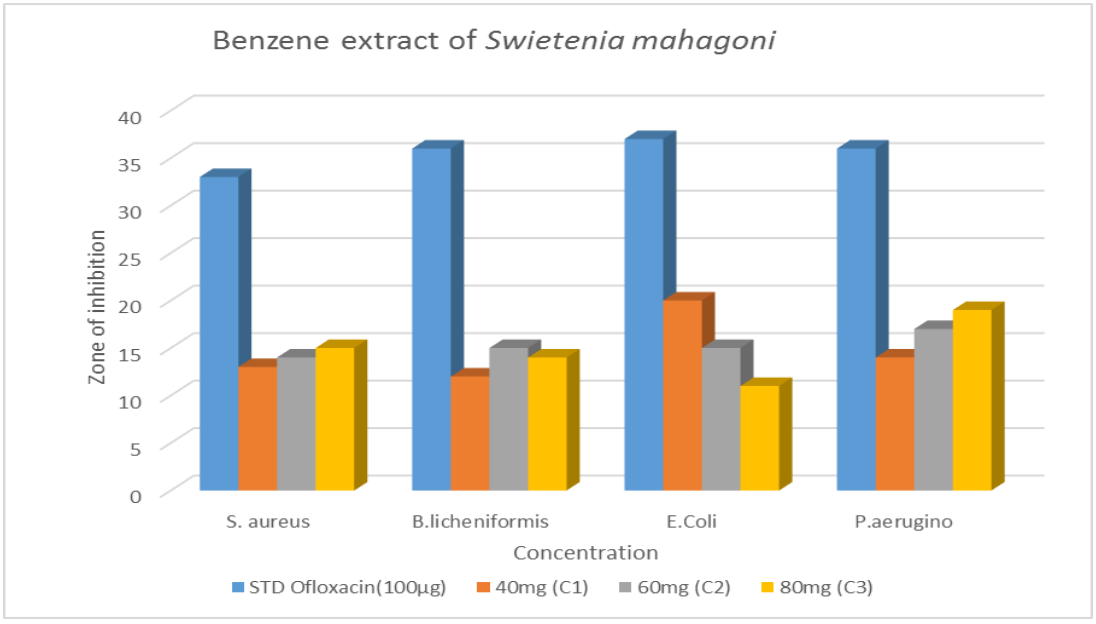

Fig.2 Comparison of antimicrobial activity of benzene extract of Swietenia *mahagoni* Jacq

Table-6 Antimicrobial activity of ethanolic extract of *Swietenia Mahagoni* Jacq

Concentration	Zone of inhibition* (mm)			
	S. aureus	***B.licheniformis***	***E.Coli***	***P.aerugino***
STD Ofloxacin(100µg)	38 ± 0.707	41 ± 0.707	40 ±0.707	38 ± 0.707
40mg (C_1)	40.3 ± 1.080	13 ± 0.707	37 ± 0.707	21.33 ± 1.47
60mg (C_2)	42 ± 0.707	11 ± 0.707	42 ± 0.707	27 ± 0.707
80mg (C_3)	41 ± 0.707	39.33 ± 1.08	19.6 ± 1.08	39 ± 1.08

**Mean ± SEM, n=3*

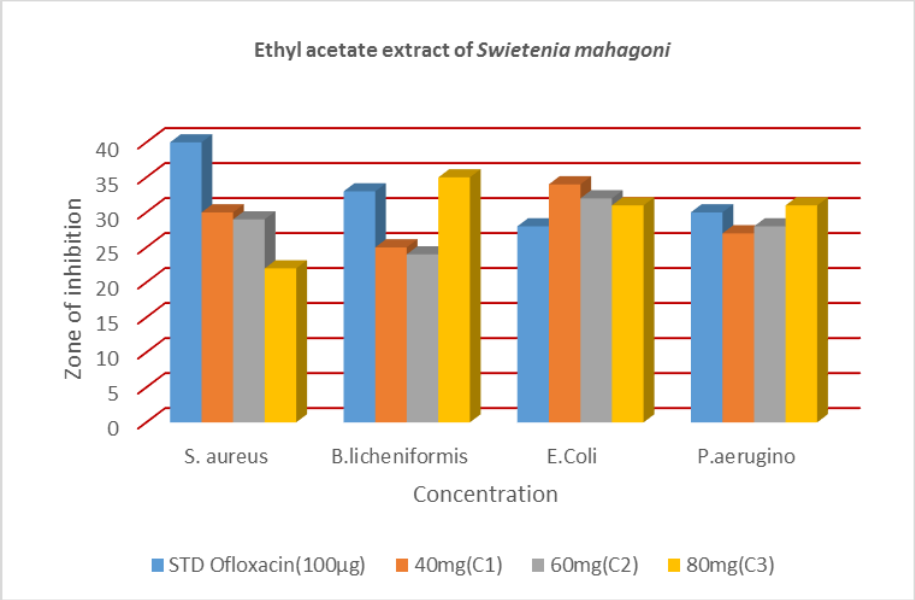

Fig.3 Comparison of antimicrobial activity of ethyl acetate extract of *Swietenia mahagoni* Jacq

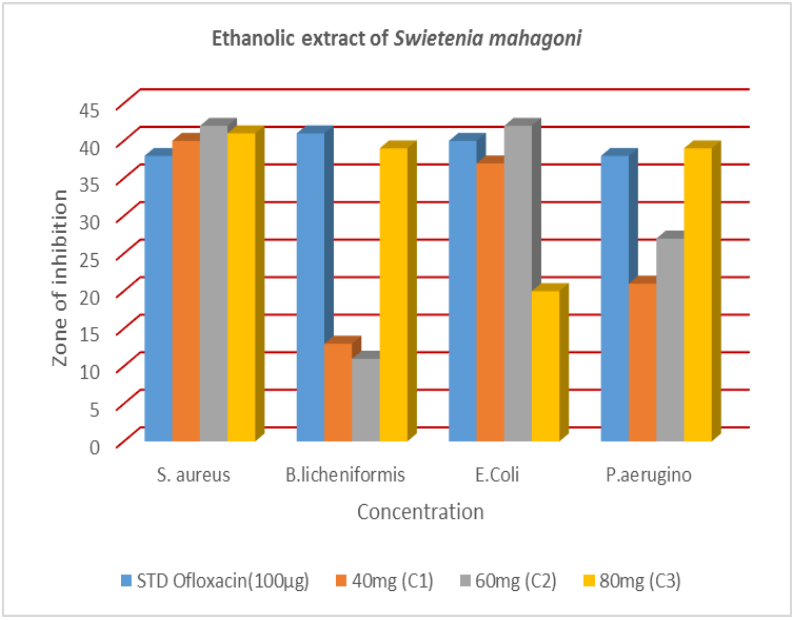

Fig.4 Comparison of antimicrobial activity of ethanolic extract of *Swietenia Mahagoni* Jacq

The zone of inhibition of microbes against standard and different concentrations of all extracts *Swietenia mahagoni* Jacq.

The zone of inhibition in diameter of the extracts were measured and reported in the tables 4-7. All the reading were interpreted by referring CLSI Guidelines 2011 based on kirbybauer method of anti microbial sensitivity analysis.

CONCLUSION

The ethanolic extract and ethyl acetate extract of *Swietenia mahagoni* Jacq. Seed at different dose (40, 60,80mg/ml) levels showed significant anti microbial activity by kirby' baueranti microbial evaluation manual. The result of ethanolic and ethyl acetate extract of *Swietenia mahagoni* Jacq. Seeds indicated that all the tested organisms were shown suceptibility against the different dose (40mg, 60mg, 80mg/ml) of ethanolic and ethyl acetate extract of *Swietenia mahagoni* Jacq. , based on CLSI Guidelines 2011(susceptible ≥ 17, intermediate susceptible 15-16 and

resistant ≤ 14 zone diameter in mm). Hence from the significant antimicrobial activity at the different concentration dose level from the above results, it may be concluded that the ethanolic and ethyl acetate extract of *Swietenia mahagoni* Jacq. , Seed is a nontoxic and safe herbal drug. The plant can be considered as low cost, potent, herbal medicine for good anti-microbial activity.

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