Original Research Article

Phytochemical and Antimicrobial Screening of *Tephrosia calophylla* Bedd.

ABSTRACT:

Tephrosia calophylla, (Fabaceae) a perennial woody under shrub endemic to south India. It is one of 13 rare or threatened *Tephrosia* species. Commonly it is known as Adavivempali. The various species of *Tephrosia* is ascribed to have many medicinal and therapeutic uses. The importance of this study was to preliminary screening of different phytochemical constituents for the detection of various secondary metabolites and evaluation of antibacterial, antifungal activity and Minimum Inhibitory Concentration of the different crude extracts of tuber and leaf. Tuber and leaf both yielded more number of secondary metabolites like alkaloids, phenols, flavonoids tannins, saponins and glycosides with high quantity when compared with the leaf, consisting low quantities of phyto-constituents as steroids and in tuber consisting only tannins. Antibacterial activity of *T. calophylla* tuber and leaf aqueous and alcohol extracts at 10 mg/well are showing more effective activity on *Bacillus subtilis* (MTCC-441), *Escherichia coli* (MTCC-443), *Pseudomonas aeruginosa* (MTCC-741), *Klebsealla neumoniae*, *Proteus vulgaris* strains than the control drug *Ampicillin* 10 mg/well with 30.25-15.00 mm zone of inhibition. Minimum inhibitory concentration (MIC) with leaf and tuber extracts was 0.312 to 2.50 mg compared to that of the 10 mg of *Ampicillin*. Antifungal screening of aqueous leaf extract was more effective on *Candida albicans* with 19.25 mm zone of inhibition than *Aspergillus niger* at 10 mg/well compared to *Nystatin* the control drug at 10 mg/well with 10.2 to 12.1 mm of zone of inhibition. Fungal MIC on both organisms with leaf and tuber extracts ranges from 0.612 mg to 3 mg compared to 10 mg of *Nystatin*.

Comment [H1]: The title must specify the type of extracts

Comment [H2]: It is a concentration, but here there is a a weight
The minimum not minimum

Comment [H3]: We say inhibition zone

KEY WORDS: *Tephrosia calophylla*, Phytochemical, Antimicrobial screening, Minimum Inhibitory Concentration.

1. INTRODUCTION

The different crude extracts of medicinal plants parts are used with their phytochemical compounds of known antimicrobial activities, can be of great importance in the different therapeutic treatments. In the present years, a number of works have been conducted in various countries to prove such efficiency. Number of species has been used for their antimicrobial traits, which are due to the secondary metabolites synthesized from the plants. These products are known by their active compounds like, phenolic compounds, alkaloids, flavonoids etc. The screening of plant parts for their antimicrobial activity has shown that the most of the plants represents a potential source of novel antibiotic effect. The **Fabaceae or Leguminosae** commonly known as the pea, legume, or bean family, is a large and economically important family of flowering plants. *Tephrosia* is a genus of plant which is of Indian origin. The number species of *Tephrosia* are medicinally proved for their various pharmacological activities [1, 2]. As *T. calophylla* belongs to same genus and also an important in traditional system of medicine like ayurveda and used as antimicrobial [3], heapatoprotective [4], antihyperlipidemic [5], cytotoxic [6], antiprotozoal [7], anticancer and anti –HIV [8], anthelmintic [9] and antiulcer drug [10]. It is also used as alternative cure for diseases of the liver, spleen, heart and blood. In this attempt, study had been conducted to determine phytochemical and anti microbial potentials of tuber and leaf extracts of *T. calophylla*.

2. MATERIAL AND METHODS

Collection and identification of plant material:

The tuber and leaf material of *T. callophyla* were collected during September - December 2017 from Talakona forest in Tirupati, Andhra Pradesh, India. The taxonomic identification of the plant is confirmed by Prof. N. Yasodamma. The voucher specimen B.K:3 were deposited in the herbarium, (RUK) Department of Botany, Rayalaseema University, Kurnool for future reference as per standard methods [11]. The present work was also carried out in the same Department. Plant material was thoroughly washed and then shade

dried for one week. The dried parts were ground in a mixer grinder and sieved. The powder was stored in air tight containers at room temperature for further use.

Phytochemical study.

Comment [H4]: Is it a title?

Preliminary Photochemical Screening:

To detect the different classes of secondary metabolites in the crude extracts of tuber and leaf of *T. callophyla*a preliminary phytochemical analysis was undertaken by adopting standard qualitative methods [12, 13, 14 &15].

Crude drug preparation of aqueous and organic solvent extracts:

Dried tuber and leaf powder (50 g in 250 ml) were extracted with aqueous, acetone, alcohol, benzene, chloroform, ethyl acetate, methanol and petroleum ether. The drug was soaked for 72 hrs. and the filtered extract was dried on water bath than stored at 4^0C in refrigerator.

Preparation of test solutions:

The preliminary tests for the detection of secondary metabolites was carried out for all the extracts (Methanol, ethanol, ethyl acetate, chloroform, benzene, acetone, petroleum ether and aqueous) of tubers and leaves. 500 mg of each extract was dissolved in 100 ml of the respective solvent and filtered through Whatman filter paper No.1. Thus the filtrate obtained was used as test solution for the following preliminary phytochemical screening tests.

Tests for Alkaloids: The test sample (crude extract) was dissolved in chloroform and the solution was extracted with dil. H_2SO_4 or dil. HCl and acid layer was taken and tested for presence of alkaloids.

1) Mayer's test: To the acidic solution, Mayer's reagent (Potassium mercuric iodide solution) was added. Cream colored precipitate indicates the presence of alkaloids.
2) Wagner's test: To the acidic solution, Wagner's reagent (Iodine in potassium iodide) was added. The formation of reddish brown precipitate indicated the presence of alkaloids.

Tests for Flavonoids: The test solution of the extract was dissolved in one ml of alcohol and then subjected to the following tests:

1) Ferric Chloride test: A few drops of neutral ferric chloride solution were added to one ml each of above alcoholic solution. Formation of blackish red colour indicates the presence of flavonoids.

2) Shinoda's test: To one ml of alcoholic extract, a small piece of magnesium ribbon or magnesium foil was added and few drops of conc. HCl were added. Change in colour (from red to pink) shows the presence of flavonoids.

3) Zinc-HCl reduction test: A pinch of zinc dust and a few drops of conc. HCl were added to alcoholic extract. Magenta colour indicates the presence of flavonoids.

4) Lead acetate test: To one ml of alcoholic extract, a few drops of aqueous basic lead acetate solution was added. Reddish brown bulky precipitate indicates the presence of flavonoids.

Test for Phenols:

1) Phenol test: A positive reaction is the development of intense colour by the addition of ferric chloride solution to the test solution.

2) Ellagic acid test: Test solution of the crude extract was treated with a few drops of 5% acetic acid and few drops of 5% sodium nitrate solution. Formation of muddy or niger brown precipitate indicates the presence of phenols.

Test for Glycosides:

1) Kellar Kilani test: The test solution of the extract was dissolved in glacial acetic acid and after cooling, 2 drops of ferric chloride solution is added to it. These contents are transferred to a test tube containing 2 ml of concentrated sulphuric acid. A reddish brown colour ring was observed at the junction of two layers.

Test for Tannins: The test solution of the extract was dissolved in minimum amount of water, filtered and the filtrates were thus subjected to the following test:

1) Ferric chloride test: To the filtrate, a few drops of ferric chloride solution were added. A blackish precipitate indicates the presence of tannins.

2) Gelatin test: To the filtrate, gelatin (Gelatin dissolves in warm water immediately) solution was added. Formation of white precipitate indicates the presence of tannins.

3) Lead acetate test: To the filtrate, a few drops of aqueous basic lead acetate solution were added. Formation of reddish brown bulky precipitate indicates the presence of tannins.

Test for steroids: The test solution of the extract was dissolved in 5ml of chloroform separately and was subjected to the following tests:

1) Salkowski test: One ml of conc. sulphuric acid was added to the above solution and allowed to stand for 5 minutes after shaking. Lower layer turning into golden yellow colour indicates the presence of steroids.

2) Liebermann Burchard test: To one ml of the extract treated with chloroform, a few drops of acetic anhydride, one ml of conc. H_2SO_4 were added from the sides of the test tube and allowed to stand for 5 minutes. Formation of brown ring at the junction of the two layers and the upper layer turning green indicates the presence of steroids.

Test for Quinones: The test solution of the extract was treated separately with alcoholic potassium hydroxide solution. Quinones give coloration from red to blue.

Test for Lignins:

1) Labat test: The test solution is mixed with gallic acid, it develops olive green colour indicating the positive reaction for lignins.

2) Lignin test: Formation of red colour, when 2% (W/V) furfuraldehyde is added to the test solution indicates the presence of lignin.

Test for Saponins: The test solution was separately mixed with 20 ml of distilled water and then agitated in a graduated cylinder for 15 minutes. Foam formation indicates the presence of saponins.

Test for Fixed oils:

1) The test solution of the extract was pressed separately between two filter papers. Formation of transparent spot indicates the presence of fixed oils.

2) A few drops of 0.5N alcoholic potassium hydroxide were added to the solution of the extract with a few drops of phenolphthalein as indicator and mixture was heated for 1 - 2 hrs. Soap formation shows the presence of fixed oils.

Antimicrobial activity

Test organisms:

Comment [H5]: What is this?

Comment [H6]: a whole section without a reference !!!

Comment [H7]: •Not "Test organisms" but Antimicrobial test

Pure bacterial cultures of *B. subtilis* (MTCC-441), *E. coli* (MTCC-443), *P. aeruginosa* (MTCC-741), *K. pneumoniae*, *P. vulgaris* (Clinical isolates) and fungal cultures of *C. albicans* (ATCC-10231) and *A. niger* (ATCC-16404) were procured from department of microbiology, S.V. University and Sri Venkateswara institute of medical sciences, Tirupati. These were further maintained on nutrient agar slants at 4^0C until further use.

Comment [H8]: What is MTCC?

Preparation of the bacterial medium:

To prepare 1 lit of nutrient agar medium 5 gm of beef extract, 3 gm of sodium chloride, 3g of peptone, 15 gm of agar were accurately weighed using digital electronic balance and dissolved in 1 litre of distilled water before the addition of agar, the P^H of the medium was adjusted to 7.2 by adding few drops of 0.1N NaOH/HCl using digital P^H meter. Later this medium was transferred to conical flasks and plugged with non-absorbent cotton. These were then sterilized by autoclaving at 15 lbs for 20 minutes, cooled to 40^0C and used for the study.

Preparation of the fungal medium:

To prepare 1 lit of potato dextrose sugar medium 200 g of potato slices were boiled with distilled water. The potato infusion was used as water source of media preparation. 20 g of dextrose was mixed with potato infusion. 20 grams of agar was added as a solidifying agent. These constituents were mixed and autoclaved at 15 lbs for 20 minutes cooled to 40^0C and used for further study.

Comment [H9]: It is not necessary to put the preparation of the mediums because they are very known

Agar well diffusion method:

Antibacterial and antifungal activities of the leaf and tuber extracts were determined by using agar well diffusion method with slight modifications [16]. Nutrient agar was inoculated with the selected microorganisms by spreading the bacterial and fungal inoculums on the media. Four agar wells (9 mm, diameter) were made in each plate equidistantly by cutting out the media using sterile broad end (8.5 mm) of micropipette tip, in order to load test solutions and are filled with 10 mg/well of the extracts in quadruplicates. Control wells containing pure solvents (negative control) or standard antibiotic (positive control) viz., *Ampicillin*10 mg/well, *Nistatin* 10 mg/well. The plates were incubated at 37^0C for 24 hrs for bacterial and 25^0C for 48 hours for fungal activity. The antimicrobial activity was

assessed by measuring the diameter of the zone of inhibition for the respective drug. The relative antimicrobial activity was calculated by comparing its zone of inhibition with that of the standard drug. The data of crud drug activity is given the mean of quadruplicates along with the standard error.

Comment [H10]: No references!!!!

Statistical analysis:

The results were analyzed for statistical significance using One way ANOVA followed by Dunnet[t]'s test. The $p < 0.01$ and $p < 0.05$ was considered significant.

Evaluation of minimum inhibitory concentration (MIC):

Minimum Inhibitory Concentration was determined by broth dilution method [17, 18]. Extracts to be tested were taken ranging from 10 mg/ml. It involves a series of nine tubes for each test extract against each strain. To the first assay tube 4 ml of broth was transferred and then 4 ml of test extracts of 10 mg/4 ml was added and mixed thoroughly. To the remaining nine assay tubes, from the first tube 4 ml of the content was pipette out into second test tube and this was mixed thoroughly. This twofold serial dilution was repeated up to ninth tube. 0.2 ml of the inoculums was added to all test tubes and also to the control tubes were taken aseptically and incubated for 24 hrs. Next day the absorbance was measured by calorimeter at 600 nm for bacterial and at 530 nm for fungal broth cultures. Bacterial MIC was compared with the control *Ampicillin* (10 mg/ml) and for fungal MIC was compared with the control *Nystatin* (10 mg/ml) and minimum inhibitory concentration mg/ml was determined.

Comment [H11]: ???

Comment [H12]: Bacterial density must be red at 620nm

Comment [H13]: No references

3. RESULTS AND DISCUSSION

Phytochemical studies

Preliminary Phytochemical screening (Table-1)

Tuber and leaf both yielded highest quantity and more number of secondary metabolites like alkaloids, phenols, flavonoids tannins, saponins and glycosides, followed by leaf consisting low quantities of phyto-constituents as steroids, whereas tuber consisting low quantities of tannins. Quinones and lignins are totally absence in benzene extract of tuber.

212 **Table-01: Preliminary phytochemical screening of leaf and tuber extracts of *T.***

213 ***calophylla***

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TEST	Leaves								Tubers							
	AC	**AQ**	**AL**	**BE**	**CH**	**EA**	**ME**	**PE**	**AC**	**AQ**	**AL**	**BE**	**CH**	**EA**	**ME**	**PE**
Alkaloids																
Mayers	+	++	-	+	++	-	-	+	++	+	+	++	++	+	+	-
Wagner's	++	+	-	+	-	-	-	-	++	-	-	++	++	+	++	+
Flavonoids																
Shin dons	++	++	-	-	-	-	++	-	-	++	-	-	-	-	++	-
$FeCl_3$	-	+	-	-	-	-	-	+	++	++	-	+	+	++	++	-
Phenols																
$FeCl_3$	++	++	++	++	++	-	++	++	++	+	++	-	++	+	-	-
Ellagic acid	-	-	-	-	++	-	-	-	-	-	++	++	-	-	-	-
Glycosides																
Keller – Kilani	++	++	+	+	-	-	+	+	++	++	++	++	-	++	++	++
Tannins																
$FeCl_3$	+	+	-	-	++	-	-	-	++	++	-	-	-	-	-	-
Steroids																
Salkowski	+	+	-	++	++	-	++	-	-	+	++	-	++	++	++	++
Quinones	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-
Lignins																
Labat test	++	-	++	+	+	-	-	-	-	-	-	-	-	-	-	-
Saponins	++	-	++	++	-	-	++	++	-	+	-	-	++	-	-	++

"++" indicates -Abundant presence; **" +" indicates** -(Slightly presence); **"– "indicates** - Absent

AC: Acetone,**AL:** Alcohol,**AQ:** Aqueous, **BE:** Benzene, **CH:** Chloroform, **EA:** Ethyl acetate,**ME:** Methanol, **PE:** Petroleum ether

Antibacterial Activity (Zone of inhibition in mm) (Plate-1Table-2, Figure-1)

Antibacterial activities of leaf and tuber aqueous extracts were showing more effective activity with 30.25 mm zone of Inhibition on *E. coli* than other extracts. It is also observed that there is no activity of leaves and tubers with petroleum ether extracts on all organisms. Antibacterial activity of *T. calophylla* leaf and tuber aqueous and alcohol extracts at 10 mg/well are showing more effective activity on all selected gram + ve and gram – ve bacterial strains than the control drug *Ampicillin* 10 mg/well with 30.25-15.00 mm zone of inhibition.

Table-2: Antibacterial Activity (Zone of Inhibition in mm) of leaf and tuber extracts of *T. calophylla*

Organism	***B. subtilis***	***E. coli***	***K. neumoniae***	***P. aeruginosa***	***P. vulgaris***
Leaves					
Ac	0.0±0.0	0.0±0.0	8.25±0.43**	16.75±0.82*	0.0±0.0
Al	0.0±0.0	0.0±0.0	15.00±0.00	0.0±0.0	0.0±0.0
Aq	0.0±0.0	30.25±0.43**	20.25±0.43	19.75±0.82**	15.00±0.00
Be	0.0±0.0	0.0±0.0	15.25±0.43	0.0±0.0	0.0±0.0
Ch	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	12.00±0.00

Comment [H14]: This word is to remove

Comment [H15]: to remove

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Ea	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
Me	17.50±0.50***	25.00±0.70**±	20.25±0.43***	20.50±0.50	19.50±0.50***
Pe	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
Tubers					
Ac	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
Al	25.50±0.50***	17.75±0.43**	24.50±0.50***	19.75±0.43***	15.5.±0.50
Aq	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	12.25±0.50*
Be	0.0±0.0	9.50±0.50**	0.0±0.0	8.50±0.50**	12.50±0.50*
Ch	11.75±0.43***	12.25±0.23**	10.50±0.50***	13.50±0.50	11.5±0.50**
Ea	0.0±0.0	10.25±0.43**	0.0±0.0	8.25±0.43**	0.0±0.0
Me	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	10.25±0.43***
Pe	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
***Amp* (CON)**	14.83±0.11	20.33±0.23	19.2±0.43	13.66±0.06	16.23±0.23

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235 **AC:** Acetone, **AL:** Alcohol, **AQ:** Aqueous, **BE:** Benzene, **CH:** Chloroform, **EA:** Ethyl acetate,

236 **ME:** Methanol, **PE:** Petroleum ether

237

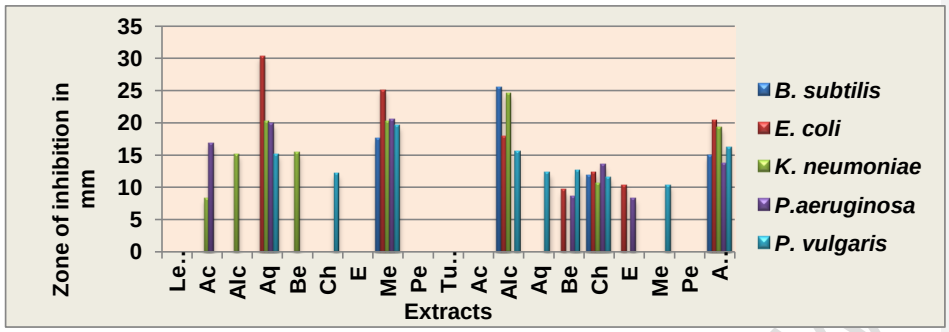

Figure-1: Antibacterial Activity of leaf and tuber extracts of *T. calophylla*

Comment [H18]: the table and the figure represent the same result, it is a repetition

MIC for Antibacterial Activity

Minimum Inhibitory Concentrations with leaf and tuber extracts at 0.312 to 2.50 mg compared to that of the 10 mg of *Ampicillin*.

Antifungal Activity (Plate-2, Table-3, Figure-2)

Antifungal activity of leaves aqueous extracts was more effective on *C. albicans* with 19.25 mm zone of inhibition than *A. niger* at 10 mg/well when compared to *Nystatin* the control drug at 10 mg/well with 10.2 to 12.1 mm of zone of inhibition. Benzene extracts has not shown any antifungal activity on both organisms.

Table-3: Antifungal Activity (Zone of Inhibition in mm)

Comment [H19]: to remove

Organism	*A. niger*	*C. albicans*
Leaf		
Ac	12.5±0.50**	9.5±0.50**
Al	8.5±0.50	10.5±0.50*
Aq	0.0±0.0	19.25±0.43**

Comment [H20]: what do these asterisks represent? explain

Be	00.0±0.0	10.0±0.00**
Ch	10.5±0.50	00.0±0.0
Ea	00.0±0.0	00.0±0.0
Me	9.25±0.43	5.25±0.43**
Pe		
Tuber		
Ac	10.5±0.50**	9.5±0.50
Al	11.75±0.43	10±0.0**
Aq	00.0±0.0	00.0±0.0
Be	00.0±0.0	10.25±0.43**
Ch	11.50±0.50**	12.0±0.00*
Ea	9.5±0.50**	8.5±0.50**
Me	8.25±0.43*	00.0±0.0
Pe	5.50±0.50**	00.0±0.0
***Nys*(CON)**	10.20±	12.10

AC: Acetone, **AL:** Alcohol, **AQ:** Aqueous, **BE:** Benzene, **CH:** Chloroform, **EA:** Ethyl acetate, **ME:** Methanol, **PE:** Petroleum ether

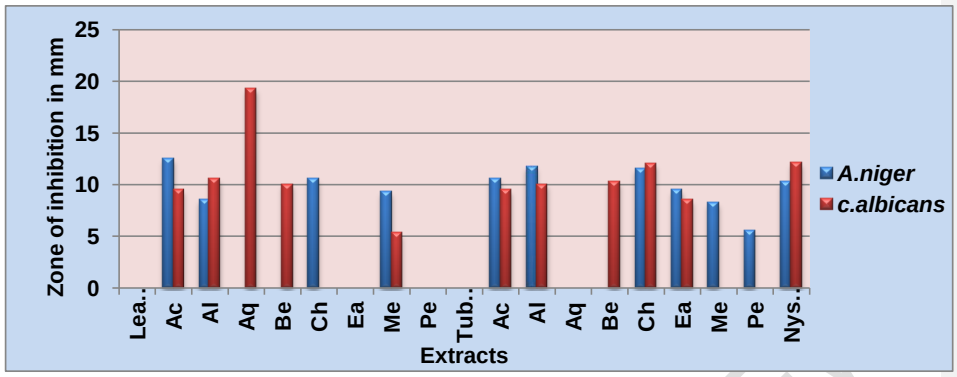

Figure-2: Antifungal Activity of leaf and tuber extracts of *T. calophylla*

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MIC for Antifungal Activity

Fungal Minimum Inhibitory Concentrations on both organisms with leaf and tuber extracts ranges from 0.612 mg to 3 mg compared to 10 mg of *Nystatin*.

DISCUSSION

Now a day's plant based drug development is very useful in primary health care because of most of the synthetic drugs creates different side effects. *T. calophylla* leaf and root different extracts were capable of suppressing the test organisms such as *B. subtilis, E. coli, S. aures, K. pneumonia, P. aeuriginosa, P. vulgaris*, *C. albicans* and *A. niger*. This work was also agrees with the work of (Rama devi, 2014). By conducting qualitative analysis I found that alkaloids, phenols, flavonoids, tannins, saponins, steroids and glycosides are present in the different extracts of root and leaf of *T. calophylla*. This work also supports the traditional use of this plant in therapeutic use against microbial infections. The antibiotic principles of plants may be the presence of phytoconstituents like alkaloids, flavanoids and glycosides (Hafiza, 2000).

Comment [H22]: to be rephrased

Comment [H23]: we never use personal pronouns

Comment [H24]: does not exist in the list

4. CONCLUSION

The results revealed that the methanol, aqueous and ethyal acetate extracts were showing more effective zone of inhibition than the standard drug *Ampicillin.* Acetone and methanol

extracts which were showing 0.312 mg of Minimum Inhibitory Concentration (Bacterial, Fungal) are advised as the drug dosages for the preparation of standard drugs against bacterial pathogens, especially ***P. vulgaris*** (causing urinary track infections) and fungal pathogens like ***C. albicans*** (causing mucous membrane infections).

NOTE:

The study highlights the efficacy of "Ayurved" which is an ancient tradition, used in some parts of India. This ancient concept should be carefully evaluated in the light of modern medical science and can be utilized partially if found suitable.

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